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# Zoosystematics and Evolution

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# Zoosystematics and Evolution
A Bulletin of Zoology since 1898

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# Descriptions of new species of *Issikiomartyria* (Lepidoptera, Micropterigidae) and a new genus *Melinopteryx* gen. n. with two new species from Japan

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*http://zoobank.org/7D111266-5F9F-4D1E-BEAC-7E405F379DB9*

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## Abstract

Micropterigidae is considered to be the sister group of all other extant Lepidoptera. In Japan, 17 species of five genera have been recorded including three endemic genera, *Issikiomartyria* Hashimoto, 2006, *Kurokopteryx* Hashimoto, 2006 and *Neomicropteryx* Issiki, 1931, all of which are associated with the liverwort genus *Conocephalum* Hill. We discovered four new species of *Issikiomartyria* from snowy regions in Northeastern Japan, and two new species of a new genus *Melinopteryx* **gen. n.** from the subalpine zone of the Akaishi Mountain Range. All these new taxa, *I. hyperborea* **sp. n.**, *I. leptobelos* **sp. n.**, *I. catapasta* **sp. n.**, *I. trochos* **sp. n.**, *M. coruscans* **sp. n.** and *M. bilobata* **sp. n.** are also associated with *Conocephalum* liverworts. Furthermore, females of *I. akemiae* Hashimoto, 2006 and *I. plicata* Hashimoto, 2006 are described here for the first time. Our extensive surveys revealed that the fine-scale endemism of *Issikiomartyria* restricted to the fragmented area facing the Japan Sea. Keys to *Issikiomartyria* species based on the adult morphology are provided.

## Introduction

Micropterigidae represents one of the branches in the first splitting event among extant Lepidoptera (Kristensen 1999, Kristensen et al. 2007). The fossil record of Micropterigidae extends to the Early Cretaceous (Whalley 1978) and likely originated during or before the Late Triassic (Zhang et al. 2013, van Eldijk et al. 2018). The Micropterigidae is a non-glossatan moth family, which along with Agathiphagidae and Heterobathmiidae are devoid of a coilable proboscis and instead retain functional mandibles which in micropterigids only are functional in the adult (Kristensen 1984, 1999, Kristensen et al. 2015, Regier et al. 2015). The Micropterigids are species-rich, comprising about 160 described species in 21 genera from all biogeographic regions (Gibbs 2010, van Nieukerken et al. 2011), including four fossil genera (Whalley 1978, Kozlov 1988, Skalski 1995, Engel and Kinzelbach 2008), and more than 100 species have been found which are still undescribed (van Nieukerken et al. 2011). The geographic ranges of the micropterigids are patchy, with a concentration of endemic genera in the Australian ecozone and the Japanese Archipelago, where four and three genera are known respectively (Gibbs 2010, 2014, Hashimoto 2006). The global diversity and high endemism of the micropterigids provide us with a wealth of opportunities for understanding of the biogeographic patterns and processes suggested by primitive moths (Gibbs 1983, 2006, Gibbs and Lees 2014) whose evolution and ecological associations largely predates the diversification of angiosperms (Imada et al. 2011).

Micropterigidae in Japan comprise 17 described species in five genera: namely, *Micropterix* Hübner, 1825, *Paramartyria* Issiki, 1931, *Neomicropteryx* Issiki, 1931, *Kurokopteryx* Hashimoto, 2006, and *Issikiomartyria* Hashimoto, 2006. *Micropterix* is a genus distributed

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over the Palearctic ecozone as far as the Himalayan foothills (Lees et al. 2010); in Japan, *M. aureatella* (Scopoli, 1763) patchily inhabits Hokkaido and subalpine zones of Honshu (Issiki 1953; Hashimoto 2006, Imada et al. 2011). Among East Asian *Paramartyria*, two species are known only from Japan (Issiki 1931): *P. immaculatella* Issiki, 1931 and *P. semifasciella* Issiki, 1931. Three genera, *Neomicropteryx*, *Kurokopteryx*, and *Issikiomartyria*, are endemic to Japan and the species ranges are often restricted in small geographic areas in a strongly allopatric fashion (Hashimoto 2006, Imada et al. 2011).

All species of Japanese endemic genera, comprising at least 14 species, feed exclusively on *Conocephalum* Hill. liverworts and also seem to occupy similar ecological niches (Hashimoto 2006, Imada et al. 2011). Remarkably, the liverwort-feeding micropterigids in Japan have experienced diversification without changing host-plants, during or after the Miocene (Imada et al. 2011), which represent one of the largest radiations known to have taken place on a single host-plant genus. These *Conocephalum*-feeders are widely distributed across the major islands of Japan (i.e. Honshu, Shikoku, Kyushu) yet the species do not co-occur with one another (Imada et al. 2011).

Our thorough field surveys for molecular phylogenetic analysis have revealed that the micropterigid fauna in the Northeastern Japan is unexpectedly diverse. We reveal a new genus that inhabits an elevational zone of ca. 1500–1800 m in the Akaishi Mountain Range, which is sister to *Issikiomartyria* in the molecular phylogenetic analysis of Imada et al. (2011). In addition, we newly discovered that four new species of *Issikiomartyria* were distributed across the area ranging from the northern end of Honshu to the northern part of Yamagata Prefecture, which has not sufficiently been investigated. *Issikiomartyria* is a genus consisting of five species, and each species tends to be found from geographically fragmented areas facing the Japan Sea (Issiki 1953; Hashimoto 2006). Furthermore, we describe females of *I. akemiae* Hashimoto, 2006 and *I. plicata* Hashimoto, 2006, for the first time.

In this study, males and females of four *Issikiomartyria* species new to science and females of two known species are described. Also, a new genus is established based on two species new to science. An updated account of the distribution of micropterigids in the northeastern Japan based on the detailed additional sampling records is provided.

# Methods

Adults and larvae of micropterigid moths were collected from temperate forests in Japan, and larvae were reared in plastic cases with their host-plants. A total of 226 adult pinned specimens were used for this study. For genital dissections, the whole abdomen was removed and macerated for 30 min in 10 % KOH. Residual scales and tissues were then washed in distilled water to remove KOH, immersed in 50 % ethanol and dehydrated in an ethanol series. Genitalia were then stained with 5 % chlorazol black E for 10 min, and dehydrated in a series of 70−100 % ethanol. After washing, specimens were mounted and stored in 70 % glycerol. For some specimens, to examine the wing veins, wings were removed and scales were removed by brushing in 70 % EtOH. Observation and measurements were made under an Olympus BX53F microscope at 10–40× with the aid of a micrometer scale.

All the specimens examined in this study are deposited in the following collections: National Museum of Nature and Science (NMNS), Graduate School of Human and Environmental Studies, Kyoto University (KUHE).

Terminology follows Gibbs and Kristensen (2011) and Davis and Landry (2012). Author's names are abbreviated: YI and MK stand for Yume Imada and Makoto Kato, respectively.

# Taxonomy

### ***Melinopteryx* gen. n.**

http://zoobank.org/54C3DAD7-B3D6-4EA7-8706-6DAFBCD57E63
Figs 1a, b, 2–7

**Type species.** *Melinopteryx coruscans* sp. n. by present designation.

**Diagnosis.** Aedeagus with three pairs of dorsal fins, a pair of lateral triangular fins, and a ventral longitudinal fin. Genital chamber with a large genital sclerite with four paddle-shaped accessory sclerites at posterior end.

**Description.** The generic description is based on *M. coruscans* sp. n. and *M. bilobata* sp. n.

Head capsule densely covered by microtrichia; genal area glossy and naked; most of clypeus, frons, and vertex covered with brownish yellow piliform scales. Ocelli present. Antenna moniliform, approximately as long as forewing in male, longer than in female; densely covered with fuscous piliform scales on scape and pedicel; scape the largest segment, three times longer than most basal flagellum; pedicel small, as long as most basal flagellum. SOI (Kristensen and Nielsen 1979) about 0.4. MIOI (Hirowatari 1997) about 0.5. Interocellar sulcus complete. Postinterocellar sulcus distinct. Epicranial sulcus almost absent. Temporal sulcus as a darker line. Occipital sulcus interrupted at ventro- and dorso-lateral corner. Occipus fan-shaped. Labrum approximately pentagonal, length more or less twice that of clypeus. Mandible elongate rectangular, distal edge truncated. Proximal prelabium sclerite weakly sclerotized. Labial palp 2-segmented. Maxillary palp 5-segmented.

Foretibial epiphysis absent. Antero-lateral processes of pronotum present, weakly sclerotized.

Wing venation as shown in Fig. 3A, B. Fore- and hindwings obtuse at apex, forewing with brown to purple luster, cilia shining grayish brown. Forewing with Sc forked, R1 unforked; R3 stalked with R4+5. Hindwing with a

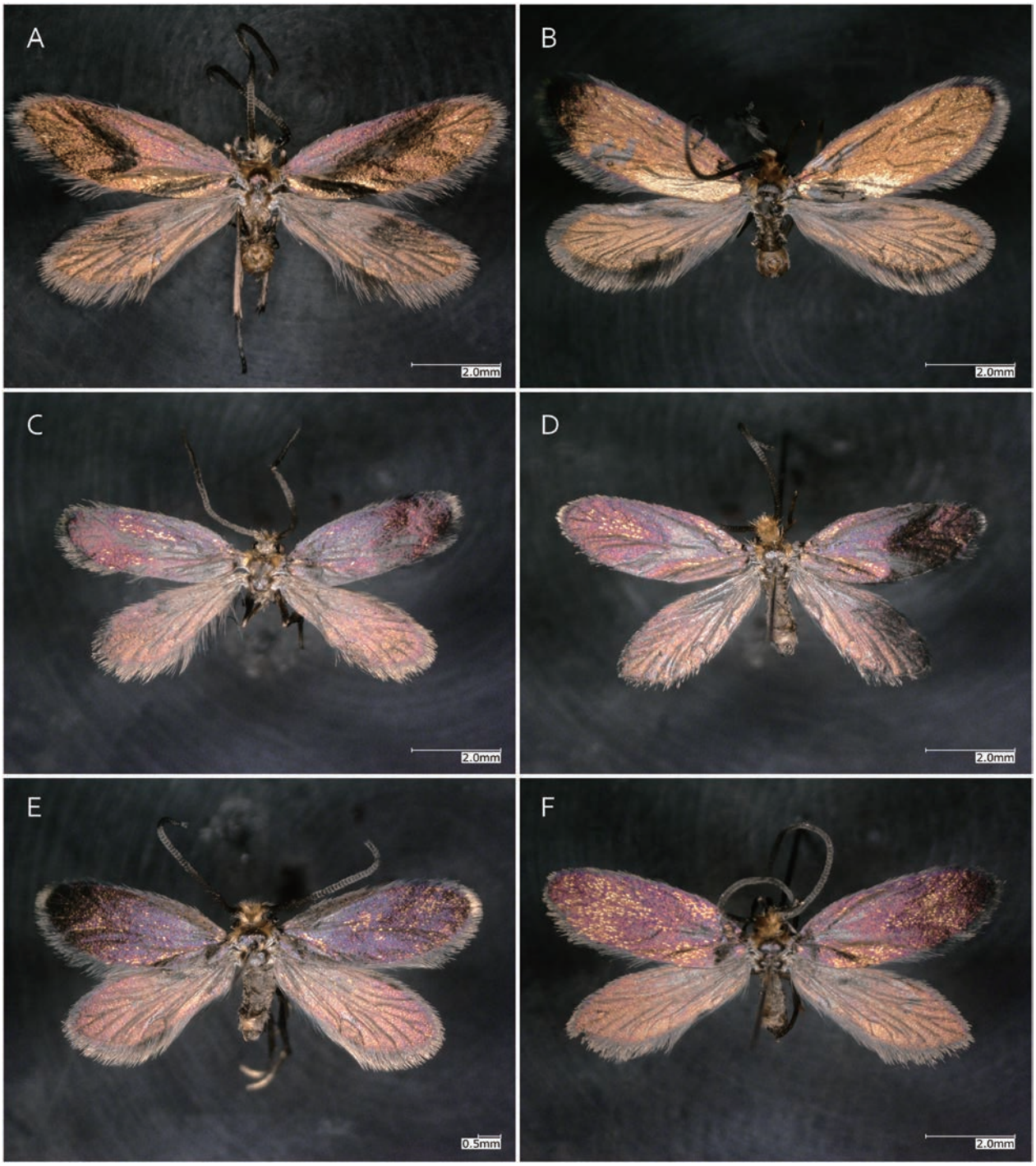


**Figure 1.** Habitus of adult males of *Melinopteryx* and *Issikiomartyria*. **A:** *Melinopteryx coruscans* sp. n. [holotype; MC 0204]; **B:** *M. bilobata* sp. n. [holotype: MC 0221]; **C:** *Issikiomartyria hyperborea* sp. n. [holotype: MC0252]; **D:** *I. leptobelos* sp. n. [MC 0250]; **E:** *I. catapasta* sp. n. [MC 0241]; **F:** *I. trochos* sp. n. [MC 0231]

main stem of R absent; most anterior vein of hindwing forked near terminal end (Sc1 and Sc2+R1). Abdomen grayish brown, covered with piliform and lamellar scales, scattered with dark orange piliform scales on venter and genital segments in male. Sternum V gland present; orifice of gland a narrow slit.

*Male abdomen and genitalia*. Sternum VIII membranous. Segment IX a complete ring, well sclerotized, with a posterior expansion dorsally. Valva triangular, broadly membranous at proximo-dorsal surface, with a proximo-ventral ridge; anterior portion fused with median plate; median plate large, roughly fan-shaped. Phallobase strongly curved, without ventral longitudinal ridge. Aedeagus stout at caudal end, with three pairs of fins dorso-medially; a pair of lateral triangular fins extending horizontally; a pair of ventral fin extending vertically; dorsal apex of aedeagus acute and ventral one slightly forked, longer than dorsal one; gonopore opening horizontally; vesica with

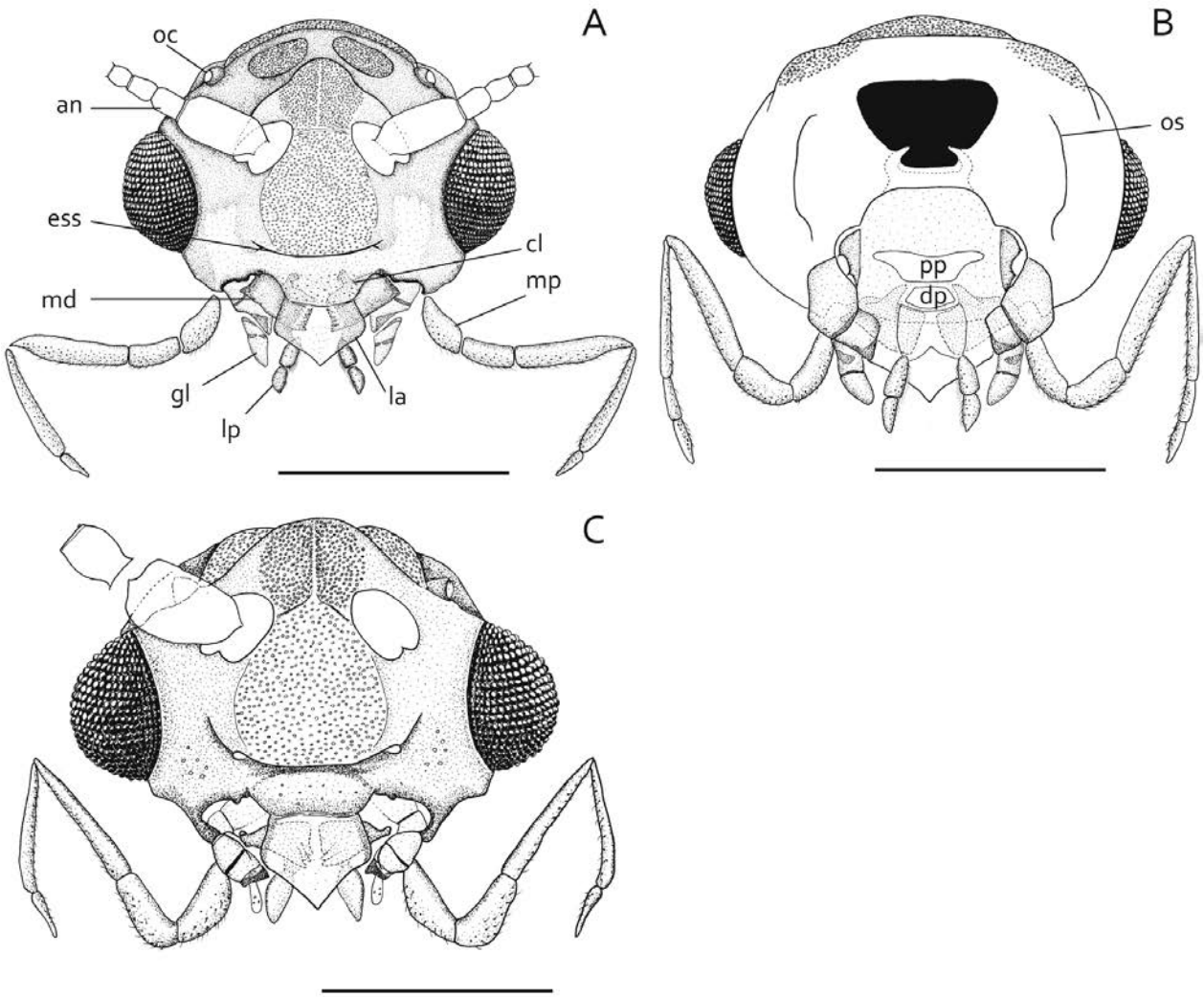


**Figure 2.** Adult heads of *Melinopteryx* and *Issikiomartyria*. **A:** *Melinopteryx bilobata* sp. n., frontal view; **B:** ditto, posterior view; **C:** *Issikiomartyria hyperborea* sp. n., frontal view. Abbreviations: **an** = antenna; **cl** = clypeus; **dp** = distal prelabium; **ess** = epistomal sulcus; **gl** = galea; **la** = labium; **lp** = labial palp; **md** = mandible; **mp** = mandibular palp; **oc** = ocellus; **os** = occipital sulcus; **pp** = proximal prelabium. Scale bars: 0.5 mm.

serrate minute projections. Tergum X broader than long, with a pair of long ventral plates (venter X plates) extending antero-ventrally at base of terminal processes.

*Female abdomen and genitalia*. Segment IX forming a complete ring, strongly sclerotized, with a dorso-lateral concavity, without lateral protrusion. Segment X composed by lateral sclerites and one or two dorsal sclerotized plates; lateral sclerites simple, broader than long, having digitate projections with an apical seta at terminal inner margin. Corpus bursae large, globular, membranous, with signa composed of three or four sclerites near caudal end. Genital chamber with a large sclerite (genital sclerite) and a few tiny sclerites; genital sclerite deeply furcated posteriorly into four paddle-shaped accessory sclerites.

**Remarks.** The genetic distance between *Melinopteryx coruscans* sp. n. (labelled as '*Issikiomartyria*' sp. in Imada et al. 2011) and its sister clade *Issikiomartyria* spp. (6.3 ± 0.9%, based on the Kimura 2-parameter model) was almost as large as the intergeneric COI divergence among other Japanese micropterigid genera (for example, 7.8 ± 0.1% between *Neomicropteryx* and *Kurokopteryx*), indicated by Imada et al. (2011). *Melinopteryx* has a 2-segmented labial palp, while 1-segmented in *Issikiomartyria*, except for *I. bisegmentata* Hashimoto, 2006 which is 2-segmented. *Melinopteryx* is characteristic in its aedeagus with three pairs of dorsal fins, a pair of lateral triangular fins, a ventral longitudinal fin or protrusion extending vertically in the male. In female, a large sclerite with more than four accessory sclerites in genital chamber is also unique in *Melinopteryx*. Fore- and hindwing without a radial cell and large bulbous corpus bursae in female are shared with *Issikiomartyria*.

**Etymology.** The genus name is a compound noun derived from the Greek words transliterated into Latin, "melinos" (honey-color) and "pteryx" (wing), referring to the adult wing color of the species of this genus. The gender is feminine.

***Melinopteryx coruscans* sp. n.**

http://zoobank.org/EB7BCFF1-B58E-4896-ADF1-C1674D21016A
Figs 1a, 3, 4, 5
Japanese name: to-o-yama-takane-kobane

**Material examined.** Holotype: JAPAN [HONSHU] 1♂collected by MK on 29.VII.2007 at Shirabiso-touge (1800m), Iida-shi, Nagano Pref (Fig. 19:1), NMNS.

Paratypes: JAPAN [HONSHU] 13♂1♀ collected by MK on 29.VII.2007 at same locality as holotype, KUHE.

Additional materials: JAPAN [HONSHU] 1♀ emerged on 9.VI.2014 from a larva collected by YI on 27.IV.2014 at same locality as holotype, KUHE; 1♂1♀ collected by MK on 13.VII.2009 at Irisawai (1115m), Ohshika-mura, Nagano Pref (Fig. 19:2), KUHE.

**Type locality.** Japan, Nagano Pref: Shirabiso-touge (Honshu).

**Diagnosis.** Aedeagus with a pair of ventral longitudinal fins, extending more than half of aedeagal length; female corpus bursae with two forms of signa consisting of two semicircular sclerite and a long rectangular sclerite.

**Description.** Head dark brown, naked and glossy on both sides, sparsely covered with brownish yellow piliform scales with dark yellow scales on vertex. Antenna about same length of forewing in male, about 4/5 in female; with 67 (60–74) flagellomeres in males (n=7). Labial palp 2-segmented. Thorax grayish brown, sparsely covered with purple and brownish gold scales on prothorax with blue metallic scales, with dark yellow piliform scales on tegula. Forewing with brownish purple luster tinged with coppery, densely covered with golden luster over basal half of dorsum; cilia grayish brown, pale yellow on apex; ventral surface glossy grayish purple. Forewing length 5.1 mm (4.8–6.0, n=8) and 5.1 mm (n=1) in male and female, respectively. Hindwing glossy brownish purple scattered with piliform scales on basal half; cilia grayish brown; ventral surface same as forewing. Abdomen sparsely covered with grayish brown piliform scales.

*Male abdomen and genitalia* (Fig. 4). Mid-dorsal length of segment IX ring about 1/6 of ventral length. Valva with obtuse apex, with a very small proximo-ventral ridge; inner ventral margin broad without concavity. Aedeagus with a ventral longitudinal fin extending vertically more than half of aedeagal length; three pairs of dorsal fins present; a pair of lateral triangular fins extending horizontally. Tergum X with longitudinal convex at medial part, with a pair of triangular lobes disto-dorsally.

*Female abdomen and genitalia* (Fig. 5). Segment IX ring strongly sclerotized, deeply concave dorso-laterally; mid-dorsal length about 1/3–1/2 of mid-ventral length, without lateral protrusion. Dorsal plate between segment X sclerites large, well sclerotized. Corpus bursae membranous, bulbous; signa consisting of two semicircular sclerites and a ribbon-shaped sclerite. Genital chamber armed with a large sclerite with four bar-shaped accessory sclerites.

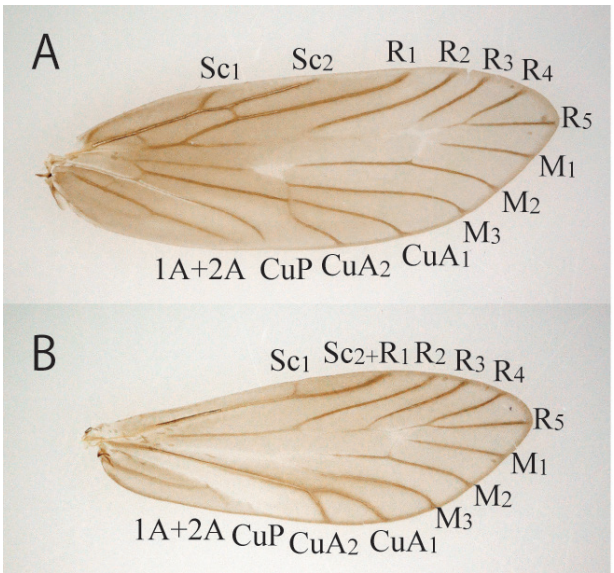


**Figure 3.** Adult wings of *Melinopteryx coruscans* sp. n. **A:** right forewing; **B:** right hindwing.

**Variations.** Geographic variation is recognizable between individuals in the populations at Irisawai and Shirabiso-touge. In the populations of Irisawai, the proximal portion of the male tergum X is stouter and much more developed than that of Shirabiso-touge; wing color tinged with strongly purplish scales at Irisawai population, whereas wing color of the individuals at Shirabiso-touge tend to be more tinged with coppery and golden scales.

**Remarks.** *Melinopteryx coruscans* sp. n. is distinguished from *M. bilobata* sp. n. based on the following character states: aedeagus with a ventral longitudinal fin extending more than half of aedeagal length; female segment X with a rectangular plate of dorsal sclerites; corpus bursae with two different forms of signa consisting of a pair of semicircular sclerites and a ribbon-shaped sclerite. This species corresponds to " '*Issikiomartyria*' sp." in Imada et al. (2011).

**Etymology.** The specific name is a participle in the nominative singular from the Latin word "coruscans", which stands for flashing.

**Distribution.** The Western mountain range of the Akaishi Mountain Range of Japan (Honshu: Nagano Pref.).

**Bionomics.** There is a single generation per year; however, there may be one generation per two years in some populations at high elevation, where larvae exhibit two significantly different size during the same period of time. The habitat is the peak or valley of sub-alpine forests at approximately 1100–1820 m of the Akaishi Mountain

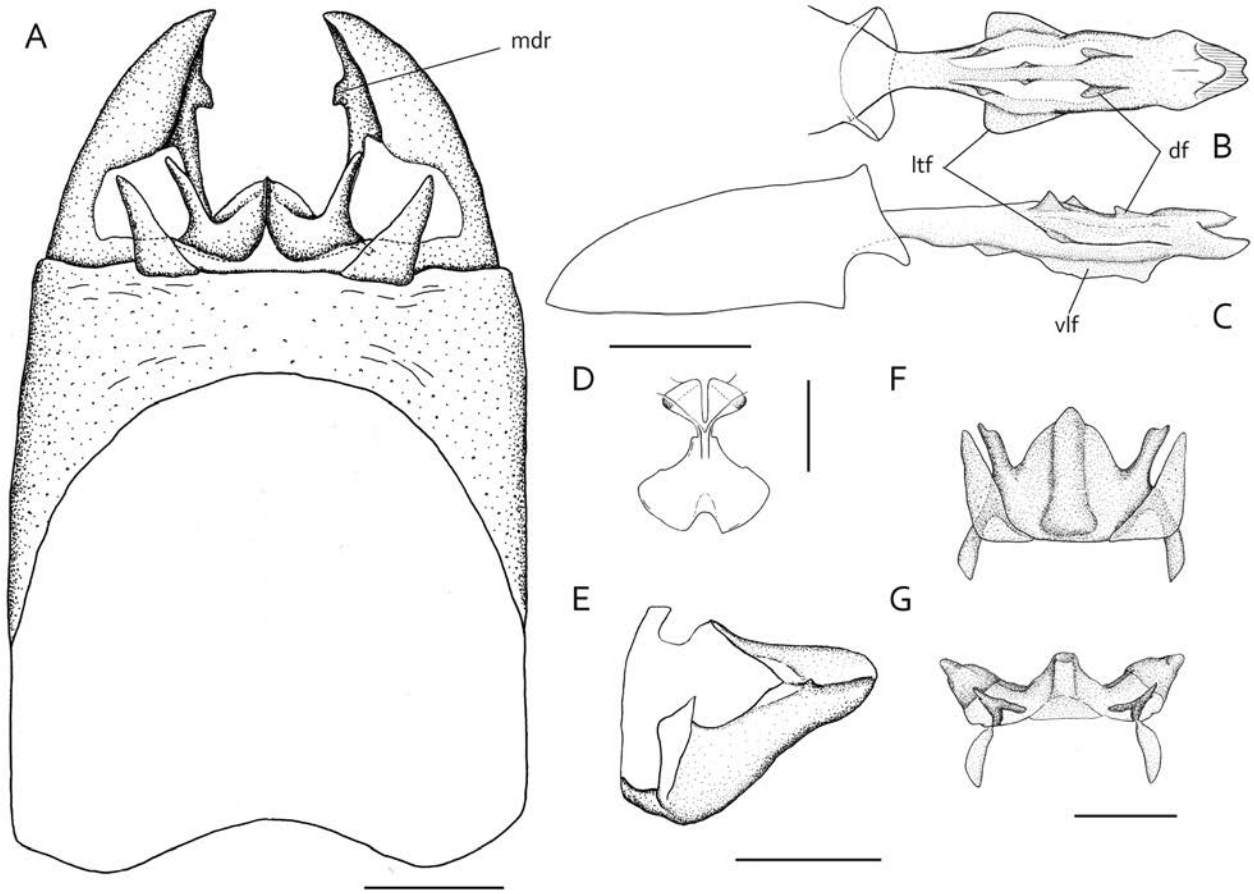


**Figure 4.** Male genitalia of *Melinopteryx coruscans* sp. n. [holotype]. **A:** genitalia capsule, dorsal view; **B:** phallus, dorsal view; **C:** ditto, lateral view; **D:** median plate; **E:** left valva, inner view; **F:** tergum X and venter X plate, dorsal view; **G:** ditto, oblique view; **H:** genital capsule, lateral view. Abbreviations: **df** =dorsal fin; **ltf** = lateral triangular fin; **mdr** = mid-dorsal ridge; **vlf** = ventral longitudinal fin. Scale bars: 0.2 mm.

Range of Japan. The dominant arboreal species of their habitat are *Tsuga diversifolia* (Maxim.) Mast., *Abies veitchii* Lindl., and *Picea jezoensis* (Sieb. & Zucc.) Carriére var. *hondoensis* (Mayr) Rehde (Pinaceae). The larvae feed on the thalli of *Conocephalum* liverworts.

### *Melinopteryx bilobata* sp. n.

http://zoobank.org/17C1F904-9FD2-4A5A-82AB-8CE02B7C7E58
Figs 1b, 2a, b, 6, 7
Japanese name: ikawa-takane-kobane

**Material examined.** Holotype: JAPAN [HONSHU] 1♂ collected by YI on 3.VII.2016 at Ikawa-touge (1559m), Tatsuno-cho, Shizuoka Pref (Fig. 19: 3), NMNS.

Paratypes: JAPAN [HONSHU] 5♂1♀ collected by T. Kato on 10.VII.2016 at same locality as holotype, NMNS.

Additional materials: JAPAN [HONSHU] 1♂ emerged on 15.VI.2015 from larva collected by MK on 10.V.2015 at same locality as holotype, NMNS; 10♂ collected by YI on 3.VII.2016 at same locality, KUHE.

**Type locality.** Japan, Shizuoka Pref: Ushikubi-touge (Honshu).

**Diagnosis.** Aedeagus with a short ventral fin in male genitalia; female segment X with two reduced lobes of dorsal sclerite.

**Description.** Head dark brown, naked and glossy on both sides, sparsely covered with brownish yellow piliform scales with dark yellow scales on vertex. Antenna slightly longer than forewing in male; with 67 (64–73) flagellomeres in males (n=8). Labial palp 2-segmented. Thorax grayish brown, sparsely covered with purple and brownish gold scales on prothorax with blue metallic scales, with dark yellow piliform scales on tegula. Legs covered with glossy fuscous scales. Forewing with brownish purple luster tinged with coppery, densely covered with golden luster over basal half of dorsum; cilia grayish brown, pale yellow on apex; ventral surface glossy grayish purple. Forewing length 4.9 mm (4.6–5.0, n=10) in male. Hindwing glossy brownish purple scattered with piliform scales on basal half; cilia grayish brown; ventral surface same as forewing. Abdomen sparsely covered with grayish brown piliform scales.

*Male abdomen and genitalia* (Fig. 6). Mid-dorsal length of segment IX ring about 1/5 of ventral length.

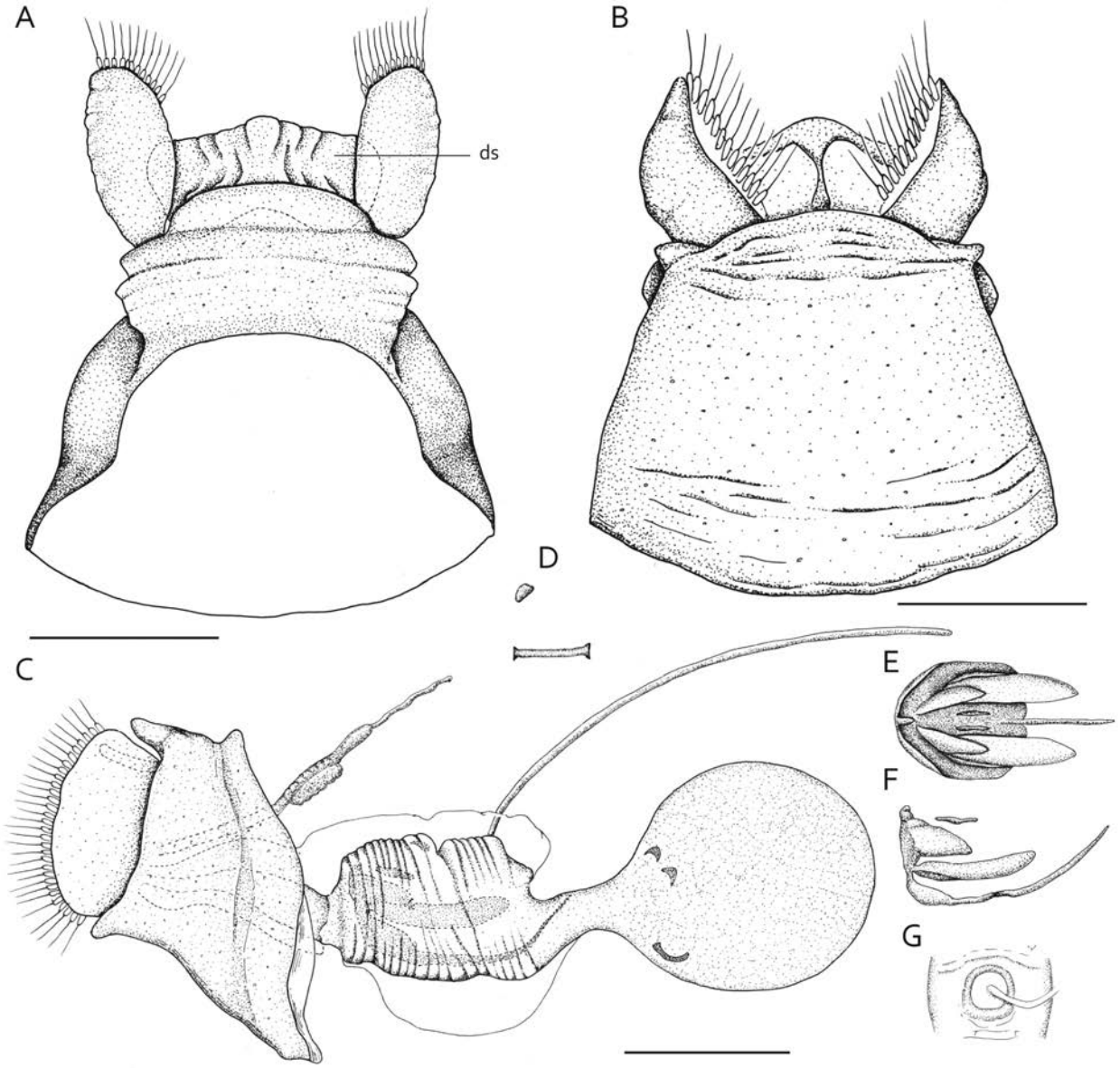


**Figure 5.** Female genitalia of *Melinopteryx coruscans* sp. n. [paratype]. **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** female genitalia, lateral view; **D:** signa; **E:** genital sclerite, dorsal view; **F:** ditto, lateral view; **G:** arising part of ductus spermathecae, dorsal view. Abbreviation: **ds** = dorsal sclerite. Scale bars: 0.2 mm.

Valva with a small proximo-ventral ridge; inner ventral margin broad without concavity; apical end obtuse. Aedeagus with a ventral protrusion at base; with three pairs of short dorsal fins; with a pair of small, lateral triangular fins extending horizontally. Tergum X with squarish medial part, with a pair of spines disto-dorsally.

*Female abdomen and genitalia* (Fig. 7). Segment IX ring strongly sclerotized, shallowly concave dorso-laterally; mid-dorsal length about 1/2 of mid-ventral length, without lateral protrusion. Segment X consisting of a pair of lateral sclerites and two dorsal sclerotized plates; dorsal plates small, well sclerotized, being behind dorsal side of segment IX. Corpus bursae membranous, bulbous, possessing four semicircular signa. Genital chamber armed with a large sclerite with four plate-shaped accessory sclerites.

**Remarks.** *Melinopteryx bilobata* sp. n. is distinguished from *M. coruscans* sp. n. based on the following characteristics: aedeagus with a ventral protrusion at base; female segment X with a pair of small dorsal sclerites.

**Etymology.** The specific name is a compound adjective in the nominative singular from the Latin words, "bi-" (two) and "lobatus" (having diminutive lobes), referring to a pair of small dorsal sclerites of the female genitalia (Fig. 7).

**Distribution.** The Eastern mountain range of the Akaishi Mountain Range of Japan (Honshu: Shizuoka Pref.).

**Bionomics.** The larvae feed on the thalli of *Conocephalum conicum* (L.) Dum. The habitat is a forest path along the mountain ridge of sub-alpine or cool-temperate for-

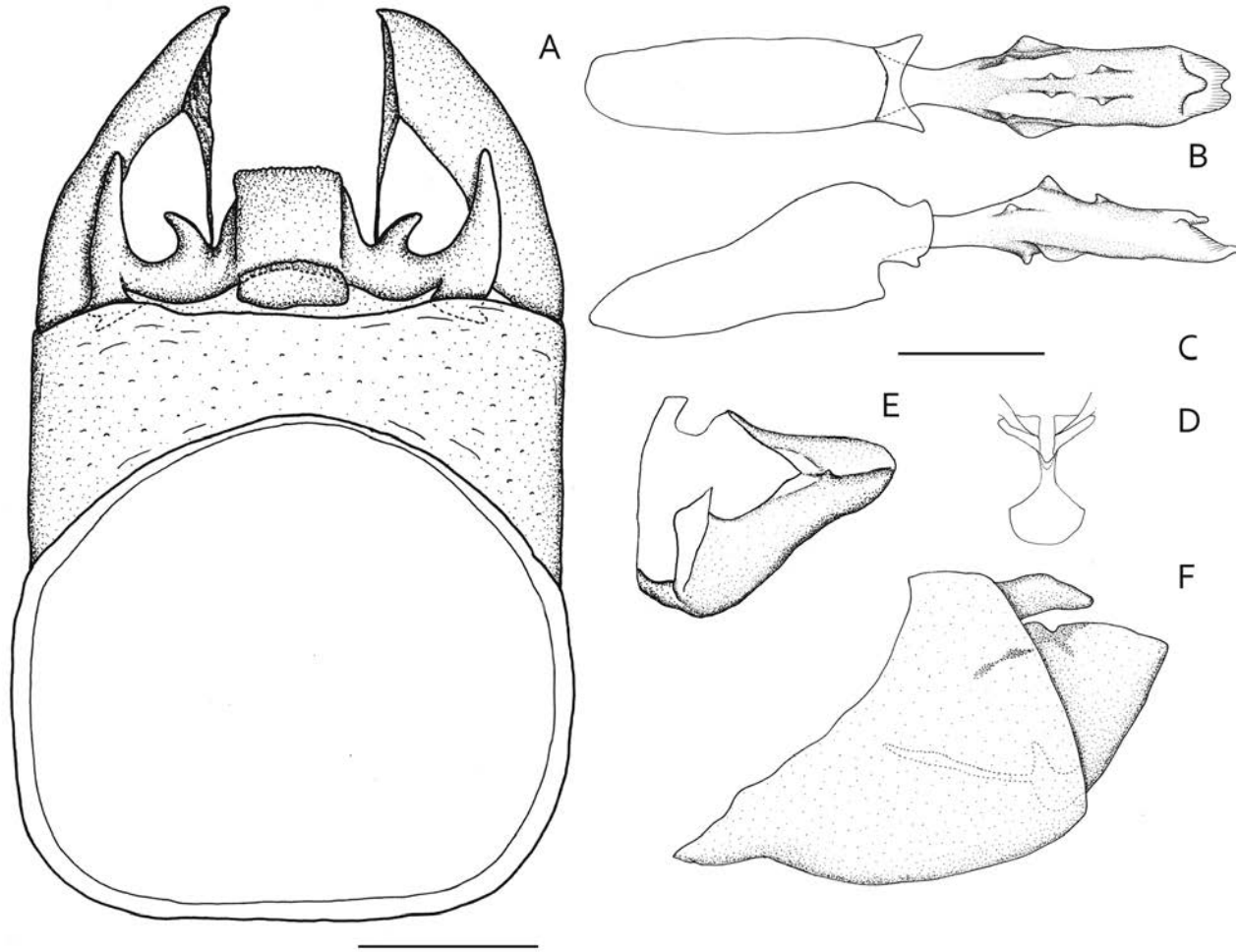


**Figure 6.** Male genitalia of *Melinopteryx bilobata* sp. n. [holotype]. **A:** genitalia capsule, dorsal view; **B:** phallus, dorsal view; **C:** ditto, lateral view; **D:** median plate; **E:** left valva, inner view; **F:** genitalia capsule, lateral view; **G:** ditto, ventral view. Scale bars: 0.2 mm.

ests at approximately 1500 m of the Akaishi Mountain Range of Japan, where *Fagus crenata* Blume (Fagaceae) and *Abies firma* (Sieb. & Zucc.) (Pinaceae) dominate.

### Genus *Issikiomartyria* Hashimoto, 2006

**Type species.** *Neomicropteryx nudata* Issiki, 1953, fixed by original designation.

**Diagnosis.** Aedeagus with two pairs of dorsal fins.

**Description.** The generic description is based on *I. nudata* (Issiki, 1953), *I. akemiae*, *I. plicata*, *I. distincta* Hashimoto, 2006, *I. bisegmentata*, *I. hyperborea* sp. n., *I. leptobelos* sp. n., *I. catapasta* sp. n., *I. trochos* sp. n., and on the previous studies on this group (Issiki 1953, Hashimoto 2006).

Head capsule densely covered by microtrichia, apart from genal area where glossy and naked; most of clypeus, frons, and vertex covered with brownish yellow piliform scales. Ocelli present. Antenna moniliform, approximately as long as forewing in male, longer than in female; scape the largest segment, twice longer than most basal flagellum; pedicel bulbous, larger than most basal flagellum; basal one or two flagellomeres cylindrical. SOI about 0.4. MIOI about 0.5. Interocellar sulcus almost complete. Postinterocellar sulcus distinct. Epicranial sulcus distinct between occipital foramen and postinterocellar sulcus, being as a short distance anterior to interocellar sulcus. Temporal sulcus as a darker line. Occipital sulcus almost complete, but slightly indistinct on dorso-lateral corner. Occipus fan-shaped. Mandibular teeth greatly reduced. Labial palp 1- or 2-segmented. Maxillary palp 5-segmented. Proximal prelabium obscure. Foretibial epiphysis absent. Antero-lateral processes of pronotum present, strongly sclerotized. Fore- and hindwings obtuse at apex, forewing with brown to purple luster, without any distinct maculation. Forewing with R1 unforked; R3 stalked with R4+5. Hindwing with a main stem of R absent; most anterior vein of hindwing forked near terminal end (Sc1 and Sc2 + R1). Sternum V gland present; orifice of gland a narrow slit.

*Male abdomen and genitalia.* Sternum VIII membranous. Segment IX a complete ring, well sclerotized, with a posterior expansion dorsally; posterior margin gradually expanded from dorsum to venter. Valva triangular, broadly

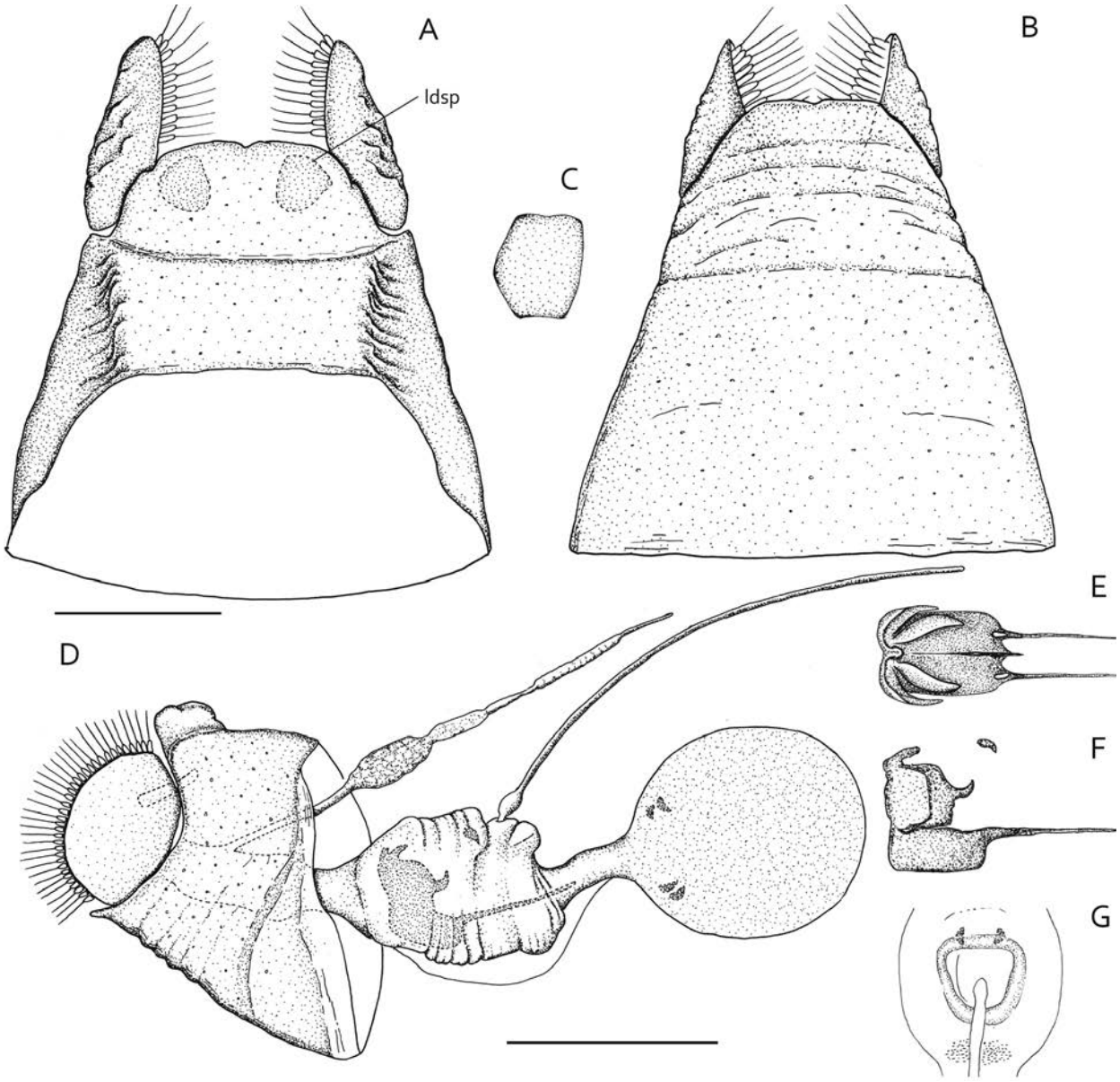


**Figure 7.** Female genitalia of *Melinopteryx bilobata* sp. n. [paratype]. **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** a lobe of dorsal sclerotized plate of Segment X, dorsal view; **D:** female genitalia, lateral view; **E:** genital sclerite, dorsal view; **F:** ditto, lateral view; **G:** arising part of ductus spermathecae, dorsal view. Scale bars: 0.2 mm. Abbreviation: **ldsp** = two lobes of dorsal sclerotized plate of segment X.

membranous at inner surface, with a proximo-ventral ridge whose anterior portion fused with median plate; median plate large, roughly fan-shaped. Phallobase strongly curved, with or without longitudinal ventral ridge(s) on midline. Aedeagus with acute apex, ventrally forked slightly at caudal end; with two pairs of basal fins dorso-medially and a pair of lateral triangular fins; gonopore opening horizontally; vesica with serrate minute projections. Tergum X, broader than long, with a pair of long ventral plates (venter X plates) extending antero-ventrally at base of terminal processes.

*Female abdomen and genitalia*. Segment IX forming a complete ring, strongly sclerotized; anterior margin gradually expanded anteriorly from dorsum to venter; mid-dorsal length generally shorter than 2/5 of mid-ventral length; laterally protruded in some species. Segment X consisting of a pair of lateral sclerites and a dorsal sclerotized plate; lateral sclerites simple, broader than long, with digitate projections having an apical seta at terminal inner margin. Corpus bursae large, globular, membraneous, with signa composed of four sclerites. Ductus spermathecae arising from a hexagonal or round concavity. Genital chamber with small sclerite(s).

**Comparative Remarks.** The following characters are regarded as synapomorphies of *Issikiomartyia*: aedeagus with two pairs of hornlike dorsal projections and without any protrusion vertically in male: sclerite in female genital chamber greatly reduced.

***Issikiomartyria hyperborea* sp. n.**

http://zoobank.org/823D926C-6B02-452A-A2CD-0F4B76A62337
Figs 1c, 8, 9
Japanese name: tsugaru-issiki-kobane

**Material examined.** Holotype: JAPAN [HONSHU] 1♂ emerged on 24.V.2012 from larva collected by T. Kato on 4.V.2012 at Tairadate (240m), Sotogahama-machi, Aomori Pref (Fig. 19:4), NMNS.

Paratype: JAPAN [HONSHU] 1♀ emerged on 24.V.2012 from larva collected by MK on 24.V.2012 at same locality, NMNS.

Additional materials: JAPAN [HONSHU] 1♀ emerged on 24.V.2014 from larva collected by YI on 10.V.2014 at same locality, KUHE.

**Type locality.** Japan, Aomori Pref: Tairadate (Honshu).

**Diagnosis.** Aedeagus with a pair of lateral triangular fins arising from ventral margin, extending horizontally. Female segment IX with a strong concavity extending from lateral to ventral sides.

**Description.** Head dark brown, naked and glossy on both sides, sparsely covered with yellow piliform scales with dark yellow scales on vertex. Antenna slightly longer than forewing in male; densely covered with fuscous piliform scales on scape and pedicel. Labial palp 1-segmented. Forewing length 3.8 mm (n=1) and 3.9 mm (n=1) in male and female.

*Male abdomen and genitalia* (Fig. 8). Mid-dorsal length of segment IX ring about 1/4 of ventral length. Valva sharply tapered apically, with a tiny proximo-ventral ridge whose anterior portion fused with median plate. Aedeagus with a pair of short distal fins and a pair of longer, proximal fins extending vertically, arising from dorsal side; a pair of lateral triangular fins ventrally-oriented, extending horizontally. Tergum X with a small medial part; shorter than half of valva; with a pair of spines disto-dorsally.

*Female abdomen and genitalia* (Fig. 9). Segment IX ring strongly sclerotized, concave both at lateral and ventral sides; mid-dorsal length about 2/5 of ventral length. Dorsal plate between segment X sclerites large, well sclerotized, enlarged dorso-caudally. Corpus bursae large, globular, membranous,

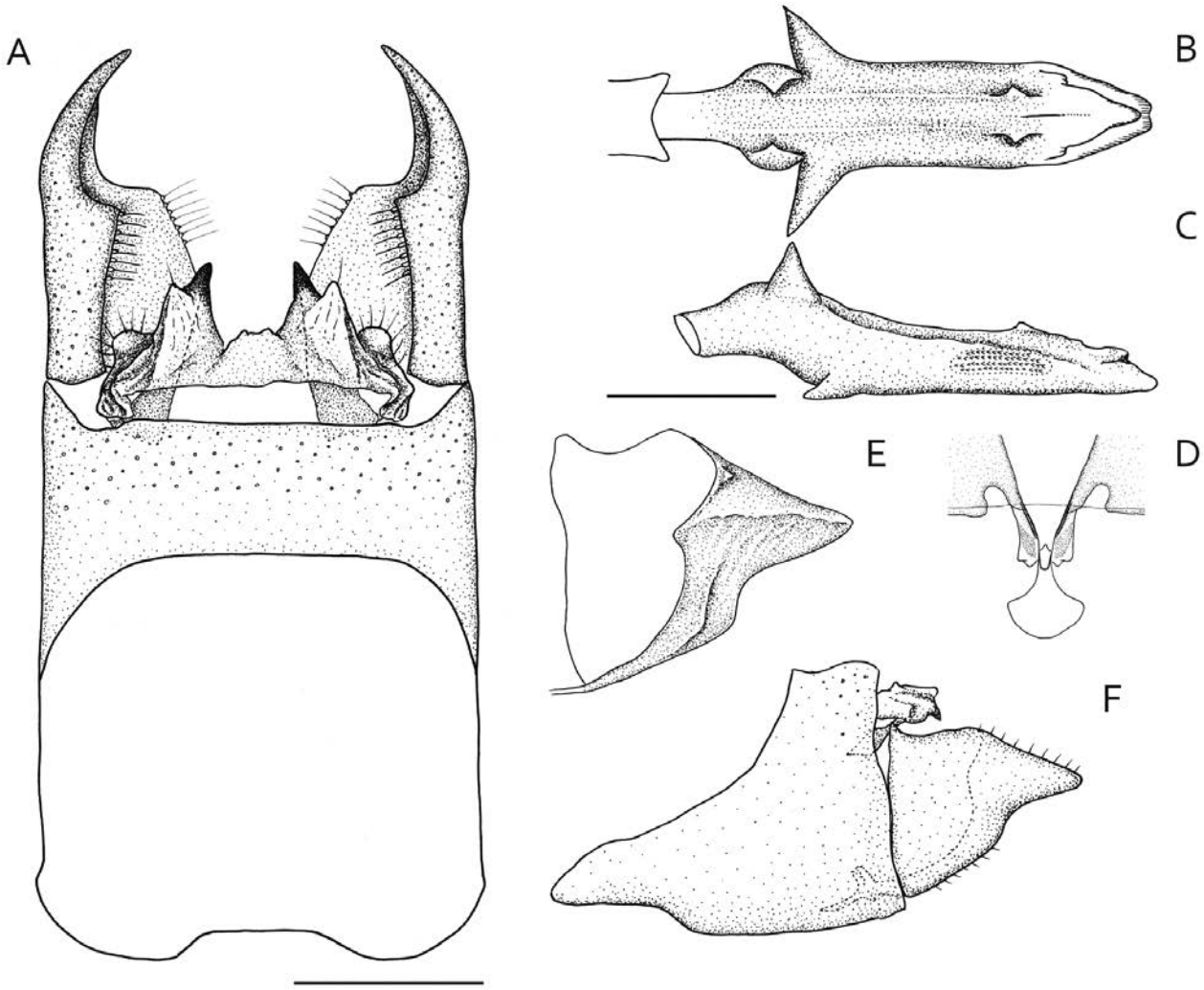


**Figure 8.** Male genitalia of *Issikiomartyria hyperborea* sp. n. [holotype]. **A:** genitalia capsule, dorsal view; **B:** phallus, dorsal view; **C:** ditto, lateral view; **D:** median plate; **E:** left valva, inner view; **F:** genital capsule, lateral view. Scale bars: 0.2 mm.

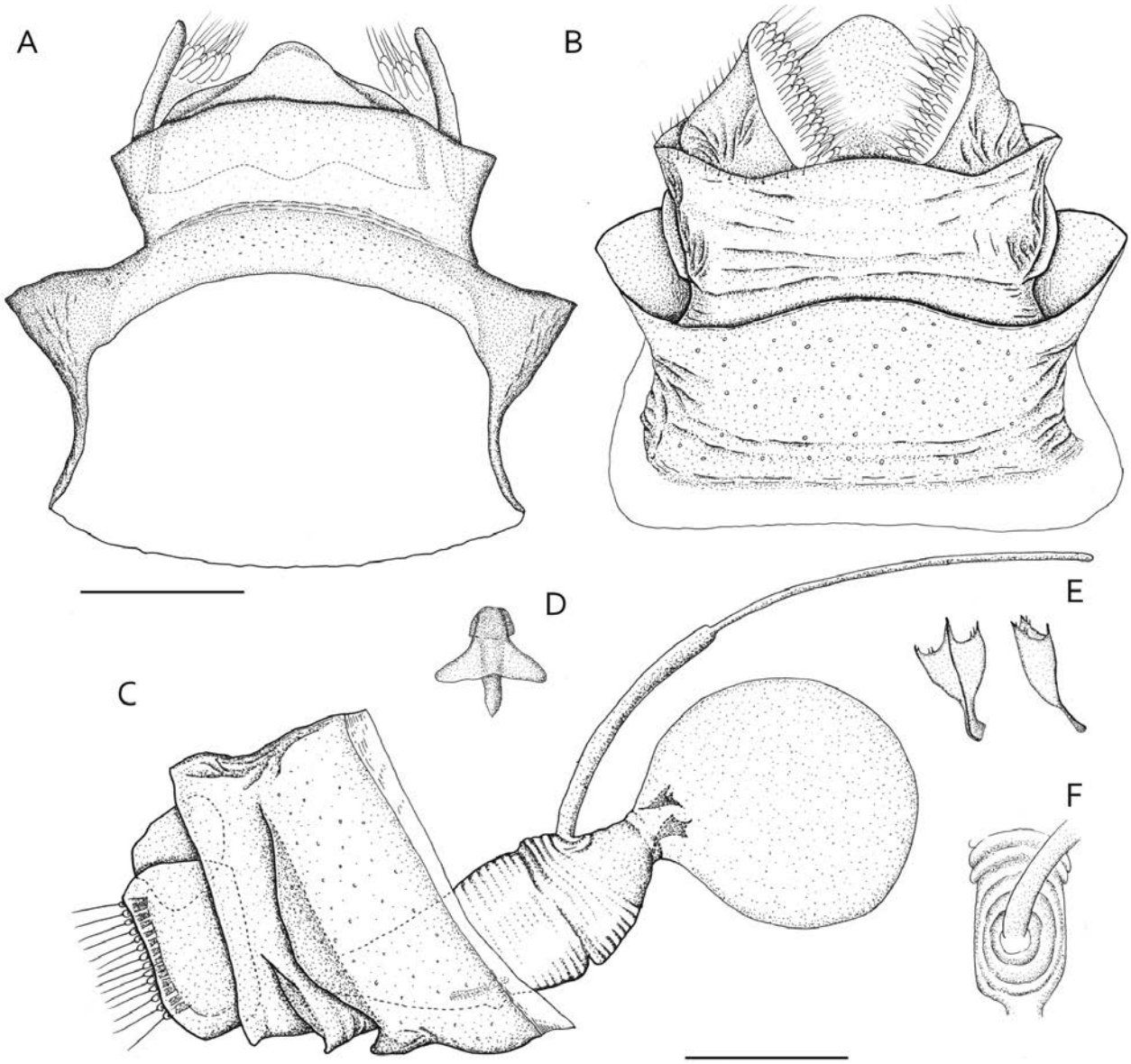


**Figure 9.** Female genitalia of *Issikiomartyria hyperborea* sp. n. [paratype]. **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** female genitalia, lateral view; **D:** genital sclerite; **E:** signa; **F:** arising part of ductus spermathecae, dorsal view. Scale bars: 0.2 mm.

with signa composed of four tridenta-form sclerites near proximal end. Ductus spermathecae arising from a round concavity. Genital chamber with a small, triangular sclerite.

**Remarks.** *Issikiomartyria hyperborea* sp. n. is distinguishable from the known *Issikiomartyria* species by the following characters. In the male, aedeagus with a pair of latero-basal fins arising from ventral side. In the female, segment IX with a deep concavity extending from lateral to ventral sides; dorsal sclerite of segment X convex vertically in the middle.

**Etymology.** The specific name is an adjective in the nominative singular derived from the Greek word transliterated into Latin, "hyperboreus", indicating the mythical people of Greek mythology who lived "Beyond the North Wind".

**Distribution.** This species has only been found from Tsugaru peninsula of Japan (Fig. 14:1; Honshu: Aomori Pref).

**Bionomics.** Larvae feed on the thalli of *Conocephalum conicum*. The locality is a forest path along a stream in the cool-temperate forests at approximately 250 m of Tsugaru peninsula, where *Fagus crenata* and *Quercus crispula* Blume (Fagaceae) dominate.

### *Issikiomartyria leptobelos* sp. n.

http://zoobank.org/28714D8E-2B21-482F-B372-CFCF435FCFC4
Figs 1d, 10, 11
Japanese name: shirakami-issiki-kobane

**Material examined.** Holotype: JAPAN [HONSHU] 1♂ collected by YI on 15.VI.2015 at Hachimori (100 m), Happo-cho, Akita Pref (Fig. 19:5), NMNS.

Paratype: JAPAN [HONSHU] 9♂2♀ collected by YI on 15.VI.2015 at same locality, NMNS.

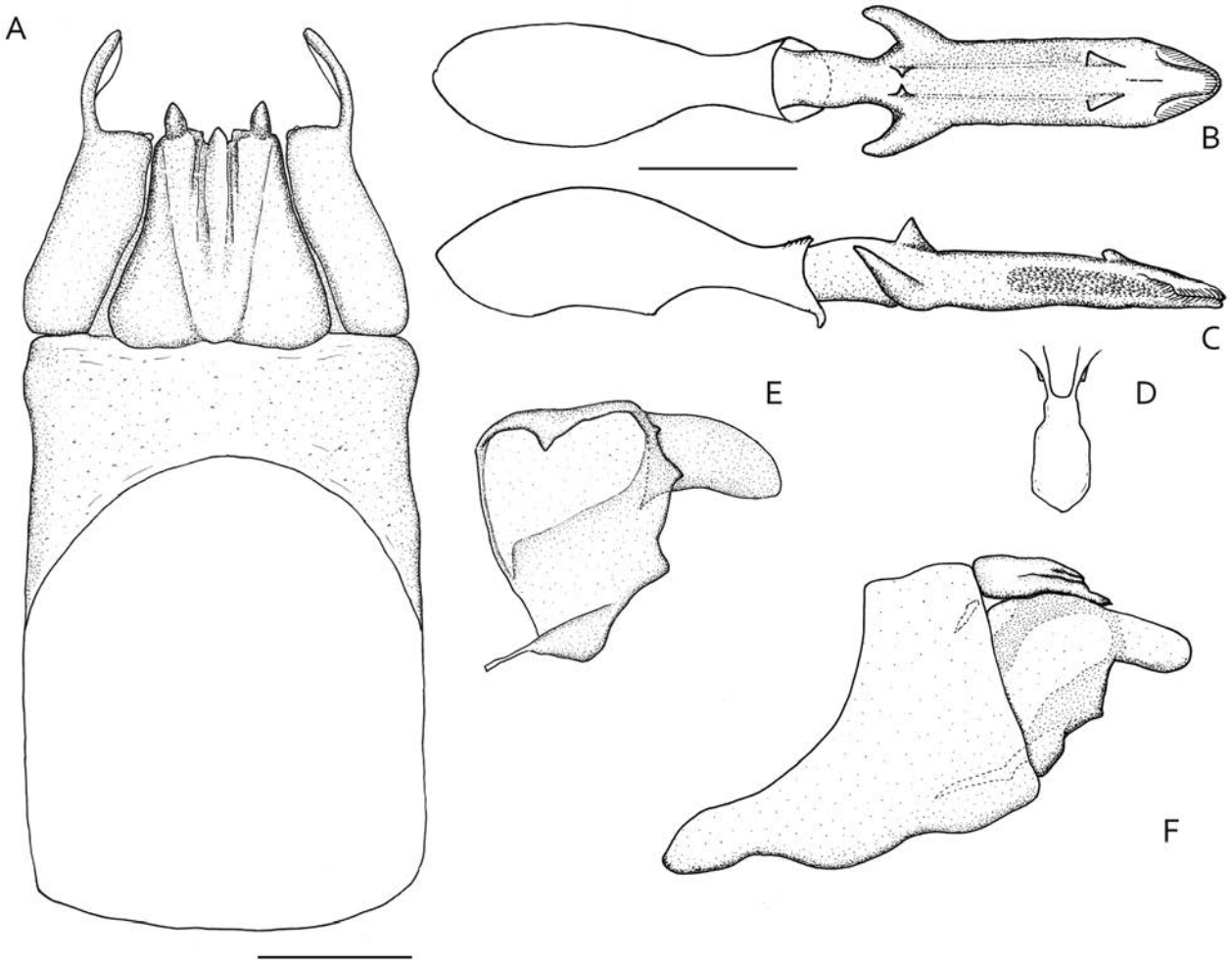


**Figure 10.** Male genitalia of *Issikiomartyria leptobelos* sp. n. [holotype]. **A:** genitalia capsule, dorsal view; **B:** phallus, dorsal view; **C:** ditto, lateral view; **D:** median plate; **E:** left valva, inner view; **F:** genital capsule, lateral view. Scale bars: 0.2 mm.

**Type locality.** Japan, Akita Pref: Hachimori (Honshu).

**Diagnosis.** Apical part of valva digitiform. Tergum X longer than 3/4 of valva.

**Description.** Head dark brown, naked and glossy on both sides, sparsely covered with yellow piliform scales with dark yellow scales on vertex. Antenna longer than forewing in male; with 56 (45–61) flagellomeres in males (n=5). Labial palp 1-segmented. Forewing length 4.5 mm (4.0–4.7, n=9) in male.

*Male abdomen and genitalia* (Fig. 10). Mid-dorsal length of segment IX ring about 1/4 of ventral length. Valva with digitiform, elongated apex; with a tiny proximo-ventral ridge. Aedeagus straight, with two pairs of dorsal fins: a pair of shorter, distal fins and a pair of longer proximal fins extending vertically; with a pair of lateral triangular fins extending upwards. Tergum X with squarish medial part, longer than 3/4 of valva; with a pair of protrusions disto-dorsally.

*Female abdomen and genitalia* (Fig. 11). Segment IX forming a complete ring, strongly sclerotized; strongly concave dorso-laterally. Segment X consisting of a pair of lateral sclerites and a dorsal sclerotized plate: lateral sclerite simple, broader than long; dorsal sclerite rounded at apex. Corpus bursae large, globular, membranous, with signa composed of four sclerites near proximal end. Ductus spermathecae arising from a round concavity. Genital chamber with a small, umbrella-shaped sclerite.

**Remarks.** *Issikiomartyria leptobelos* sp. n. is unique in valva with the digitiform apex in the male. This species is most similar to *I. hyperborea* sp. n., but can be distinguished by the segment IX without a concavity at lateral and ventral sides in the female.

**Etymology.** The specific name is a compound noun in apposition derived from the Greek words transliterated into Latin, "leptos" (fine) and "belos" (divine, arrow), referring to the digitiform valva of this species.

**Distribution.** This species has only been found from Hachimori-cho (Honshu: Akita Pref).

**Bionomics.** Larvae feed on the thalli of *Conocephalum conicum*. The locality is a forest path along a stream in the cool-temperate forests at 100–340 m.

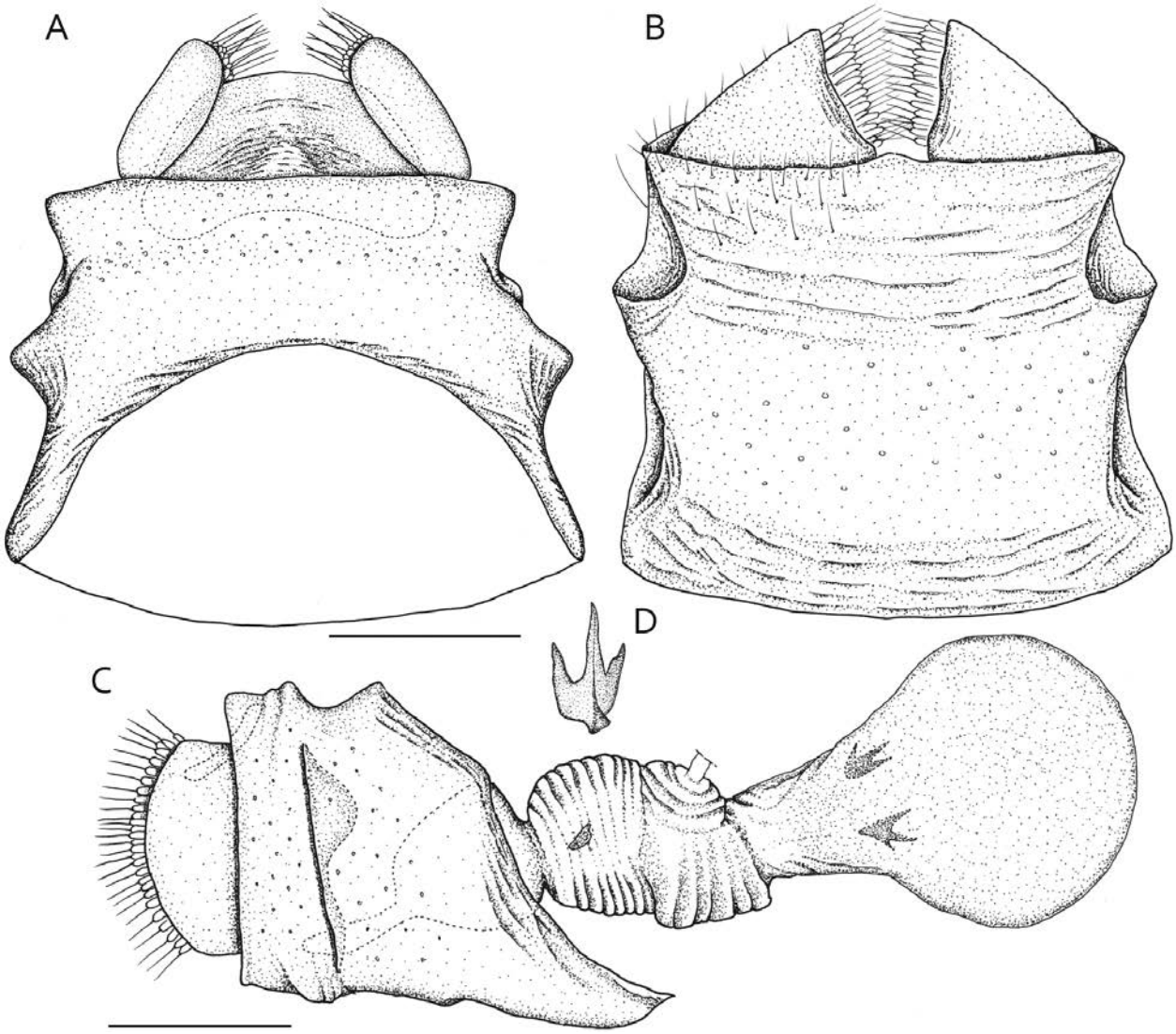


**Figure 11.** Female genitalia of *Issikiomartyria leptobelos* sp. n. [paratype]. **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** female genitalia, lateral view; **D:** signa. Scale bars: 0.2 mm.

### *Issikiomartyria catapasta* sp. n.

http://zoobank.org/55CCF04A-D282-4619-B886-535EB184527C
Figs 1e, 12, 13
Japanese name: ani-issiki-kobane

**Material examined.** Holotype: JAPAN [HONSHU] 1♂ collected by YI on 21.VI.2015 at Tachimata-keikoku (620 m), Kitaakita-shi, Akita Pref (Fig. 19:6), NMNS.

Paratypes: JAPAN [HONSHU] 1♀ collected by YI on 13.VI.2016 at same locality as holotype (Fig. 19:6), NMNS.

Additional materials: JAPAN [HONSHU] 3♂ collected by YI on 21.VI.2015 at same locality as holotype, KUHE; 3♂ 1♀ collected by YI on 13.VI.2016 at same locality, KUHE; 1♂ collected by YI on 14.VI.2016 at Mt. Moriyoshi (380 m), Kitaakita-shi, Akita Pref (Fig. 19:7), KUHE.

**Type locality.** Japan, Akita Pref: Tachimata-keikoku (Honshu).

**Diagnosis.** Middle portion of tergum X in male undeveloped, approximately half of lateral portions. Female genital chamber with numerous sclerites at proximo-dorsally.

**Description.** Head dark brown, naked and glossy on both sides, sparsely covered with yellow piliform scales with dark yellow scales on vertex. Antenna longer than forewing in male, with 60 flagellomeres in female (n=1). Labial palp 1-segmented. Forewing length 4.3 mm (4.1–4.6, n=9) in male.

*Male abdomen and genitalia* (Fig. 12). Mid-dorsal length of segment IX ring about 1/4 of ventral length. Valva with elongated apex; with a very small proximo-ventral ridge. Aedeagus with two pairs of dorsal ridges: a pair of shorter distal fins and a pair of longer proximal fins extending vertically; with a pair of lateral triangular fins extending upwards. Tergum X with squarish medial part, with a pair of triangular lobes disto-dorsally.

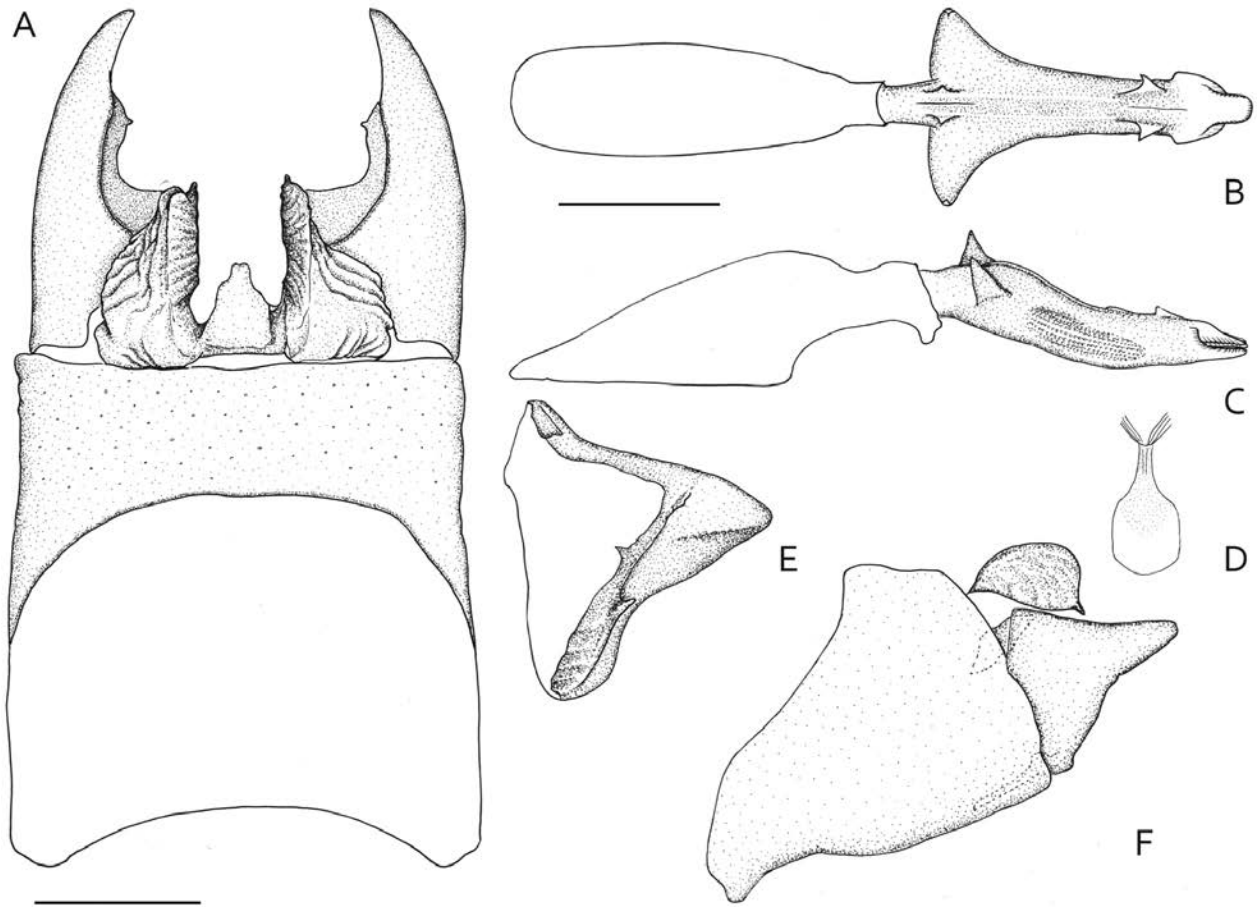


**Figure 12.** Male genitalia of *Issikiomartyria catapasta* sp. n. [holotype]. **A:** genitalia capsule, dorsal view; **B:** phallus, dorsal view; **C:** ditto, lateral view; **D:** median plate; **E:** left valva, inner view; **F:** genitalia capsule, lateral view. Scale bars: 0.2 mm.

*Female abdomen and genitalia* (Fig. 13). Segment IX forming a complete ring, strongly sclerotized; with a pair of dorso-lateral protrusions near base. Segment X consisting of a pair of lateral sclerites and a dorsal sclerotized plate; dorsal sclerite longer than width, projected upward at caudal end. Corpus bursae large, globular, membranous, with long narrow neck region anteriorly; with signa composed of four saggitate sclerites near proximal end. Ductus spermathecae arising from a round concavity. Genital chamber with numerous tiny sclerites dorsally and a large, fan-shaped sclerite ventrally.

**Remarks.** *Issikiomartyria catapasta* sp. n. is most similar to *I. trochos* sp. n. in that lateral parts of tergum X extending dorsally, but can be distinguished by the following traits: two basal pairs of dorsal and lateral aedeagal fins closer to each other; numerous tiny sclerites scattered dorsally in female genital chamber; corpus bursae without tiny sclerites.

**Etymology.** The specific name is a noun in the genitive singular derived from a Latin word, "catapastus" (patchwork), referring to the numerous tiny sclerites in the female genital chamber of this species (Fig. 13).

**Distribution.** This species has only been found from Kitaakita-shi (Honshu: Akita Pref).

**Bionomics.** Larvae feed on the thalli of *Conocephalum conicum*. The localities are forest paths along mountain streams of cool-temperate forests at 380–620 m, where *Fagus crenata* Blume (Fagaceae), *Pterocarya rhoifolia* Sieb. Et Zucc. (Junglandaceae) and *Aesculus turbinate* Blume (Sapindaceae) predominately occur.

### *Issikiomartyria trochos* sp. n.

http://zoobank.org/6D2D9E1C-04D0-405B-A00A-5F4E965C8654
Figs 1f, 14, 15
Japanese name: waga-issiki-kobane

**Material examined.** Holotype: JAPAN [HONSHU] 1♂ collected by YI on 19.VI.2015 at Mahirudake-rindou (410 m), Nishiwaga-cho, Iwate Pref (Fig. 19:8), NMNS.

Paratypes: JAPAN [HONSHU] 3♂1♀ collected by YI on 19.VI.2015 at same as holotype locality, NMNS.

Additional materials: 1♂ collected by YI on 11.VI.2016 at Kodera-keikoku (580 m), Ohemachi, Yamagata Pref (Fig. 19:9), KUHE; 2♂ collected by YI on 12.VI.2016

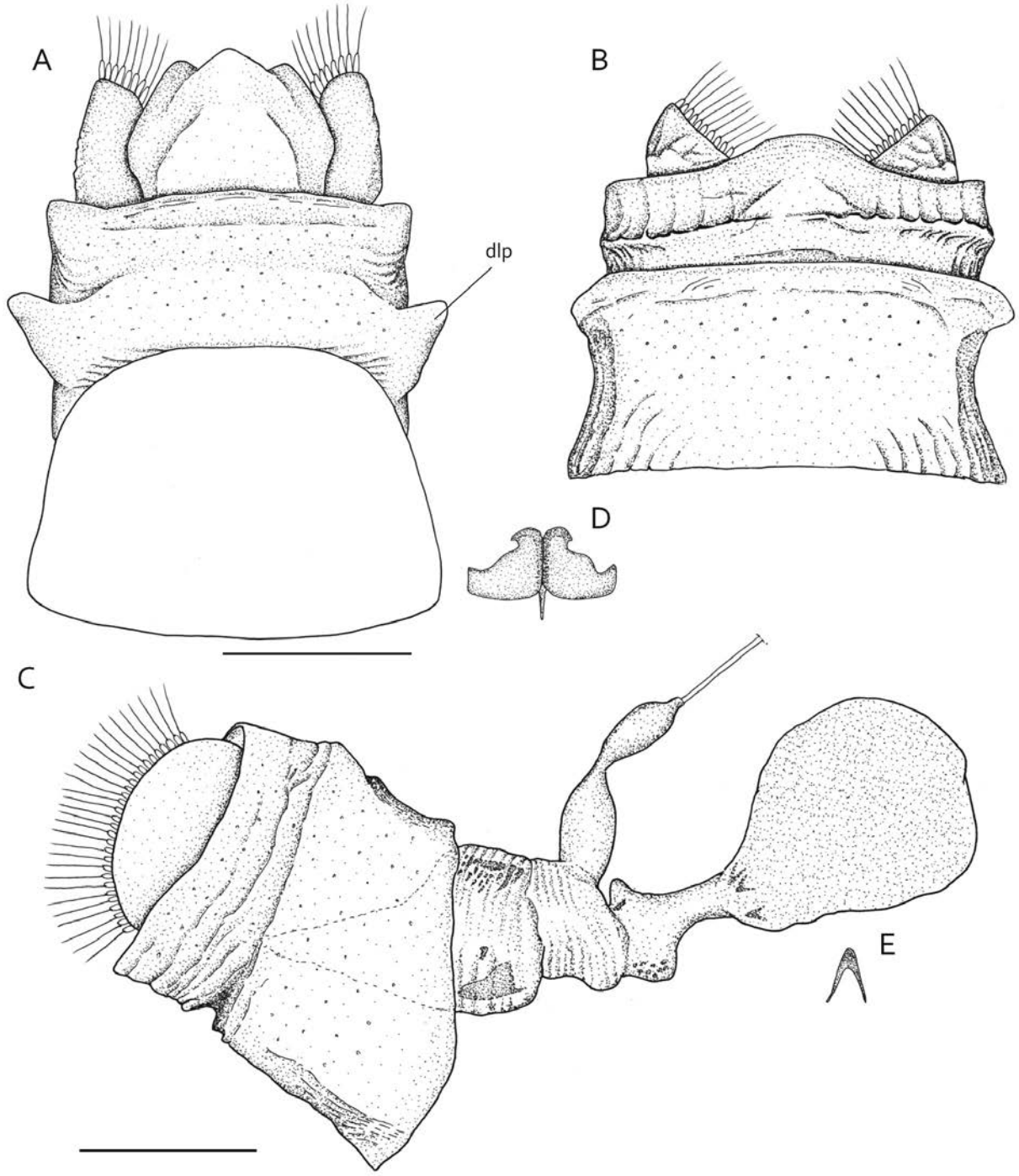


**Figure 13.** Female genitalia of *Issikiomartyria catapasta* sp. n. [paratype]. **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** female genitalia, lateral view; **D:** genital sclerite; **E:** signa. Abbreviation: **dlp** = dorso-lateral protrusion. Scale bars: 0.2 mm.

at Aosawa Sakata-shi, tunnel (340 m), Yamagata Pref (Fig. 19:10), KUHE.

**Type locality.** Japan, Iwate Pref: Mahirudake-rindou (Honshu).

**Diagnosis.** Male tergum X with middle portion as large as lateral portions, lateral portions extending dorsally. Female corpus bursae with tiny sclerites on membrane surface near caudal end.

**Description.** Head dark brown, naked and glossy on both side, sparsely covered with yellow piliform scales with dark yellow scales on vertex. Antenna longer than forewing in male; with 62 (61–65) flagellomeres in males (n= 3). Labial palp 1-segmented. Forewing length 4.4 mm (4.2–4.7, n=8) in male.

*Male abdomen and genitalia* (Fig. 14). Mid-dorsal length of segment IX ring about 1/4 of ventral length. Valva with a proximo-ventral ridge; inner ventral margin broad without concavity. Aedeagus with two pairs of dorsal ridges: a pair

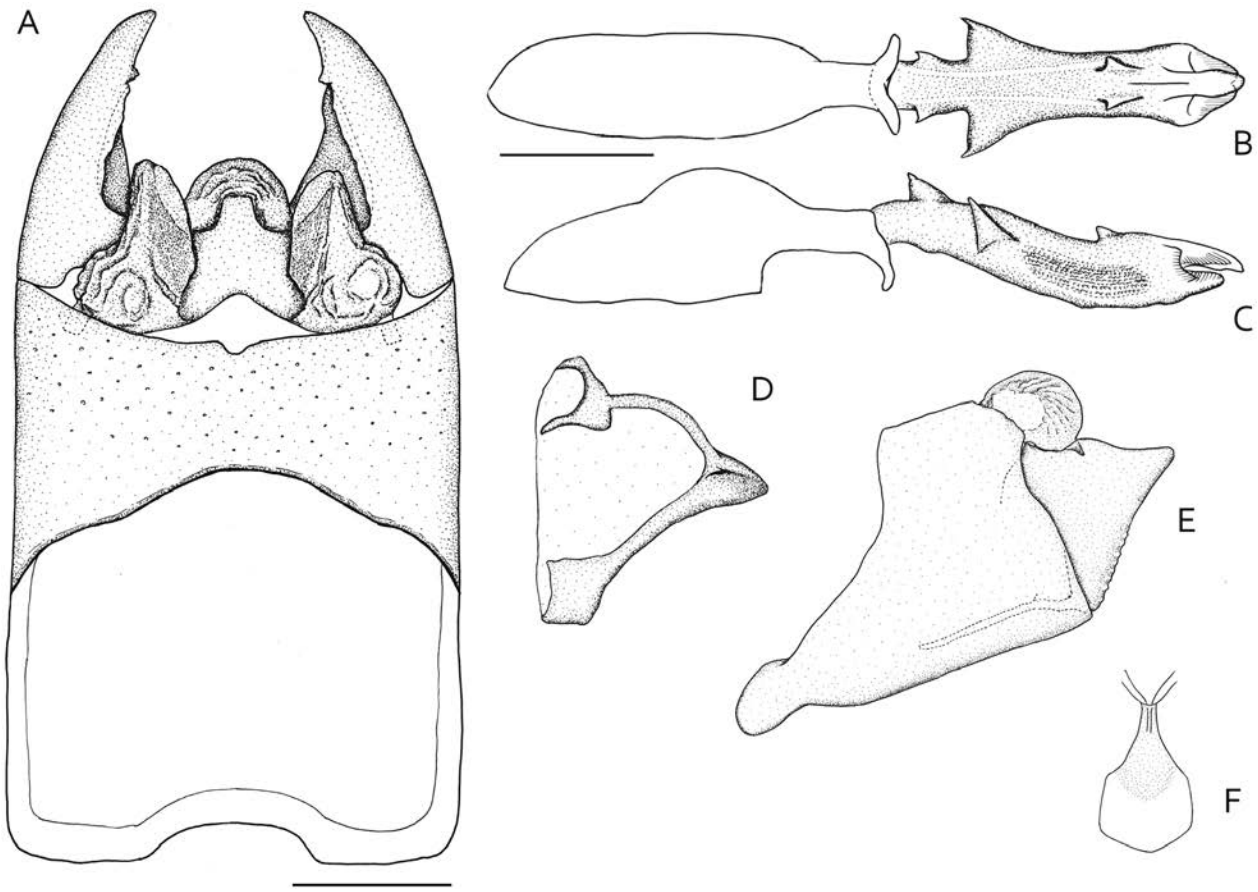


**Figure 14.** Male genitalia of *Issikiomartyria trochos* sp. n. [holotype]. **A:** genitalia capsule, dorsal view; **B:** phallus, dorsal view; **C:** ditto, lateral view; **D:** left valva, inner view; **E:** genitalia capsule, lateral view; **F:** median plate. Scale bars: 0.2 mm.

of shorter distal fins and a pair of longer proximal fins oriented about proximal quarter of aedeagal length, extending upwards; with a pair of lateral triangular fins extending horizontally. Tergum X with developed medial part, as large as lateral portions; lateral portions expanded dorsally.

*Female abdomen and genitalia* (Fig. 15). Segment IX forming a complete ring, strongly sclerotized; with a pair of dorso-lateral protrusions near base. Segment X consisting of a pair of lateral sclerites and a dorsal sclerotized plate; dorsal plate almost rectangular, slightly projected in middle. Corpus bursae large, globular, membranous, with long narrow neck region anteriorly; membrane surface with tiny sclerites scattered near caudal end; with signa composed of four saggitate sclerites near proximal end. Ductus spermathecae arising from a round concavity. Genital chamber smooth without sclerites, and a large fan-shaped sclerite ventrally.

**Remarks.** *Issikiomartyria trochos* sp. n. is most similar to *I. catapasta* sp. n. in that lateral parts of tergum X extending dorsally, but can be distinguished by the following traits: dorso- and latero-basal aedeagal fins separated from each other; female genital chamber without small sclerites; corpus bursae with tiny sclerites on membrane surface near caudal end.

**Etymology.** The specific name is a noun in apposition from the Greek word, "trochos" (wheel, disk), referring to the unusually extended form of tergum X of this species.

**Distribution.** This species has been found from the northern part of the main island of Japan (Honshu: Yamagata Pref.).

**Bionomics.** Larvae feed on the thalli of *Conocephalum conicum*. The habitat is a forest path along mountain streams of cool-temperate forests at 340–595 m, where *Fagus crenata* and *Quercus crispula* dominate.

### *Issikiomartyria akemiae* Hashimoto, 2006

Fig. 16

*Issikiomartyria akemiae* Hashimoto, 2006: 70, fig. 20.

**Material examined.** Lectotype: JAPAN [HONSHU] 5♂1♀ collected by MK on 8.VI.2009 at Kiyotsu-kyo, Niigata Pref (Fig. 19: 11), NMNS.

**Description (based on female).** Head dark brown, naked and glossy on both side, sparsely covered with yellow pili-

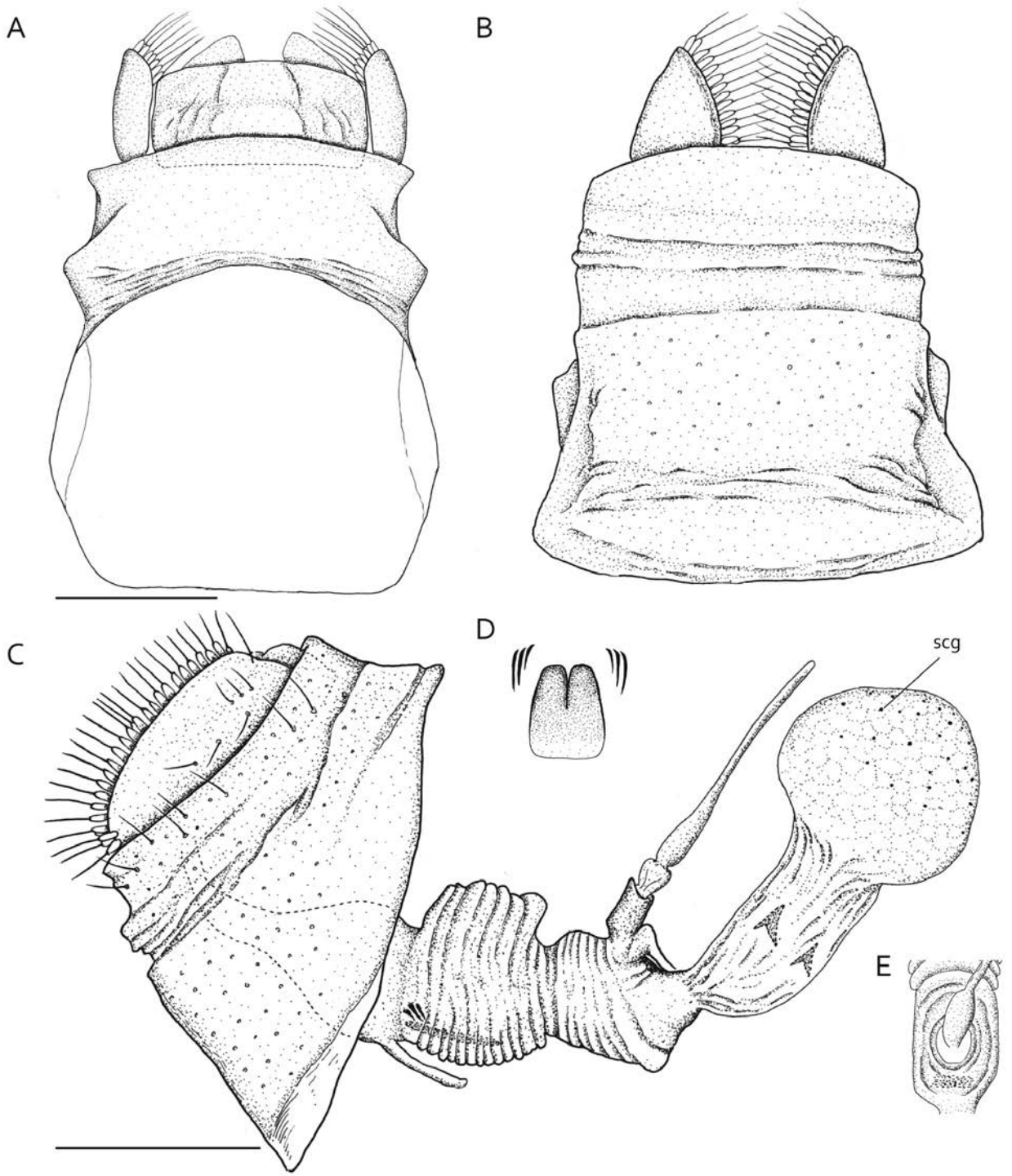


**Figure 15.** Female genitalia of *Issikiomartyria trochos* sp. n. [paratype]. **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** female genitalia, lateral view; **D:** genital sclerite; **E:** arising part of ductus spermathecae, dorsal view. Scale bars: 0.2 mm. Abbreviation: **scg** = sclerotized granules of corpus bursae.

form scales with dark yellow scales on vertex. Antenna longer than forewing in male. Labial palp 1-segmented.

*Female abdomen and genitalia* (Fig. 16). Segment IX forming a complete ring, strongly sclerotized, without lateral concavity. Segment X consisting of a pair of lateral sclerites and a dorsal sclerotized plate; lateral sclerites simple, as broad as long, with digitate projections having an apical seta at terminal inner margin. Corpus bursae large, membranous, with long narrow neck region anteriorly; with signa composed of four tridenta-form sclerites around neck region. Ductus spermathecae arising from a round concavity, forked in middle. Genital chamber with numerous tiny sclerites dorsally and a large fan-shaped sclerite ventrally.

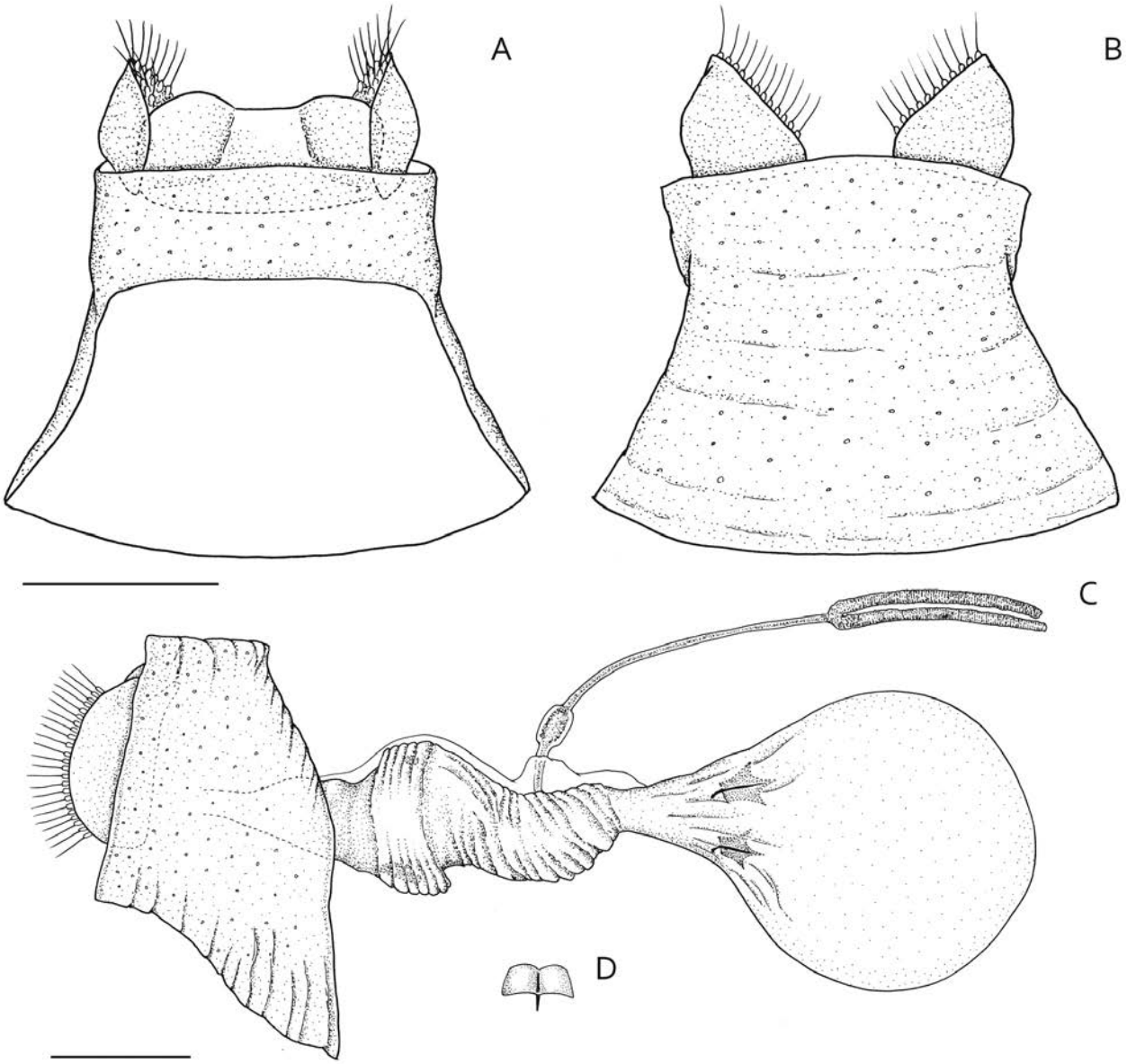


**Figure 16.** Female genitalia of *Issikiomartyria akemiae* **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** female genitalia, lateral view; **D:** genital sclerite. Scale bars: 0.2 mm.

**Remarks.** *I. akemiae* can be distinguished by female genitalia having the ductus spermathecae forked in the middle.

**Bionomics.** Larvae feed on the thalli of *Conocephalum conicum*.

### *Issikiomartyria plicata* Hashimoto, 2006

Fig. 17

*Issikiomartyria plicata* Hashimoto, 2006: 71, fig. 21.

**Materials examined.** Lectotype: JAPAN [HONSHU] 11♂2♀ collected by MK, YI on 8.VI.2009 at Amamizukoshi (495m), Matsunoyama-cho, Niigata Pref (Fig. 19:12), NMNS.

Additional materials: JAPAN [HONSHU] 2♂ collected by YI on 14.V.2008 at Shimooritateurasa, Uonuma-shi, Niigata Pref (Fig. 19:13); 11♂ collected by YI on 27.VI.2011 at Komanoyu (370m), Uonuma-shi, Niigata Pref (Fig. 19:14); 1 larva collected by YI on 13.VI.2011 at Sagurigawa-dam, Uonuma-shi, Niigata Pref (Fig. 19:15); 11♂2♀ collected by MK on 8.VI.2009 at Nishinomae, Matsunoyama-cho, Niigata Pref (Fig. 19:16); 3♂3♀ collected as larvae by YI on 6.XII.2010 at same locality; 3♂1♀ collected by YI on 13.VI.2011 at Mizunashi, Matsunoyama-cho, Niigata Pref (Fig. 19:17).

**Description (based on female).** Head dark brown, naked and glossy on both side, sparsely covered with yellow piliform scales with dark yellow scales on vertex. Antenna longer than forewing in male. Labial palp 1-segmented.

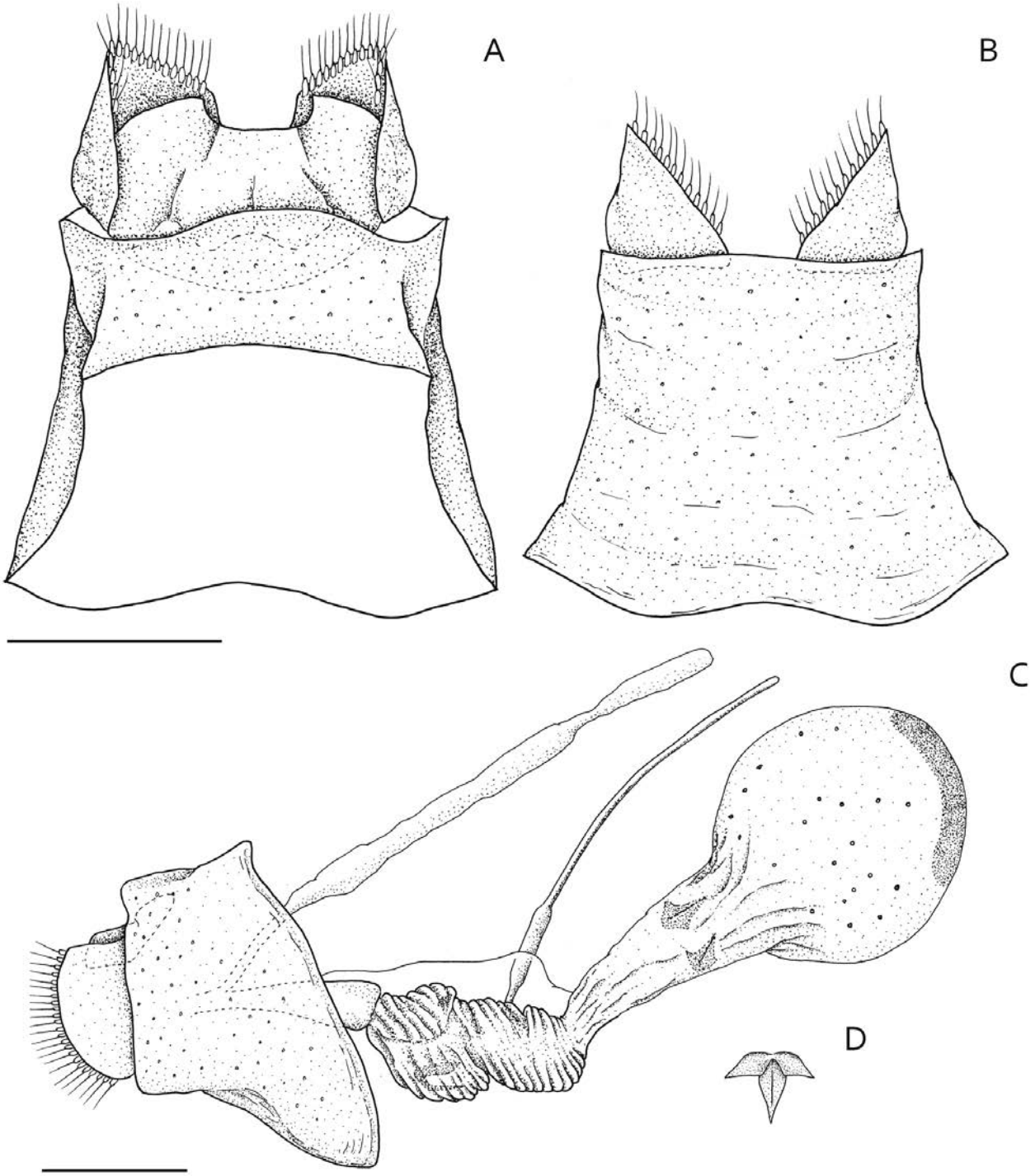


**Figure 17.** Female genitalia of *Issikiomartyria plicata* sp. n. [paratype]. **A:** genitalia capsule, dorsal view; **B:** ditto, ventral view; **C:** female genitalia, lateral view; **D:** genital sclerite. Scale bars: 0.2 mm.

*Female abdomen and genitalia* (Fig. 17). Segment IX forming a complete ring, strongly sclerotized; concave dorso-laterally. Segment X consisting of lateral sclerites and a dorsal sclerotized plate; lateral sclerites simple, as broad as long, with digitate projections having an apical seta at terminal inner margin. Corpus bursae large, membranous, with long narrow neck region anteriorly; with signa composed of four fan-shaped sclerites at distal of neck region; distal end densely sclerotized. Ductus spermathecae arising from a round concavity. Genital chamber with a sclerite ventrally.

**Remarks.** *I. plicata* can be distinguished from other species of *Issikiomartyria* based on the female genitalia in that having densely sclerotized zone at distal end of corpus bursae.

**Bionomics.** Larvae feed on the thalli of *Conocephalum conicum*.

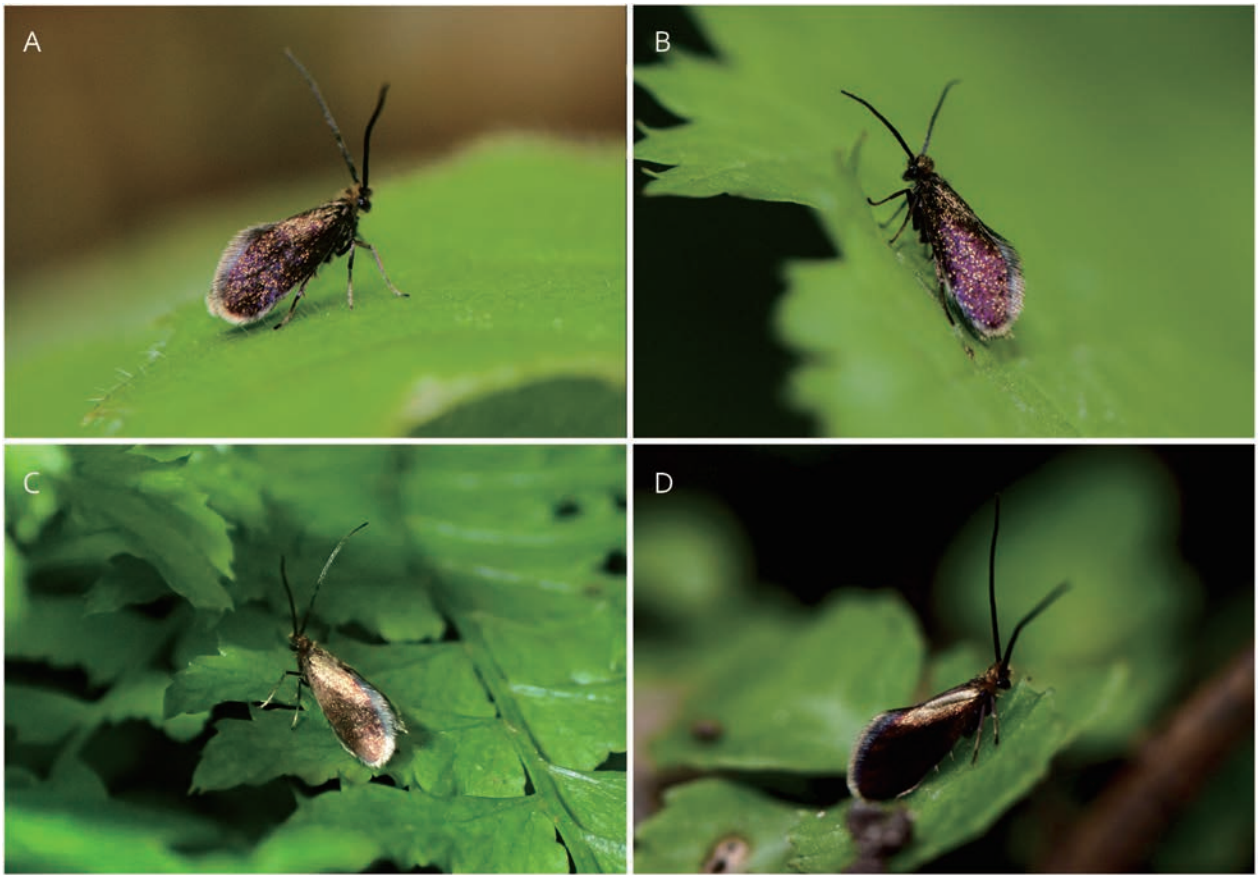


**Figure 18.** Ecology. **A:** *Issikiomartyria catapasta* sp. n. at Tachimata-keikoku, Akita Pref; **B:** *I. trochos* sp. n. at Jintsu-kyo, Yamagata Pref; **C:** *I. trochos* sp. n. at Mahirudake-rindo, Iwate Pref; **D:** *Melinopteryx bilobata* sp. n. at Ushikubi-touge, Shizuoka Pref.

## Key to the Japanese genera of Micropterigidae (modified from Hashimoto 2006)

1 Forewing with RI vein deeply bifurcate, except for *Austromartyria porphyrodes* Turner, 1932.....Southern Hemisphere genera
– Forewing with RI vein unforked (rarely shallowly bifurcate as an individual variation) ..... (Northern Hemisphere genera)...2
2 Fore- and hindwings with an acute apex; hindwing usually with a complete stem vein of RI ..... *Micropterix*
– Fore- and hindwings with an obtusely round apex; hindwing with an incomplete stem vein of RI ..... 3
3 Head covered with yellow or orange piliform scales ..... 4
– Head covered with black piliform scales..... 8
4 Foretibia with epiphysis ..... 5
– Foretibia without epiphysis ..... 6
5 Fore- and hindwings with a radial cell; aedeagus with about 20 to 50 minute serrate projections; female segment X without a dorsal sclerite ..... *Paramartyria*
– Fore- and hindwings without a radial cell; aedeagus with a few minute serrate projections; female segment X with a dorsal sclerite .....*Palaeomicroides* Issiki, 1931
6 Fore- and hindwings without a radial cell..... 7
– Fore-and hindwings with a radial cell ..... 8
7 Pedicel as large as the most basal flagellum; labial palp developed, 2-segmented; aedeagus with three pairs of dorsal ridges, a pair of lateral triangular fins, a ventral longitudinal fin extended vertically ..... *Melinopteryx* gen. n.
– Pedicel twice as large as the most basal flagellum; labial palp weakly developed, usually 1-segmented (except for *I. bisegmentata*); aedeagus with two pairs of dorsal ridges and a pair of lateral triangular fins, without ventral fins ..... *Issikiomartyria*
8 A basal stalk of each flagellomere distinct; aedeagus divided into dorsal and ventral branches; corpus bursae large, with four distinct tridentaform signa .....*Epimartyria* Walsingham, 1898
– A basal stalk of each flagellomere distinct; aedeagus not divided; corpus bursae small, with or without four minute signa.....*Vietomartyria* Hashimoto & Mey, 2000
9 Forewing slender; valva with a costal long projection curved ventro-mesally; aedeagus without dorsal and ventral longitudinal ridges; female segment X without a dorsal sclerotized plate ..... *Kurokopteryx*
– Forewing rather broad and oval; valva without such a long projection; aedeagus with dorsal and ventral longitudinal ridges; female segment X with a dorsal sclerotized plate.....*Neomicropteryx*

### Key to *Issikiomartyria* species based on male genitalia

1 Valva with digitiform apex; tergum X longer than 3/4 of valva ..... *I. leptobelos* sp. n.
– Valva gradually tapering toward apex; tergum X shorter than 3/4 of valva ..... 2
2 Tergum X with a pair of lateral flanges or protrusions exerted vertically; aedeagus with broad, triangular latero-basal fins ..... 3
– Tergum X without lateral flanges; aedeagus with narrow latero-basal fins ..... 4
3 A pair of latero-basal fins of aedeagus acute at base, arising from ventral side ..... *I. hyperborea* sp. n.
– A pair of latero-basal fins of aedeagus broad at base, arising from lateral side ..... 5
4 Valva with a mid-dorsal ridge; aedeagus with a pair of lateral triangular fins at or near middle ..... 6
– Valva without mid-dorsal ridge; aedeagus with a pair of lateral triangular fins near terminal end ..... *I. bisegmentata*
5 Valva with a mid-dorsal ridge; middle portion of tergum X smaller than lateral portion; dorso- and latero-basal aedeagal fins and flanges close to each other ..... *I. catapasta* sp. n.
– Valva without mid-dorsal ridge; middle portion of tergum X almost same size as lateral portion; dorso- and latero-basal aedeagal fins and flanges separated from each other ..... *I. trochos* sp. n.
6 Dorso-basal margin of valva strongly expanded posteriorly; a mid-dorsal ridge of valva a thin plate expanding ventro-mesally; dorso- and latero-basal aedeagal fins rather broadly separated from each other; lateral triangular fins of aedeagus small ..... *I. distincta*
– Dorso-basal margin of valva not expanded; a mid-dorsal ridge of valva either hornlike or a rather thick plate; dorso- and latero-basal aedeagal ridges close to each other; lateral triangular fins of aedeagus relatively large ..... 7
7 A mid-dorsal ridge of valva a thick plate, rounded; phallobase without a longitudinal ventral ridge; proximo-lateral fins of aedeagus short, rather stout; medial part of tergum X broadly concave ..... *I. nudata*
– A mid-dorsal ridge of valva hornlike, acute ventrally; phallobase with longitudinal ventral ridges; proximo-lateral fins of aedeagus slender; medial part of tergum X not concave ..... 8
8 Phallobase with two longitudinal short ridges running parallel each other; proximo-lateral fins of aedeagus extending antero-dorsally ..... *I. akemiae*
– Phallobase with a longitudinal ridge; proximo-lateral projections extending horizontally ..... *I. plicata*

### Key to *Issikiomartyria* species based on female genitalia (female unknown in *distincta*)

1 Segment IX with a deep concavity on ventral side ..... *I. hyperborea* sp. n.
– Segment IX without concavity on ventral side ..... 2
2 Segment IX with a dorso-lateral protrusion ..... 3
– Segment IX without protrusion ..... 4
3 Genital chamber with numerous small sclerites scattered; distal surface of corpus bursae smooth, without sclerotization ..... *I. catapasta* sp. n.
– Genital chamber without sclerites on dorsal side but with a large one on ventral side; corpus bursae densely covered with weakly sclerotized granules on distal surface ..... *I. trochos* sp. n.
4 Mid-dorsal length of segment IX ring shorter than 1/3 of ventral length ..... *I. leptobelos* sp. n.
– Mid-dorsal length of segment IX ring longer than 1/3 of ventral length ..... 5
5 Distal zone of corpus bursae densely sclerotized ..... *I. plicata*
– Distal zone of corpus bursae without sclerotization ..... 6
6 Signa composed of four reduced sclerites in corpus bursae ..... *I. nudata*
– Signa composed of four tridenta-form sclerites in corpus bursae ..... 7
7 Sclerites in signa shallowly bifurcated; ductus spermathecae forked in middle ..... *I. akemiae*
– Sclerites in signa deeply bifurcated; ductus spermathecae without forked ..... *I. bisegmentata*

### Additional sampling records of micropterigids in the Northeastern Japan

All specimens described below are stored in KUHE.

#### *Issikiomartyria bisegmentata* Hashimoto, 2006

JAPAN [HONSHU] 2♂ collected by YI on 20.X.2013 at Aosawa tunnel, Sakata-shi, Yamagata Pref (Fig. 19:10); 1♀ emerged on 9.VII.2015 collected by MK on 28.VI.2015 at Okutadami dam, Uonuma-shi, Niigata Pref (Fig. 19:18); 1 larva collected by N. Yoshikawa on 9.XII.2013 at Tadami-cho, Fukushima Pref (Fig. 19:19); 2♂ emerged on 29.VI.2014 collected by YI on 17.V.2015 at Hahanaritouge, Koriyama-shi, Fukushima Pref (Fig. 19:20).

#### *Issikiomartyria distincta* Hashimoto, 2006

JAPAN [HONSHU] 2 larvae collected by YI on 20.V.2013 at Shirabu-onsen, Yonezawa-shi, Yamagata Pref (Fig. 19:21); 2♂ emerged on 19.VI.2015 collected as larvae by YI on 17.V.2015 at same locality; 3♂ collected by YI on 27.VI.2015 at Goreibitsu-touge, Konan-cho, Fukushima Pref (Fig. 19:22); 1♂ collected on 17.V.2014

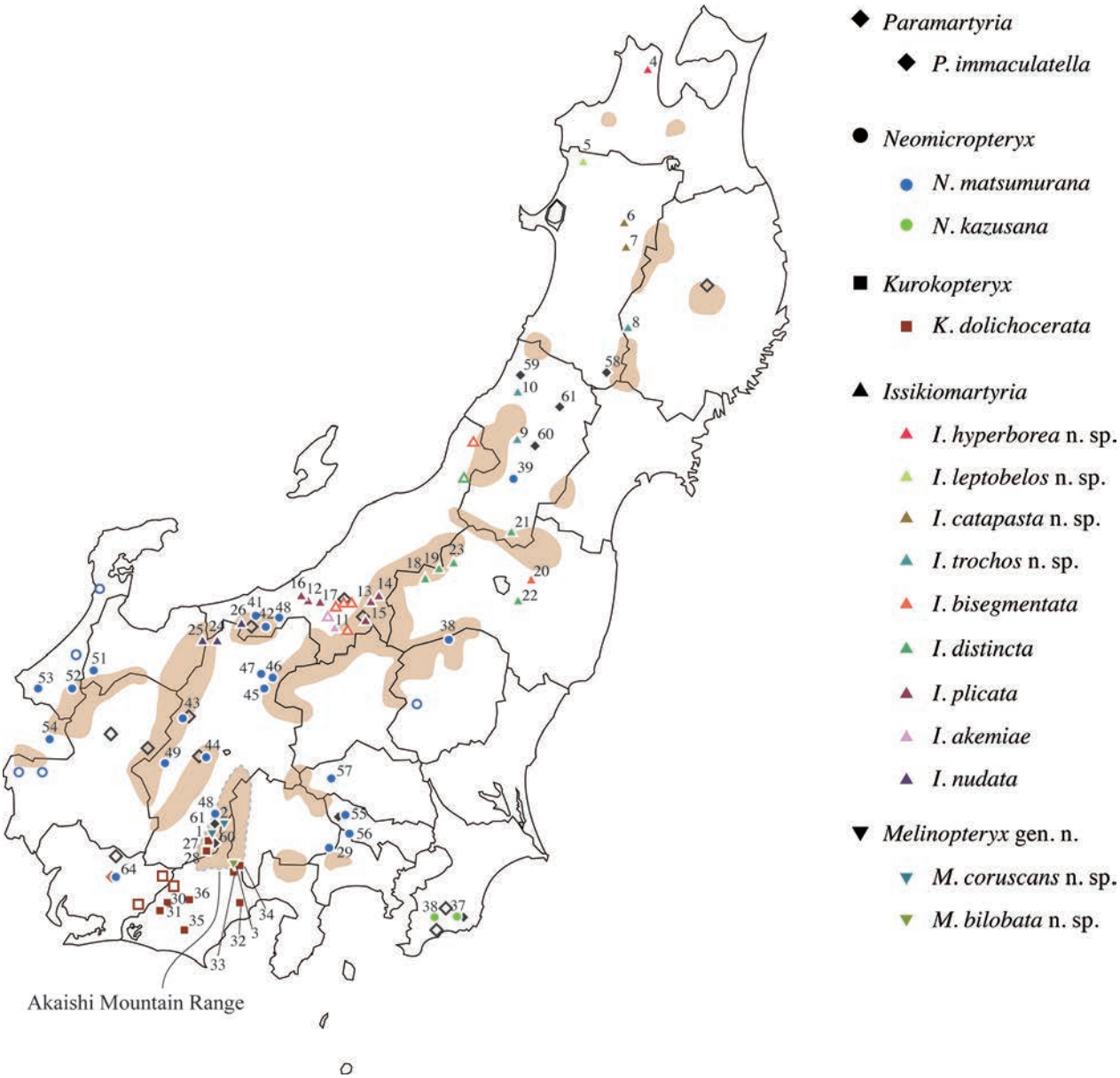


**Figure 19.** Locality records of micropterigid moths in the Northeastern Japan. Locality codes correspond to those in the text. Shaded are the areas higher than 1000 m elevation. Sampling records from Hashimoto (2006) are indicated as outlined symbols.

at same locality; 6♂ collected by YI on 9.VI.2016 at Kurosawa, Yanaidu-machi, Fukushima Pref (Fig. 19:23).

### *Issikiomartyria nudata* Hashimoto, 2006

JAPAN [HONSHU] 4♂ collected by MK on 14.VII.2009 at Renge-onsen, Otoigawa-shi, Niigata Pref (Fig. 19:24); 1♂ collected by MK on 11.VII.2008 at Mt.Shirouma, Hakuba-mura, Nagano Pref (Fig. 19:25); 2♂ collected by MK on 14.VII.2009 at Mt.Amakazari, Otari-mura, Nagano Pref (Fig. 19:26).

### *Kurokopteryx dolichocerata* Hashimoto, 2006

JAPAN [HONSHU] 4♂ collected by YI on 12.VI.2011 at Kashio, Ohshika-mura, Nagano Pref (Fig. 19:27); 2♂ collected as larvae by YI on 14.V.2008 at Uemura, Iida-shi, Nagano Pref (Fig. 19:28); 2 larvae collected by YI on 14.XI.2011 at same locality; 1 larva collected by MK on 17.II.2002 at Shibakawa, Fujinomiya-shi, Shizuoka Pref (Fig. 19:29); 2♂3♀ emerged on 24.IV.2009 collected as larvae by MK on 17.II.2009 at Ketagawa, Hamamatsu-shi, Shizuoka Pref (Fig. 19:30); 2♂2♀ emerged on 24.IV.2008 collected as larvae by MK on 20.IV.2008 at Shirakura-kyo, Hamamatsu-shi, Shizuoka Pref (Fig. 19:31); 1♂ emerged on 27.IV.2008 collected as larvae by MK on 21.IV.2008 at Ashikubo, Shizuoka-shi, Shizuoka Pref (Fig. 19:32); 1♀ collected by YI on 13.IV.2014 at same locality; 1♂ emerged on 15.V.2009 collected by YI on 22.IV.2009 at Kuchisakamoto Onsen, Shizuoka-shi, Shizuoka Pref (Fig. 19:33); 2♂ collected by MK on 15.VI.2008 at Umegashima Onsen, Shizuoka-shi, Shizuoka Pref (Fig. 19:34); 1♂ emerged on 30.IV.2008 collected as larvae by MK on 21.IV.2008 at Morimachi, Shizuoka Pref (Fig. 19:35); 1♂ emerged on 11.V.2008 collected as larvae by MK on

21.IV.2008 at Ishikiri, Hamamatsu-shi, Shizuoka Pref (Fig. 19:36); 1 larva collected by YI on 20.X.2015 at Uradani, Shitara-cho, Aichi Pref (Fig. 19:64).

***Neomicropteryx kazusana* Hashimoto, 1992**

JAPAN [HONSHU] 5♂ collected by MK on 14.V.2008 at Mt. Kiyosumi, Kamogawa-shi, Chiba Pref (Fig. 19:37); 2♂ emerged on 18.IV.2012 collected as larvae by MK on 24.I.2012 at Yoro-keikoku, Ichihara-shi, Chiba Pref (Fig. 15:38); 7 larvae collected by YI on 12.XI.2011 at same locality.

***Neomicropteryx matsumurana* Issiki, 1931**

JAPAN [HONSHU] 1♂ emerged on 20.IV.2014 from larva collected by YI on 19.X.2013 at Nogawakeikoku, Nagai-shi, Yamagata Pref (Fig. 19:39); 1♂ emerged collected as larva by YI on 20.V.2014 at Sanno-rindo, Nikko-shi, Tochigi Pref (Fig. 19:40); 1♂ collected by YI on 27.V.2014 at Mt.Yakiyama, Myoko-shi, Niigata Pref (Fig. 19:41); 1♂ collected as larva by YI on 29.IV.2014 at Myoko-kougen, Myoko-shi, Niigata Pref (Fig. 19:42); 1♂ collected by YI on 28.V.2014 at Shimashimatani, Matsumoto-shi, Nagano Pref (Fig. 19:43); 2 larvae on 7.X.2012 at Yokokawa-keikoku, Tatsuno-cho, Nagano Pref (Fig. 19:44); 1♂ collected as larva on 26.V.2014 at same locality; 1 larva on 17.X.2015 at same locality; 3 larvae collected by YI on 22.IX.2008 at Karasawanotaki, Ueda-shi, Nagano Pref (Fig. 19:45); 1 larva collected by YI on 23.IX.2008 at Zakogawa, Shigakougen, Yamauchi-machi, Nagano Pref (Fig. 19:46); 3 larvae collected by YI on 22.IX.2008 at Ohtanirindo, Suzaka-shi, Nagano Pref (Fig. 19:47); 4♂1♀ collected by MK on 11.VI.2007 at Naenataki, Myoko-shi, Nagano Pref (Fig. 19:48); 1♂ collected as larva by YI on 29.IV.2013 at same locality; 5♂ collected by MK and YI on 7.VI.2013 at Agematshu-cho, Nagano Pref (Fig. 19:49); 2 larvae collected by YI on 5.X.2012 at same locality; 6♂ collected by YI on 2.VI.2010 at Kashio, Ohshika-mura, Nagano Pref (Fig. 19:50); 2 adults collected by YI on 9.VII.2012 at same locality; 4♂ collected by MK at Bunaotouge, Nanto-shi, Toyama Pref (Fig. 19:51); 3♂ collected by MK on 3.V.2013 at Mt. Hakusan, Hakusan-shi, Ishikawa Pref (Fig. 19:52); 1♂ collected by MK as larva on 1.IX.2011 at Takedagawa, Sakai-shi, Fukui Pref (Fig. 19:53); 2 larvae collected by YI on 22.IV.2015 at Ifuriyama, Ohno-shi, Fukui Pref (Fig. 19:54); 3♂2♀ emerged on 21.IV.2016 collected as larvae by MK on 15.III.2016 at Nippara, Okutama-cho, Tokyo Metropolitan (Fig. 19:55); 2♂1♀ emerged on 9.V.2011 collected as larvae by YI on 5.IV.2011 at Kogesawa, Hachioji-shi, Tokyo Metropolitan (Fig. 19: 56); 2♂ emerged on 21.IV.2015 collected as larvae by MK on 28.XI.2014 at Okuchichibu, Chichibu-shi, Saitama Pref (Fig. 19:57); 1 larva collected by MK on 19.VIII.2002 at same locality; 2♂ emerged on 21.IV.2015 collected by MK as larvae on 28.XI.2014 at same locality.

***Paramartyria immaculatella* Issiki, 1931**

JAPAN [HONSHU] 5♂ collected by YI on 12.VI.2016 at Sanzugawa-keikoku, Yuzawa-shi, Akita Pref (Fig. 19:58); 1 larva collected by YI on 20.X.2013 at Shimoaosawa, Sakata-shi, Yamagata Pref (Fig. 19:59); 1♂ emerged on 20.IV.2014 collected as larva by YI on 19.X.2013 at Kodera-keikoku Jintsukyo, Ooema-chi, Yamagata Pref (Fig. 19:9); 1 larva collected by YI on 20.X.2013 at Onumanoukishima, Asahi-cho, Yamagata Pref (Fig. 19:60); 1 larva collected by YI on 20.X.2013 at Mt. Mokuzousan, Sakata-shi, Yamagata Pref (Fig. 19:61); 1♂ collected by YI on 7.VI.2013 at Agematsu-cho, Kiso, Nagano Pref (Fig. 19:49); 7♂ collected by YI on 12.VI.2011 at Uemura, Iida-shi, Nagano Pref (Fig. 19:28); 6 larvae feeding on *Calypogeia tosana* (Steph). Steph. (Calypogeiaceae) collected by YI on 14.XI.2011 at same locality; 1 larva collected by YI on 18.X.2015 at same locality; 1 larvae collected by YI on 19.X.2015 at Hodonooike, Iida-shi, Nagano Pref (Fig. 19:62); 1 larvae collected by YI on 18.X.2015 at Wazo, Ohshika-mura, Nagano Pref (Fig. 19:63); 2♂ collected by MK on 14.V.2008 at Mt.Kiyosumi, Kamogawa-shi, Chiba Pref (Fig. 19:37); 7 larvae feeding on *Pellia endiviifolia* (Dicks.) Dumort. (Pelliaceae) collected by YI on 12.XI.2011 at Yoro-keikoku, Ichihara-shi, Chiba Pref (Fig. 15:38); 1 larva collected by YI on 20.X.2015 at Uradani, Shitara-cho, Aichi Pref (Fig. 19:64).

## Discussion on the distribution of *Issikiomartyria*

Our extensive field surveys have revealed that the distribution of *Issikiomartyria* is extended from northernmost to the central region of Honshu. *Issikiomartyria* species tend to be found from the geographically fragmented area facing the Japan Sea but not from the Pacific Ocean sides, although we conducted a census with considerable efforts throughout northeastern Honshu. The host-plant species is not likely to be the limiting factor of their distribution, because *Conocephalum* liverwort is widespread in the mainland of Japan (Imada Y and Kato M, pers. obs.). The Japan Sea side of the Japanese archipelago corresponds to the largely snow-covered area (Suzuki 1962) and harbors unique plant and animal species and it is called "Japan Sea elements" (Fukuoka 1966). The factors contributing the distribution of *Issikiomartyria* are unclear, they may be associated with heavy-snow conditions in winter.

To our knowledge, *Issikiomartyria* offers a largest example of regional diversification of insects in the northeastern Japan. Especially, it should be noted that *I. hyperborea* sp. n. may be the only insect species endemic to the Tsugaru peninsula so far known, which can be morphologically well-differenciated from the rest of *Issikiomartyria* spp. Several groups of animals represent genetic variations among the geographic populations in the northeastern Japan: terrestrial animals (Suzuki et al.

2004); *Carabus* ground beetles (Sota et al. 2001), freshwater fish (Yamamoto et al. 2004), and amphibious salamanders, *Onychodactylus nipponoborealis* (Poyarkov et al. 2012) and *Hynobius lichenatus* Boulenger, 1883 (Yoshikawa et al. 2008, Aoki et al. 2013). In addition, pronounced morphological differenciation is detected in a sinistral land snail group, *Euhadra grata* (Gude, 1900), occurring in the northeastern region. *E. grata* group consists of four morphologically distinct subspecies (Nishitani 1996, Kawana 2007), each of which range is allopatric one another. However, the geographic area where the genetic differentiations detected in these lineages are not correlated with the species ranges of *Issikiomartyria.*

Furthermore, we have discovered two species of *Melinopteryx* gen. n., *M. coruscans* sp. n. and *M. bilobata* sp. n., from highlands of the Akaishi Mountain Range. *Melinopteryx* gen. n. is sister to *Issikiomartyria* in a molecular phylogenetic analysis (Imada et al. 2011), and the species of both genera favor snowy regions of the Chubu and Tohoku regions of Japan, whereas some of the geographic populations are in proximity with either *Neomicropteryx matsumurana* or *Kurokopteryx dolichocerata*, which tend to occur in lower mountains. The species range of *M. coruscans* approximates two Japan endemic microptergid species: at the Akaishi Mountain Range, *N. matsumurana* (Fig. 19:48) and *K. dolichocerata* (Fig. 19:27,28) respectively inhabit northern and southern regions of Bunkui touge, a mountain ridge along the Median Tectonic Line, and *M. coruscans* has only been found in between narrow area higher than 1500 m. Likewise, *M. bilobata* sp. n. has been found from the southeastern area of the Akaishi Mountain Range (Fig. 19:3), where some populations of *K. dolichocerata* colonizes in adjacent but lower area (Fig. 19:33,34). Hence, this study supports prior findings that each *Conocephalum*-feeding micropterigid species belonging to the Japanese endemic micropterigid genera (*Issikiomartyria, Kurokopteryx, Neomicropteryx*) does not co-occur with one another (Hashimoto 2006, Imada et al. 2011).

The Japanese patterns of allopatry is even more extreme than that found in New Zealand *Sabatinca* yet contrasts markedly with a pattern of sympatry found in New Caledonia (Gibbs 1983; Gibbs and Lees 2014). Our study reinforces the potential research interest of Micropterigidae in differentially reflecting geological and ecological diversification processes at different spatial and temporal scales.

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## References

Aoki G, Matsui M, Nishikawa K (2013) Mitochondrial cytochrome b phylogeny and historical biogeography of the Tohoku salamander, *Hynobius lichenatus* (Amphibia, Caudata). Zoological Science 30: 167–173. https://doi.org/10.2108/zsj.30.167

Davis DR, Landry J-F (2012) A review of the North American genus *Epimartyria* (Lepidoptera, Micropterigidae) with a discussion of the larval plastron. ZooKeys 183: 37–83. https://doi.org/10.3897/zookeys.183.2556

Engel MS, Kinzelbach RK (2008) A primitive moth from the earliest Eocene Fur Formation ("Mo-clay") of Denmark (Lepidoptera: Micropterigidae). Linzer biologische Beiträge 40: 1443–1448.

Fukuoka N (1966) On the distribution patterns of the so-called Japan Sea elements confined to the Japan Sea region. The Journal of Geobotany 15: 63–80. https://doi.org/10.1007/BF00218523

Gibbs GW (1983) Evolution of Micropterigidae (Lepidoptera) in the SW Pacific. GeoJournal 7: 505–510.

Gibbs GW (2006) Ghosts of Gondwana. Craig Potton Publishing, Nelson, New Zealand, 232 pp.

Gibbs GW (2010) Micropterigidae (Lepidoptera) of the Southwestern Pacific: A revision with the establishment of five new genera from Australia, New Caledonia and New Zealand. Zootaxa 2520: 1–48.

Gibbs GW (2014) Micropterigidae (Insecta: Lepidoptera). Fauna of New Zealand 72: 1–127.

Gibbs GW, Kristensen NP (2011) *Agrionympha*, the long-known South African jaw moths: a revision with descriptions of new species (Lepidoptera, Micropterigidae). Zootaxa 2764: 1–21.

Gibbs GW, Lees DC (2014) New Caledonia as an evolutionary cradle: a re-appraisal of the jaw-moth genus *Sabatinca* (Lepidoptera: Micropterigidae) and its significance for assessing the antiquity of the island's fauna. In: Guilbert É, Robillard T, Jourdan H, Grandcolas P (Eds) Zoologia Neocaledonica 8. Biodiversity studies in New Caledonia. Muséum national d'Histoire naturelle, Paris, 239-266.

Hashimoto S (2006) A taxonomic study of the family Micropterigidae (Lepidoptera, Micropterigoidea) of Japan, with the phylogenetic relationships among the Northern Hemisphere genera. Bulletin of the Kitakyushu Museum of Natural History and Human History Series A 4: 39–109.

Hirowatari T (1997) A taxonomic revision of the genus *Adela* Latreille (Lepidoptera, Adelidae) from Japan. Transactions of the Lepidopterological Society of Japan 48: 271–290.

Imada Y, Kawakita A, Kato M (2011) Allopatric distribution and diversification without niche shift in a bryophyte-feeding basal moth lineage (Lepidoptera: Micropterigidae). Proceedings of the Royal Society B: Biological Sciences 278: 3026–3033. https://doi.org/10.1098/rspb.2011.0134

Issiki S (1931) On the morphology and systematics of Micropterygidae (Lepidoptera Homoneura) of Japan and Formosa, with some considerations on the Australian, European, and North American forms. Proceedings of the zoological society of London 1931:999–1039. https://doi.org/10.1111/j.1096-3642.1931.tb01052.x

Issiki S (1953) Micropterigoidea of Japan. Bulletin of the Naniwa University Series B Agricultural and Natural Science 3: 133–140.

Kawana M (2007) The world of land snails (Euhadra) in Japan. Kinmirai-sha, Nagoya, 332 pp. [In Japanese]

Kozlov MV (1988) Paleontology of lepidopterans and problems of the phylogeny of the order Papilionida. In: Panomarenko AG (Ed.)

The Mesozoic-Cenozoic crisis in the evolution of insects. Moscow, 16–69. [In Russian]

Kristensen NP (1984) Studies on the morphology and systematics of primitive Lepidoptera (Insects). Steenstrupia 10: 141–191.

Kristensen NP (1999) Lepidoptera: moths and butterflies, 1: evolution, systematics and biogeography, Handbook of zoology. Walter de Gruyter, Berlin, Germany, 501 pp.

Kristensen NP, Hilton DJ, Kallies A, Milla L, Rota J, Wahlberg N, Wilcox SA, Glatz RV, Young DA, Cocking G, Edwards T, Gibbs GW, Halsey M (2015) A new extant family of primitive moths from Kangaroo Island, Australia, and its significance for understanding early Lepidoptera evolution. Systematic Entomology 40: 5–16. https://doi.org/10.1111/syen.12115

Kristensen NP, Nielsen ES (1979) A new subfamily of micropterigid moths from South America. A contribution to the morphology and phylogeny of the Micropterigidae, with a generic catalogue of the family (Lepidoptera: Zeugloptera). Steenstrupia 5: 69–147.

Kristensen NP, Scoble MJ, Karsholt O (2007) Lepidoptera phylogeny and systematics: the state of inventorying moth and butterfly diversity. Zootaxa 1668: 699–747.

Lees DC, Rougerie R, Zeller-Lukashort C, Kristensen NP (2010) DNA mini-barcodes in taxonomic assignment: a morphologically unique new homoneurous moth clade from the Indian Himalayas described in Micropterix (Lepidoptera, Micropterigidae) Zoologica Scripta 39: 642–661. https://doi.org/10.1111/j.1463-6409.2010.00447.x

Nishitani H (1996) Snails in Japan. Hiroyuki Nishitani, Kobe, 100 pp.

Poyarkov NA, Che J, Min M-S, Kuro-o M, Yan F, Li C, Iizuka K, Vieites DR (2012) Review of the systematics, morphology and distribution of Asian Clawed Salamanders, genus *Onychodactylus* (Amphibia, Caudata: Hynobiidae), with the description of four new species. Zootaxa 3465: 1–106.

Regier JC, Mitter C, Kristensen NP, Davis DR, van Nieukerken EJ, Rota J, Simonsen TJ, Mitter KT, Kawahara AY, Yen S-H, Cummings MP, Zwick A (2015) A molecular phylogeny for the oldest (nonditrysian) lineages of extant Lepidoptera, with implications for classification, comparative morphology and life-history evolution. Systematic Entomology 40: 671–704. https://doi.org/10.1111/syen.12129

Skalski AW (1995) Study on the Lepidoptera from fossil resins. Part XI. *Baltimartyria*, a new genus for *Micropteryx proavittella* Rebel, 1936, with redescription of this species (Lepidoptera, Zeugloptera, Micropterigidae). Amber and Fossils 1: 26–37.

Sota T, Ishikawa R, Ujiie M, Kusumoto F, Vogler AP (2001) Extensive trans-species mitochondrial polymorphisms in the carabid beetles *Carabus* subgenus *Ohomopterus* caused by repeated introgressive hybridization. Molecular ecology 10: 2833–2847. https://doi.org/10.1046/j.1365-294X.2001.t01-1-01404.x

Suzuki H (1962) The classification of Japanese climates. Geographical Review of Japan (Chirigaku Hyoron) 35: 205–211. https://doi.org/10.4157/grj.35.205

Suzuki H, Yasuda SP, Sakaizumi M, Wakana S, Motokawa M, Tsuchiya K (2004) Differential geographic patterns of mitochondrial DNA variation in two sympatric species of Japanese wood mice, *Apodemus speciosus* and *A. argenteus*. Genes & Genetic Systems 79: 165–176. https://doi.org/10.1266/ggs.79.165

van Eldijk TJB, Wappler T, Strother PK, van der Weijst CMH, Rajaei H, Visscher H, van de Schootbrugge B (2018) A Triassic-Jurassic window into the evolution of Lepidoptera. Science advances 4: e1701568. https://doi.org/10.1126/sciadv.1701568

van Nieukerken EJ, Kaila L, Kitching IJ, Kristensen NP, Lees DC, Minet J, Mitter C, Mutanen M, Regier JC, Simonsen TJ, Wahlberg N, Yen S-H, Zahiri R, Adamski D, Baixeras J, Bartsch D, Bengtsson BÅ, Brown JW, Bucheli SR, Davis DR, Prins JD, Prins WD, Epstein ME, Gentili-Poole P, Gielis C, Hättenschwiler P, Hausmann A, Holloway JD, Kallies A, Karsholt O, Kawahara AY, Koster S, Kozlov MV, Lafontaine JD, Lamas G, Landry J-F, Lee S, Nuss M, Park K-T, Penz C, Rota J, Schintlmeister A, Schmidt BC, Sohn J-C, Solis MA, Tarmann GM, Warren AD, Weller S, Yakovlev RV, Zolotuhin VV, Zwick A (2011) Order Lepidoptera Linnaeus, 1758. In: Zhang Z-Q (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness 3148: 212–221.

Whalley PES (1978) New taxa of fossil and recent Micropterigidae with a discussion of their evolution and a comment on the evolution of Lepidoptera (Insecta). Annals of the Transvaal Museum 31(8): 71–86.

Yamamoto S, Morita K, Kitano S, Watanabe K, Koizumi I, Maekawa K, Takamura K (2004) Phylogeography of white-spotted Charr (*Salvelinus leucomaenis*) inferred from mitochondrial DNA sequences. Zoological Science 21: 229–240. https://doi.org/10.2108/zsj.21.229

Yoshikawa N, Matsui M, Nishikawa K, Kim J-B, Kryukov A (2008) Phylogenetic relationships and biogeography of the Japanese clawed salamander, *Onychodactylus japonicus* (Amphibia: Caudata: Hynobiidae), and its congener inferred from the mitochondrial cytochrome b gene. Molecular Phylogenetics and Evolution 49: 249–259. https://doi.org/10.1016/j.ympev.2008.07.016

Zhang W, Shih C, Labandeira CC, Sohn J-C, Davis DR, Santiago-Blay JA, Flint O, Ren D (2013) New Fossil Lepidoptera (Insecta: Amphiesmenoptera) from the Middle Jurassic Jiulongshan Formation of Northeastern China. PLoS ONE 8: e79500. https://doi.org/10.1371/journal.pone.0079500.t003

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# Complementary anatomy of *Actinocyclus verrucosus* (Nudibranchia, Doridoidea, Actinocyclidae) from Indo-Pacific

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## Abstract

The last review of the genus *Actinocyclus* consider only two valid species for the genus: *Actinocyclus verrucosus* Ehrenberg, 1831 (type species of the genus) and *Actinocyclus papillatus* (Bergh, 1878), both with a geographical distribution in the Indo-Pacific. The anatomy of these species is still unknown, except for some scanty anatomical information. A detailed anatomical study of *Actinocyclus verrucosus* is performed, including inedited structures such as digestive system, odontophore muscles and circulatory system, beyond complementary information on the commonly studied structures, in order to clarify the taxonomy and distribution.

## Introduction

The family Actinocyclidae comprises two genera, *Actinocyclus* Ehrenberg, 1831 [type species: *Actinocyclus verrucosus* Ehrenberg, 1831, by subsequent designation of Gray (1847)], with two valid species: the type species and *A. papillatus* (Bergh, 1878) (Rosenberg 2010); and *Hallaxa* Eliot, 1909 with 15 valid species (Bouchet 2011).

The current systematics of this family is mainly based on Gosliner and Johnson (1994) who studied the phylogenetic relationship of the *Hallaxa*, and hypothesized *Actinocyclus* as its sister taxa, considered only these two genera as members of Actinocyclidae, and this family as sister taxon of Chromodorididae (Valdés 2002). The last review of *Actinocyclus* was based on some traditional characters, such as the external morphology, the reproductive system and radular data (Valdés 2002). As have been seen in recent papers (e.g., DaCosta et al. 2007, Simone 2011, Lima and Simone 2015), the scenario of the morphological characters is an effective tool to better understanding the relationship among species and has been useful to clarify and refine their taxonomy.

In this paper the morphological data of *Actinocyclus verrucosus* are described in more details, including previously unexplored structures, such as digestive tubes, odontophore muscles and circulatory system, and builds a conceptual scenario for comparative characters in future analysis.

## Material and methods

The studied material belongs to museum collections, consisting of specimens preserved in 70% ethanol; a complete information follows description. Dissections were performed under a stereomicroscope by standard techniques (Simone 2004, 2011). The initial steps of the anatomical investigation were done through a longitudinal cut on the integument covering the dorsal visceral mass. Digestive, circulatory, excretory, reproductive and central nervous systems were investigated in detail. The terminology used

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for odontophore muscles was based on Ponder et al. (2008), Simone (2011) and Lima and Simone (2015). Drawings were done with the aid of a camera lucida. Scanning electron microscopy (SEM) was used to examine details of the radula at the Laboratório de Microscopia Eletrônica of Museu de Zoologia da Universidade de São Paulo (MZSP).

The following abbreviations are used herein: **aa**: anterior aorta; **ab**: afferent branchial vein; **am**: ampulla; **an**: anus; **ap**: posterior aorta; **au**: auricle; **at**: aortic trunk; **bc**: bursa copulatrix; **bg**: blood gland; **bl**: branchial leaves; **bs**: buccal sphincter; **cb**: buccal commissure; **ce**: cerebral ganglia; **cl**: pleural commissure; **cp**: pedal commissure; **cu**: caecum; **dd**: duct of digestive gland; **dg**: digestive gland; **dt**: digitiform tentacle; **eb**: efferent branchial vein; **es**: oesophagus; **ey**: eye; **fg**: female gland; **ft**: foot; **gb**: buccal ganglia; **gc**: gill circle; **ge**: gonopore; **gg**: gastro-oesophageal ganglia; **gp**: pedal ganglia; **hg**: hermaphrodite gland; **il**: inner lip; **in**: intestine; **mo**: mouth; **ms**: medial sinus; **m2 – m10**: odontophore muscles; **mt**: oral tube muscle; **ne**: nephrostome; **oc**: odontophore cartilage; **ol**: outer lip; **ot**: oral tube; **ov**: oviduct; **pc**: pericardium; **pe**: penis; **pl**: pleural ganglia; **pr**: prostate; **ra**: radula; **ri**: rhinophore; **rg**: rhinophoral ganglia; **rm**: gill retractor muscle; **rn**: rinophoral nerve; **rp**: reproductive system; **rs**: radular sac; **rv**: renal vesicle; **sg**: salivar gland; **sm**: subradular membrane; **sn**: nervous system; **sr**: seminal receptacle; **st**: stomach; **sy**: statocysts; **tu**: dorsal tubercles; **ud**: uterine duct; **va**: vagina; **vd**: vas deferent; **ve**: ventricle.

**Institutional abbreviation**

| | |
|---|---|
| AUS | Australian Museum Research Institute, Sydney |
| CASIZ | Invertebrate Zoology and Geology of the California Academy of Science, San Francisco |
| MHUB | Museum für Naturkunde der Humboldt-Universität zu Berlim |
| ZMUC | Zoologisk Museum, Kobenhavns Universitet, Copenhagen |
| MNHN | Muséum National d'Histoire Naturalle, Paris |

# Results

### Family Actinocyclidae O'Donoghue, 1929

### Genus *Actinocyclus* Ehrenberg, 1831

**Type species.** *Actinocyclus verrucosus* Ehrenberg, 1831, by subsequent designation of J. E. Gray (1847)

### *Actinocyclus verrucosus* Ehrenberg, 1831

Figs 1–31

*Actinocyclus verrucosus* Ehrenberg, 1831: 28.
*Sphaerodoris punctata* Bergh, 1877: 66 (*nomen nudum*); Bergh 1878: 587, pl. 65, figs 1–5.
*Sphaerodoris laevis* Bergh, 1890: 925, pl. 88, figs 3–12; Odhner 1919: 40.
*Sphaerodoris japonica* Eliot, 1913: 23.
*Aldisa nhatrangensis* Risbec, 1956: 14, pl. 20, fig. 109, pl. 22.

**Type material.** See Valdés (2002).

**Type locality.** Massawa, Eritrea.

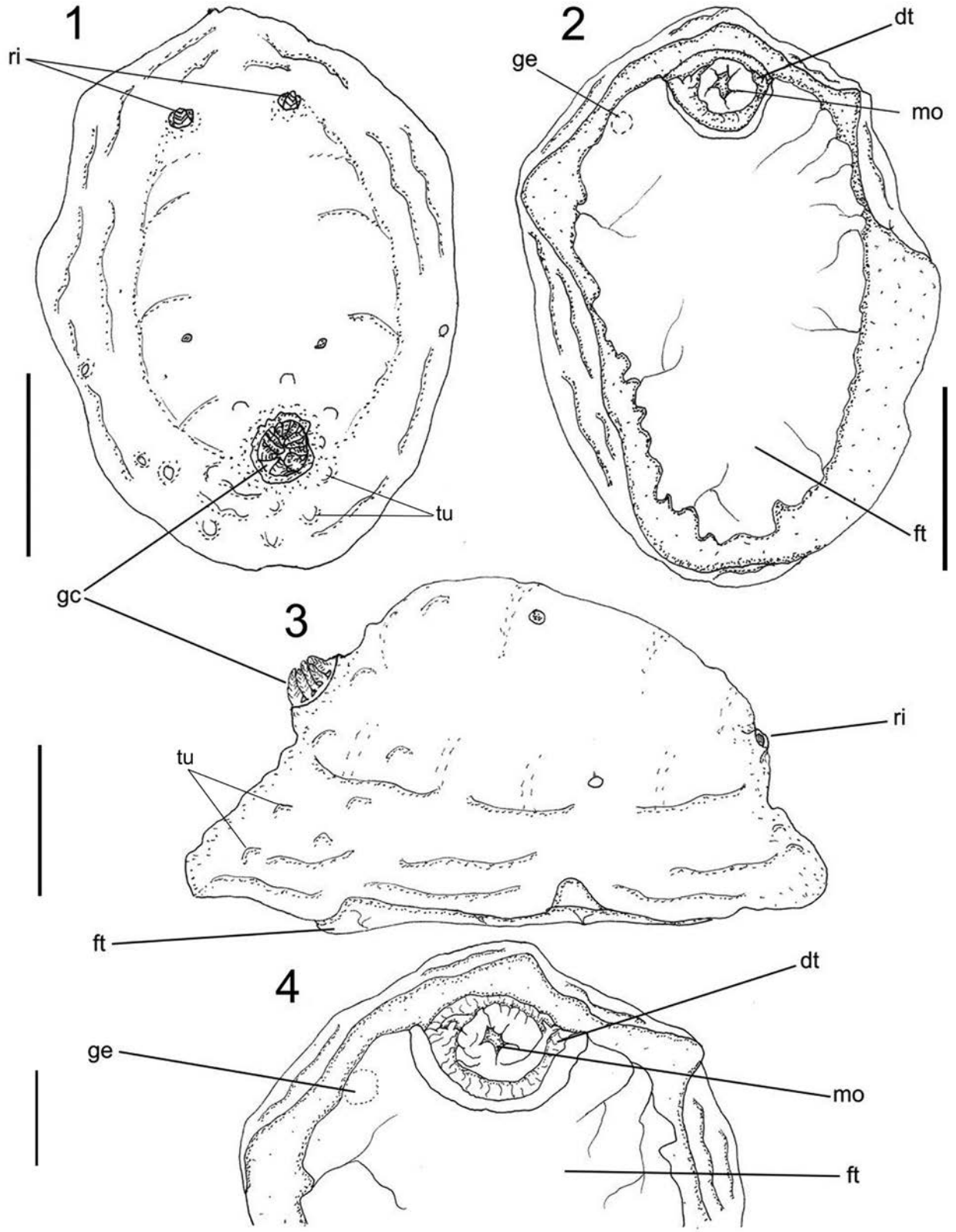
**Material examined.** AUSTRALIA, Coral Sea, North East Herald Cay, AUS 333868.001, 2 specimens (M. Preker, coll., 24/vi/1997, beach rock, SE side of cay, LT at dusk). VANUATU, Espiritu Santo Island, Palikulo Peninsula (15°28.90'S 167°15.50'E (DDM)), CAS 179791, 1 specimen (M. Pola-Perez, Y. Camacho-Garcia et al. Coll., 15/ix/2006, intertidal, soft bottom).

**Diagnosis.** Body of grayish color, with some black dots and some tubercles. Anterior border of foot concave and not bilabiate nor longitudinally notched. Presence of m7a odontophore muscles and pleural commissure.

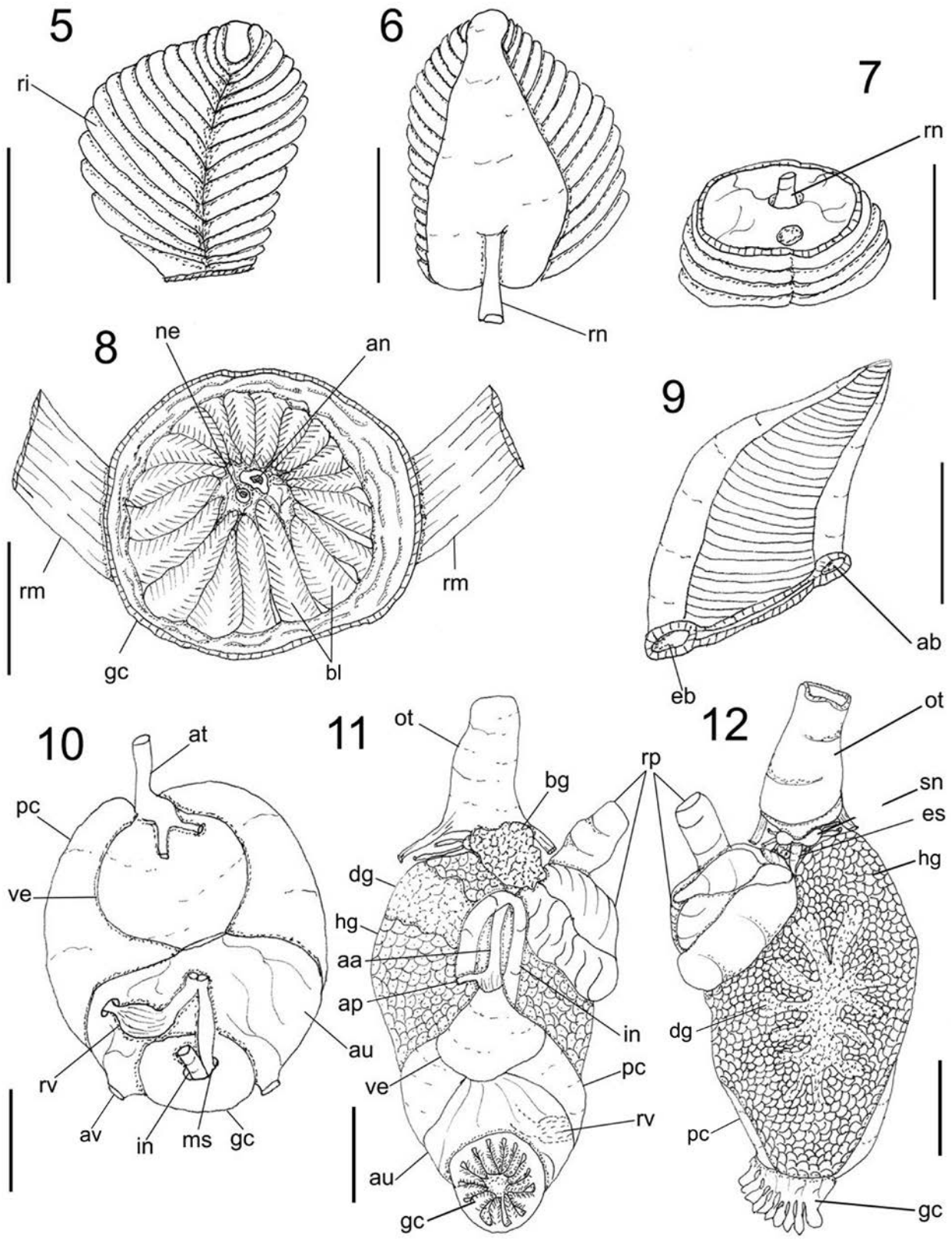
**Redescription.** *External morphology* (Figs 1–9): Size, ~12mm length, ~10mm width. Color grayish with some black dots in preserved specimen. Body rounded, oval, almost as long as wide; elevated dorsum with some several simple dorsal tubercles scattered irregularly (Figs 1–3). Mantle smooth without spicules. Rhinophores with ~17 transverse lamellae (preserved specimen with ~12mm-long CASIZ 179791) (Figs 5–7); rhinophoral sheaths smooth. Gill circle composed of 16–19 unipinnate branchial leaves surrounding anus (preserved specimen with ~12mm-long) (Figs 8–9); branchial sheaths smooth. Mouth opening in anterior ventral region, between anterior region of notum and foot (Fig. 2). Digitiform tentacles very small, one on each side of mouth (Fig. 4). Anterior border of foot concave, not bilabiate, nor longitudinally notched (Fig. 4). Foot not exceeding notum in a preserved specimen (Fig. 2).

*Haemocoel organs* (Figs 11–12): pericardium and posterior half of visceral mass occupying ~40 % of haemocoel volume. Buccal mass located anteriorly, occupying ~20 % of haemocoel volume. Nervous system dorsal to buccal mass, covered by blood gland, occupying ~10 % of haemocoel volume. Genital system on right side; occupying ~20 % of haemocoel volume. Stomach internal to digestive gland, intestine with small curve at anterior portion, both occupying ~10 % of haemocoel volume.

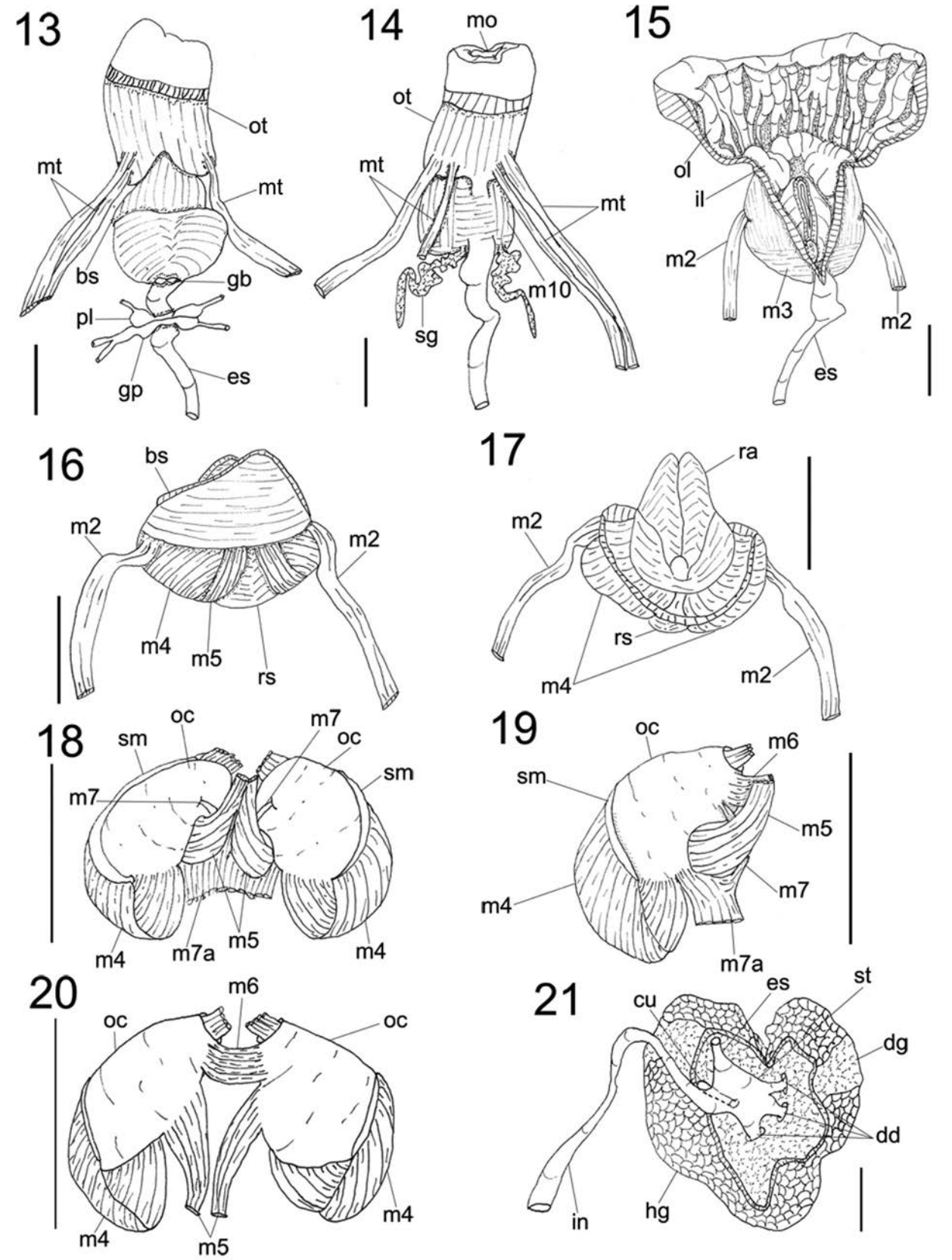
*Circulatory and excretory systems* (Figs 8–11): pericardial cavity dorsal and posterior to digestive gland, anterior to gill circle. Afferent and efferent veins located at edge of each branchial leaves (Fig. 9). Gill retractor muscle divided in two fibers originating from base of gill circle, running lateral to haemocoel longitudinally up to half of foot level, inserting into dorsal surface of foot (Fig. 8). Auricle funnel-like (wider anteriorly) with thin walls (Fig. 10). Ventricle slightly taller than wide, with thick muscular walls (Fig. 10). Aortic trunk anterior to pericardium, connected to anterior ventricular region (Figs 10–11); posterior artery branched into anterior artery irrigating reproductive system, buccal mass, odontophore and nervous system inserting on blood gland; anterior artery irrigating stomach, caecum and digestive gland



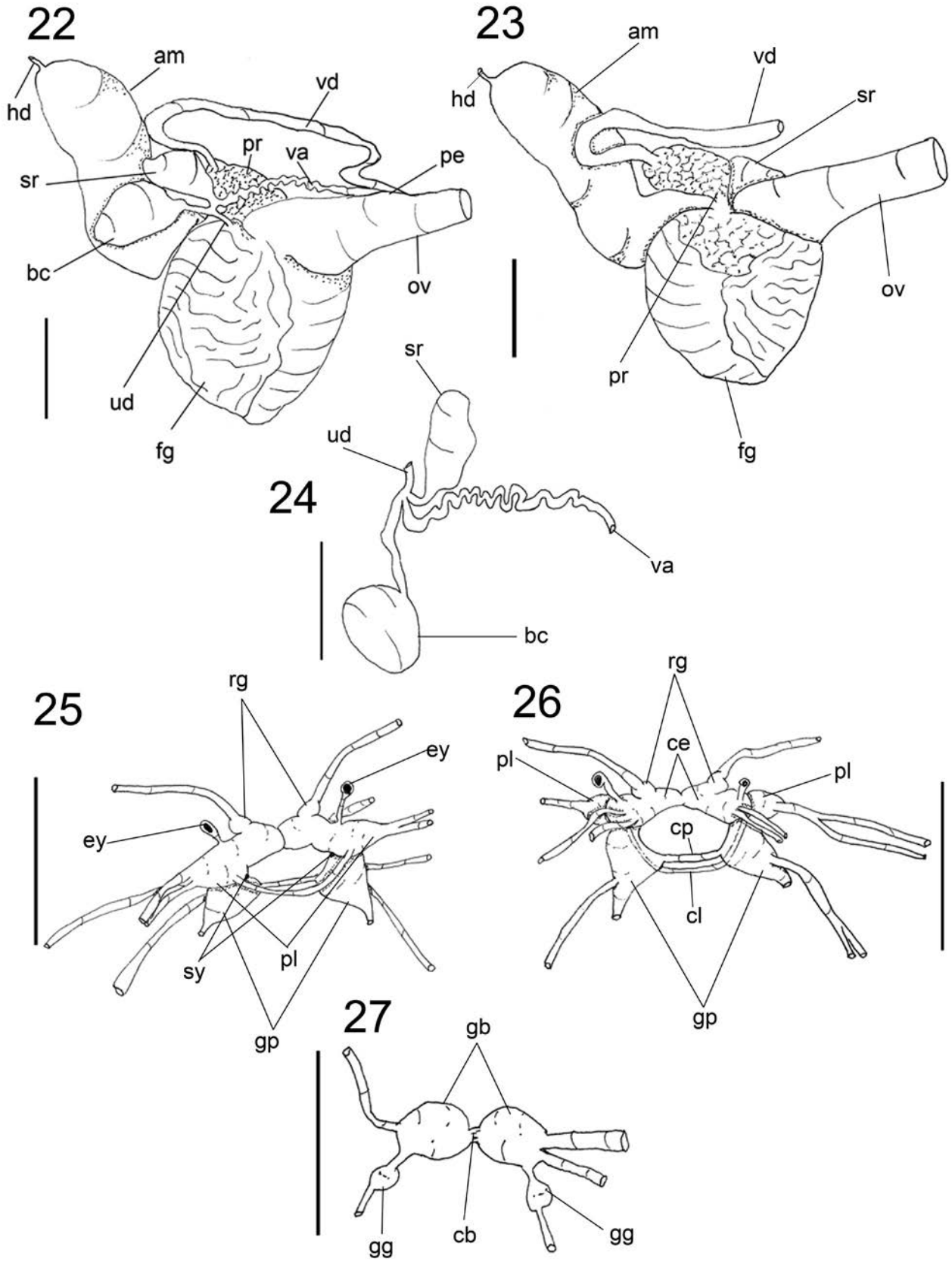

**Figures 1–4.** External morphology of *Actinocyclus verrucosus*. **1.** Whole dorsal view, scale: 5 mm. **2.** Whole ventral view, scale: 5 mm. **3.** Whole right lateral view, scale: 5 mm. **4.** Detail of anterior border of foot, ventral view, scale: 2 mm.




**Figures 5–12.** Anatomical details of *Actinocyclus verrucosus*. **5.** Rhinophore extracted, ventral view, scale: 0.5 mm. **6.** Same dorsal view, scale: 0.5 mm. **7.** Same, transverse section, scale: 0.5 mm. **8.** Gill circle with retractor muscle, dorsal view, scale: 1 mm. **9.** Detail of gill filament, scale: 1 mm. **10.** Detail of circulatory system (structures of pericardium), ventral view, scale: 2 mm. **11.** Extracted visceral mass, dorsal view, scale: 2 mm. **12.** Same, ventral view, scale: 2 mm.




**Figures 13–21.** *Actinocyclus verrucosus* details of digestive system. **13.** Foregut and nerve ring, ventral view, some adjacent structures also shown as in situ, scale: 1 mm. **14.** Same dorsal view, scale: 1 mm. **15.** Same, longitudinally sectioned, showing internal oral tube, dorsal view, scale: 1 mm. **16–20.** Odontophore anatomy, scales: 1 mm. **16.** Whole ventral view, sphincter removed. **17.** Whole dorsal view, esophagus removed. **18.** Dorsal view, radula removed, each cartilage slightly deflected. **19.** Whole right view. **20**. Ventral view, m4 and m5 folded down to expose odontophore cartilage; m7 and m7a removed. **21.** Midgut as in situ, dorsal view, scale: 2 mm.




**Figures 22–27.** *Actinocyclus verrucosus* reproductive system. **22.** Dorsal whole view, most structures uncoiled, scale: 1 mm. **23**. Same, ventral view, scale: 1 mm. **24**. Detail of uncoiled female structures, dorsal view, scale: 1 mm. **25–27.** Central nervous system. **25.** Dorsal view, scale: 1 mm. **26.** Ventral view, scale: 1 mm. **27.** Detail of buccal and gastroesophageal ganglia, ventral view, scale: 0.5 mm.

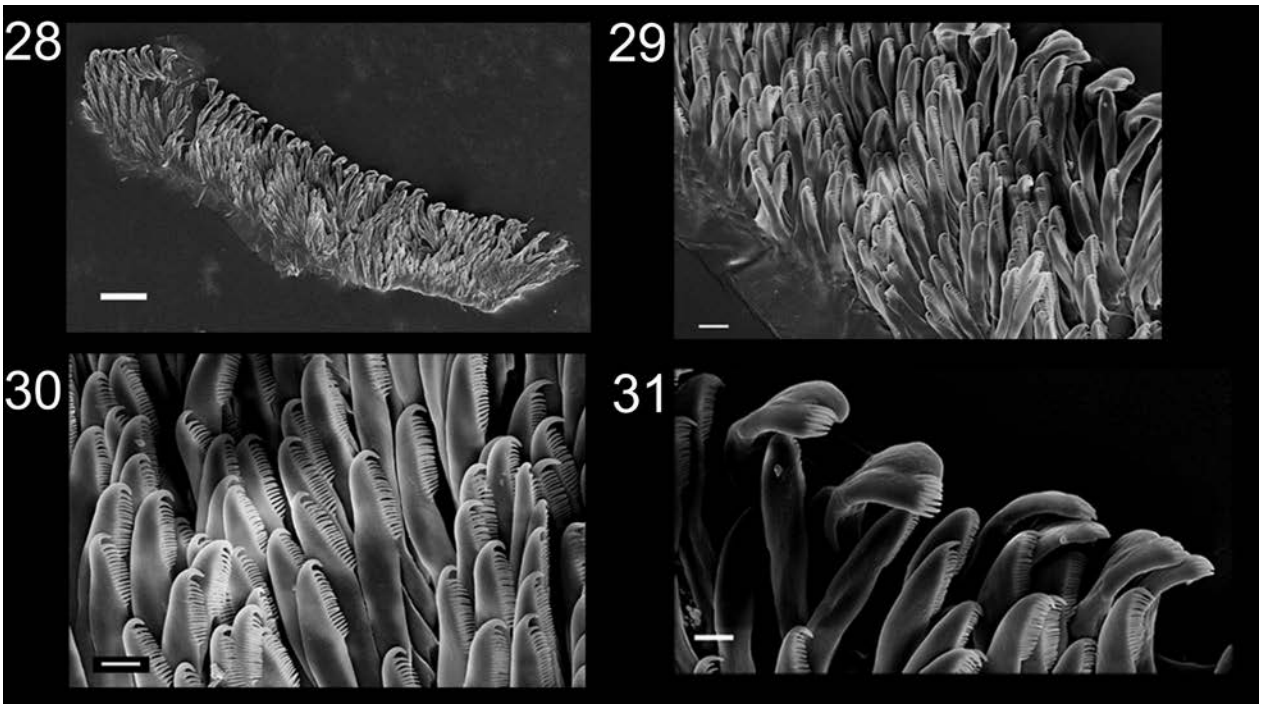
**Table 1.** Comparative table of some features between *Actinocyclus verrucosus*, *Hallaxa apefae* and *Chromodoris magnifica* (all these features of the three species was analyzed in Lima 2016).

| | Anterior border of foot | Foot | Nephrostome | Jaws | M7a | Ducts of digestive gland | Cecum | Pedal commissure |
|---|---|---|---|---|---|---|---|---|
| *Actinocyclus verrucosus* Ehrenberg, 1831 | Concave, not bilabiate nor notched | Not projected beyond the notum | Readily visible | Present | Present | 3 | Present | Present |
| *Hallaxa apefae* Er. Marcus, 1957 | Convex, bilabiated not notched | Pointed, poserior projected beyond the notum | Not readily apparent | Absent | Absent | 1 | Absent | Absent |
| *Chromodoris magnifica* (Quoy & Gaimard, 1832) | Convex, bilabiated not notched | Pointed, poserior projected beyond the notum | Readily visible | Absent | Absent | 2 | Absent | Absent |

(Fig. 11). Auricular vessels connecting lateral cavities of integument to auricle (Fig. 10). Medial sinus connected to afferent branchial ring, irrigating entire digestive gland. Renal vesicle located on right dorsal side of pericardium, near base of auricle, connected to inner surface of pericardium; renal chamber elliptical, with longitudinal folds, ~1/4 of size of ventricle (Fig. 10). Renal chamber extending from dorsal to medial sinus, previously connected to renal vesicle, extending posteriorly to center of gill circle and opening in nephrostome, next to anus pore (Fig. 8). Blood gland undivided (Fig. 11).

*Digestive system* (Figs 8, 13–21, 28–31): Oral tube composed of outer lip, with pleats lengthwise; inner lip with transverse fold; **mt**, two pairs of retractor muscles of buccal mass, originating on oral tube, running dorsally and ventrally to oral tube, inserting on body side, about three times as wide and twice as long as **m10** (Fig. 15). Odontophore oval, connected to oral tube by several fine longitudinal dorsal and ventrolateral protractors muscles of buccal sphincter, originating in anterior region of odontophore, inserting in posterior region of integument, close to oral tube (**m10**) (Fig. 14); Buccal sphincter surrounding chitinous part of oral tube. Odontophore muscles: **m2**, pair of strong retractor muscles of buccal mass, six times longer than wide, originating on anterior dorsal odontophore, running laterally to m4 and inserting ventrally on dorsal portion of foot; **m3**, two times wider than long, transverse fibers between esophagus and odontophore (Figs 15–16); **m4**, pair of dorsal tensor muscles of radula, strong and broad, 1/2 winder than long, covering externally 2/3 of cartilage, inserting on ventral portion of subradular membrane; **m5**, pair of dorsal auxiliary tensor muscles of radula, twice as long as wide, originating on most posterior region of odontophore cartilages, covering ~1/3 of posterior cavity of odontophore, as long as, but with ~1/3 of m4 width, inserting on ventral side of subradular membrane, around radular sac; **m6**, unpaired horizontal muscle, with transverse fibers connecting anterior surfaces of left and right odontophore cartilages, as long as wide, about same length and half as wide as m4 (Fig. 20); **m7**, pair of thin muscles originating each into an odontophore cartilages and inserting on **m7a** passing ventrally by **m5**, and on radular sac (Fig. 19); **m7a**, originating on posterior region of odontophore cartilage and inserting on radular sac, m7' auxiliary (Fig. 19). Pair of odontophore cartilages slender, elliptical. Subradular membrane thin, strong, translucent (Fig. 18). Radular sac ~1/6 of odontophore (Fig. 16). Jaw elements not analyzed. Radular teeth (Figs 28–31): rachidian teeth absent; formula 50 x 17.0.17 (preserved specimen, ~15 mm-long, AUS C333868001). Innermost lateral teeth broad and thick, with large and rounded cusp and about six to eight cusps along inner edge (Fig. 31). Mid-lateral teeth narrow basally and elongated, with apical cusp larger than other, twenty-one lateral cusps (Fig. 30). Outermost teeth shorter than middles laterals, about sixteen to eighteen cusps (Fig. 29). Pair of salivary glands long, tubular, about same length as esophagus; duct inserting in anterior region of esophagus, extending posteriorly to anterior region of digestive gland (Fig. 14). Esophagus simple, originating dorsally to odontophore, inserting directly in anterior region of stomach, internal longitudinal folds with same diameter along entire length (Figs 14–15). Stomach internal to digestive gland, oval, close to anterior region of intestine, with distinct digestive ducts (Fig. 21). Intestine with longitudinal folds along its entire length, diameter same as esophagus diameter. Caecum as an elongated sac, located ventrally to stomach, opening on anterior portion of stomach (Fig. 21), close to esophageal insertion, ~½ length and ~1/2 of width of stomach. Digestive gland dark brown, internal to hermaphrodite gland, cone-shaped; inner face of gland sponge-like, bearing three ducts (Fig. 21). Anus opening into pore at center of gill circle (Fig. 8); anal papilla absent.

*Genital system* (Figs 2, 11–12, 22–24): located between buccal mass and digestive gland, mainly on right and dorsal sides. Hermaphrodite gland around digestive gland, dark beige in color (Figs 11–12). Hermaphrodite duct thin, long located posterior end of ampulla (Figs 22–23). Ampulla located on female gland, elongated and tubular, about same length as oviduct, inserting distally at junction of oviduct and prostate (Fig. 23). Prostate glandular, connected to female gland, ~1/2 of ampulla's length. Vas deferens and penis muscular, cylindrical, elongated, ~1/2 of prostate's width (Fig. 22). Female gland well-developed, rounded, occupying ~40% of reproductive system volume, about same length and twice width as oviduct (Figs 22–23). Oviduct occupying ~1/3 of female gland volume (Figs 22–23). Uterine duct located at base of bursa cop-




**Figures 28–31.** *Actinocyclus verrucosus,* radula in SEM. **28.** Panoramic view, scale: 100 µm. **29.** higher magnification in central region, scale: 40 µm. **30.** Outer lateral teeth, scale: 10 µm. **31.** Detail of marginal teeth tip, scale: 10 µm.

ulatrix and seminal receptacle, inserted in female gland near oviduct, relatively short, ~1/10 of vagina's length and same diameter as vagina (Figs 22, 24). Seminal receptacle pyriform, as large as bursa copulatrix, connected to vagina near uterine duct through short stalk (Figs 22, 24). Bursa copulatrix rounded, connected to vagina after seminal receptacle, length ~1/6 of vagina's length, also through stalk three times longer than uterine duct (Figs 22, 24). Vagina cylindrical, elongated, same width and four times longer than penis, followed ventrally by prostate and located parallel to penis on gonopore (Fig. 24). Gonopore on right side, located in anterior fifth of length of animal from head, between foot and notum (Fig. 2).

*Central nervous system* (Figs 25–27): located dorsally to odontophore, mostly covered by blood gland. Pair of cerebral and pleural ganglia fused with each other dorsally and ventrally. Pedal ganglia fused with cerebral and pleural ganglia ventrally, not fused among themselves, connected by long and thin pleural commissure. Pedal commissure simple, ½ of length of pleural commissure, both surrounding esophagus and salivary glands (Fig. 26). Buccal ganglia short, located ventrally to odontophore, between radular sac and anterior portion of esophagus, connected to cerebral ganglia through long and slender connective tissue, united to gastro-esophageal ganglia by short connective tissue. Gastro-esophageal ganglia with length ~1/4 of buccal ganglia length, spherical (Fig. 27). Rhinophoral ganglia bulb-shaped, connected to anterior portion of cerebral ganglia (Fig. 25). Dorsal eyes located on cerebral ganglia with short stalk, pedunculated. Statocysts small and iridescent, located ventrally between pedal and pleural ganglia (Fig. 25).

**Distribution.** West and Central Indo Pacific [Red Sea (Ehrenberg 1831), Philippines, and Indonesia (Bergh 1878, 1890), East Africa, Malaysia and Japan (Eliot 1904, 1913), Vietnam (Ribesc 1956), Hawaii (Kay and Young 1969), Queensland (Willan and Coleman 1984), Western Australia (Wells and Bryce 1993), Madagascar and Marshall Island (Valdés 2002)].

## Discussion

The presence of a short pair of digitiform tentacles around the mouth (Fig. 4) is noteworthy, they were also reported by Gosliner and Johnson (1994). However, these digitiform tentacles have been reported as absent by Kay and Young (1969) and Valdés (2002). The most external differences of *A. verrucosus*, when compared to other Doridoidea, for example *Hallaxa apefae*, *Chromodoris magnifica* and *Doris verrucosa* (Tab. 1), is the presence of an anterior border of foot concave, not convex and not grooved, nor notched (Figs 2, 4).

The rhinophores have 17 lamellae instead of 20 described by Valdés (2002), and the number of branchial leaves ranges from 16 to 19, instead of only 16. Regarding the color of the body, no alive specimens have been analyzed.

In the circulatory system, interesting features were found in the position in relation to gill circle, the afferent and efferent vessels, the gill retractor muscle, medial sinus, renal chamber and nephrostome. Despite some of these features have already used in phylogenetics analyzes (Lima 2016), because of lack of further information, a deeper analysis is still difficult.

The oral tube is composed of a pair of retractor muscles, which attaches to the body wall (mt), in *A. verrucosus* there are two pairs of mt, while in *Hallaxa apefae*, and the most species of Doridoidea, present three pairs (Lima 2016). A buccal sphincter and the m3 (transverse muscle) involve the odontophore (Figs 15–16) that have a pair of long retractor muscles (m2) (Figs 15–17). A group of muscles are described for the first time (m4, m5, m6, m7) (Figs 16–20), with similar functions of their counterparts in other heterobranchs (Simone 2011). The odontophore cartilage is well-developed (Fig. 18) like in other nudibranchs as, e.g., *Doris verrucosa* Linnaeus, 1758 (Lima and Simone 2015). However, some differences are visible between *A. verrucosus* and *D. verrucosa* as following: m5 pair originates on the middle region of the odontophore cartilages in *A. verrucosus* (Figs 19–20) instead on the posterior region in *D. verrucosa* (Lima and Simone 2015, fig. 8B); m6 located more anteriorly in *A. verrucosus* (Fig. 20), whereas in *D. verrucosa* the m6 connects the both odontophore cartilages anteriorly and posteriorly (Lima and Simone 2015, Figs 8A-B). However, the most significant difference of odontophore muscles of *A. verrucosus* and others Doridoidea species appears to be the presence of the pair m7a.

The reproductive system seems to be similar to those described by Valdés (2002), but it has some different features from the interpretation by Kay and Young (1969) that described the prostate without the glandular portion, which was not observed in the present studied samples (Figs 23–24).

In the central nervous system, the abdominal ganglion described by Valdés (2002) was not observed, but a pleural commissure (Figs 25–26), that is not mentioned by him, was found. This last feature was uncovered as autapomorphy in a recent phylogenetic study (Lima 2016) as well as the presence of m7a – originating on posterior region of odontophore cartilage and inserting on radular sac, probably m7's auxiliary.

In the same recent phylogenetic study (Lima 2016) *Hallaxa apefae* appears more related to Chromodorididae (Tab. 1) clade and could be considered as sister group based on the posterior projection of the foot beyond the notum and the absence of integumentary spicules. In the same analysis, *A. verrucosus* resulted as sister group of a clade that united Dorididae and Discodorididae with two characters: radula with many lateral teeth and buccal commissure readily visible.

The present complementary anatomical investigation improved the species delimitation of *A. verrucosus*. In addition, allowed to evaluate the characters usually used in taxonomy and phylogenetic studies, as well as the discovery of new characters with phylogenetic signal and provided more bases for the synonymies. The evaluation of new morphological characters will improve the knowledge of the *Actinocyclus* evolutionary history, or even Doridoidea. This paper also shows the importance in investigating systems and organs beyond the traditional external features, radula and genital structures, which sometimes bear clearer data for comparative analysis as, e.g., the odontophore muscles.

## Acknowledgements

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## References

Bergh R (1877) Kritische Untersuchung der Ehrenberg'schen Doriden. Jahrbuch der Deutschen Malakozoologischen Gesellschaft 4: 45–76.

Bergh R (1878) Malacologische Untersuchungen, Theil 2, Heft 13. In: Semper C (Ed.) Reisen im Archipel der Philippinen. Kreidel, Wiesbaden, 547–602.

Bergh R (1890) Malacologische Untersuchungen, Theil 3, Heft 17. In: Semper C (Ed.) Reisen im Archipel der Philippinen. Kreidel, Wiesbaden, 873–991.

Bouchet P (2011) *Hallaxa* Eliot, 1909. MolluscaBase (2017) World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=531808 [on 2017-12-22]

DaCosta S, Cunha CM, Simone LRL, Schrödl M (2007) Computer-based 3-dimensional reconstruction of major organ systems of a new aeolid nudibranch subspecies, *Flabelinna engeli luciane*, from Brazil (Gastropoda: Opisthobranchia). Journal of Molluscan Studies 73: 339–535. https://doi.org/10.1093/mollus/eym035

Ehrenberg CG (1828–31) Symbolae physicae animalia evertebrata exclusis insectis. Series prima cum tabularum decade prima continente animalia Africana et Asiatica, Decas Prima. In: Hemprich FG, Ehrenberg CG (Eds) Symbolae physicae, seu Icones adhue ineditae corporum naturalium novorum aut minus cognitorum, quae ex itineribus per Lybiam, Aegyptum, Nubiam, Dengalam, Syriam, Arabiam et Habessiniam. Pars Zoologica, 4. Officina Academica, Berlin. [Pages unnumbered; Dates of publication: pls. 1-2 [1828], text [1831]]

Eliot CN (1904) On some nudibranchs from East Africa and Zanzibar. Part IV. Proceedings of the Zoological Society of London 1: 380–406, pls. 23–24.

Eliot CN (1913) Japanese nudibranchs. Journal of the College of Science, Imperial University of Tokyo 35: 1–47, pls. 1–2.

Gosliner TM, Johnson S (1994) Review of the genus *Hallaxa* (Nudibranchia: Actinocyclidae) with descriptions of nine new species. The Veliger 37(2): 155–191.

Kay EA, Young DK (1969) The Doridacea (Opisthobranchia: Mollusca) of the Hawaiian Islands. Pacific Science 23: 172–231.

Lima POV (2016) Análise filogenética de Cryptobranchia (Gastropoda: Nudibranchia) com base em anatomia comparada. Tese (Doutorado em Zoologia), Instituto de Biociências da Universidade de São Paulo. São Paulo-SP.

Lima POV, Simone LRL (2015) Anatomical review of *Doris verrucosa* and redescription of *Doris januarii* (Gastropoda: Nudibranchia) based on comparative morphology. Journal of Marine Biological Association of the United Kingdom, 1–18. https://doi.org/10.1017/S0025315415000296

Ponder WF, Colgan DJ, Healy JM, Nützel A, Simone LRL, Strong EE (2008) Caenogastropoda. In: Ponder WF, Lindberg DL (Eds) Molluscan phylogeny. Los Angeles, CA: University of California Press, 331–383. https://doi.org/10.1525/california/9780520250925.003.0013

Risbec J (1956) Nudibranches du Viet-nam. Archives du Musèum National d'Histoire Naturalle (7)4: 5–34, pls. 1–22.

Rosenberg G (2010) *Actinocyclus* Ehrenberg, 1831. In: MolluscaBase (2017) World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=196347 [on 2017-12-22]

Simone LRL (2004) Comparative morphology and phylogeny of representatives of the superfamilies of Architaenoglossans and Annulariidae (Mollusca, Caenogastropoda). Arquivos do Museu Nacional 62: 387–504.

Simone LRL (2011) Phylogeny of Caenogastropoda (Mollusca), based on comparative morphology. Arquivos de Zoologia 42: 161–323. https://doi.org/10.11606/issn.2176-7793.v42i4p161-323

Valdés A (2002) Review of the genus *Actinocyclus* Ehrenberg, 1831 (Opisthobranchia: Doridoidea). The Veliger 45(3): 193–202.

Wells FE, Bryce CW (1993) Sea Slugs and their relatives of Western Australia. Western Australia Museum, Perth, 184 pp.

Willan RC, Coleman N (1984) Nudibranchs of Australasia. Australian Marine Photographic Index, Sydney, 56 pp.

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# A distinctive new frog species (Anura, Mantellidae) supports the biogeographic linkage of two montane rainforest massifs in northern Madagascar

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## Abstract

We describe a new species of the genus *Gephyromantis*, subgenus *Vatomantis* (Mantellidae, Mantellinae), from moderately high elevation (1164–1394 m a.s.l.) on the Marojejy, Sorata, and Andravory Massifs in northern Madagascar. The new species, *Gephyromantis* (*Vatomantis*) *lomorina* **sp. n.** is highly distinct from all other species, and was immediately recognisable as an undescribed taxon upon its discovery. It is characterised by a granular, mottled black and green skin, reddish eyes, paired subgular vocal sacs of partly white colour, bulbous femoral glands present only in males and consisting of three large granules, white ventral spotting, and a unique, amplitude-modulated advertisement call consisting of a series of 24–29 rapid, quiet notes at a dominant frequency of 5124–5512 Hz. Genetically the species is also strongly distinct from its congeners, with uncorrected pairwise distances ≥10 % in a fragment of the mitochondrial 16S rRNA gene to all other nominal *Gephyromantis* species. A molecular phylogeny based on 16S sequences places it in a clade with species of the subgenera *Laurentomantis* and *Vatomantis*, and we assign it to the latter subgenus based on its morphological resemblance to members of *Vatomantis*. We discuss the biogeography of reptiles and amphibians across the massifs of northern Madagascar, the evidence for a strong link between Marojejy and Sorata, and the role of elevation in determining community sharing across this landscape.

## Introduction

In recent decades, the number of frog species that have been discovered in Madagascar, while steadily increasing (Köhler et al. 2005), often included species that were not immediately recognizable as new to science, though with occasional exceptions, e.g. *Boophis lichenoides* (Vallan et al. 1998), *Scaphiophryne boribory* (Vences et al. 2003), and *Tsingymantis antitra* (Glaw et al. 2006). The majority of newly discovered taxa are assignable to existing complexes and must be investigated closely before it can be confirmed whether or not they constitute new species (e.g. Vieites et al. 2012). Differing from this general pattern, on a 2012 expedition to the Sorata massif in northern Madagascar, we discovered a small green frog of the genus *Gephyromantis* that was immediately recognisable as a new species. It was not given a candidate species number at the time, and no sequences of this species were included in the barcoding assessment of Perl et al. (2014). In a 2016 survey in Andravory, near Sorata, and a 2016 survey of Marojejy National Park in northeastern Madagascar, we encountered the same species.

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At present, 44 species of *Gephyromantis* are recognized and assigned to six subgenera (*Asperomantis*, *Duboimantis*, *Gephyromantis*, *Laurentomantis*, *Phylacomantis*, and *Vatomantis*) based on molecular and morphological criteria (Glaw and Vences 2006, Vences et al. 2017). This classification is largely in agreement with the molecular multi-gene phylogeny of Kaffenberger et al. (2012). However, this phylogenetic study revealed that the subgenera *Laurentomantis* and *Vatomantis* are closely related, and that *Gephyromantis klemmeri* Guibé, 1974, morphologically similar to other species of the subgenus *Gephyromantis*, is sister to the *Laurentomantis* clade, suggesting the need for an improved classification. We here provide a description of the new species, which has potential implications for the supraspecific taxonomy of *Gephyromantis*, and the biogeographical linkage of the rainforest massifs of northern Madagascar.

## Materials and methods

### Specimen collection and morphological measurement

Specimens were collected at night using head torches along montane streams, euthanized using MS222 anaesthesia and subsequent overdose, fixed in 96 % ethanol, and deposited in 75 % ethanol for long-term storage. Tissue samples were stored in 96 % ethanol. Field numbers refer to the zoological collections of Miguel Vences (ZCMV), Frank Glaw (FGZC), and Steven Megson (SM). Specimens were deposited in the amphibian collections of the Mention Zoologie et Biodiversité Animale, Université d'Antananarivo (UADBA-A) and the Zoologische Staatssammlung München (ZSM).

Morphological measurements were taken to the nearest 0.1 mm using a digital calliper. Measurement schemes followed generally previous work on the genus (e.g. Vences et al. 2017) with modifications to decrease the risk of damaging the fragile limbs of the specimens when ascertaining limb lengths: snout–vent length (SVL), maximum head width (HW), head length from posterior edge of tympanum to snout tip (HL), horizontal eye diameter (ED), horizontal tympanum diameter (TD), distance from eye to nostril (END), distance from nostril to snout tip (NSD), distance between nostrils (NND), upper arm length from the articulation of the arm with the trunk to the elbow (UAL), lower arm length from the elbow to the base of the hand (LAL), hand length from the base of the hand to the tip of the longest finger (HAL), forelimb length (FORL*, given by the sum of UAL, LAL, and HAL), forearm length (FARL, given by the sum of LAL and HAL), thigh length from cloaca to knee (THIL), tibia length from knee to heel (TIBL), tarsus length from heel to base of foot (TARL), foot length from base of foot to tip of longest toe (FOL), hindlimb length (HIL*, given by the sum of THIL, TIBL, TARL and FOL), and length and width of femoral gland (FGL, FGW). Asterisks in this list indicate measurements that have the same abbreviation as the analogous single-measurement of previous studies (e.g. Vences et al. 2017) but are cumulative here and therefore not necessarily equivalent; comparison of such values must be done cautiously.

### Sequencing and analysis of DNA sequences

DNA was extracted from tissue samples using a Qiagen DNeasy blood & tissue kit (Qiagen, Hilden, Germany), or standard salt extraction protocols. For two samples from Sorata and one sample from Marojejy (ZCMV 15269), we amplified a fragment of the mitochondrial 16S rRNA gene (hereafter 16S) in 25 µl polymerase chain reactions with the primers 16Sra-L and 16Sb-H (Palumbi et al. 1991), 1 µl of template DNA, and the following steps: initial denaturation for 3 min at 94 °C, followed by denaturation with 35 cycles of 30 sec each at 94 °C, 30 sec of annealing at 55°C and 60 sec of elongation at 72 °C, and a final elongation step of 10 min at 72 °C. Sequencing was conducted using the BigDye Terminator v1.1 Cycle Sequencing Kit on ABI 3730 and ABI 3130xl capillary sequencers. Newly determined sequences were deposited in GenBank (accession numbers MG926811–MG926823). For an additional nine specimens from Marojejy, we sequenced a shorter, highly variable stretch of 250 bp of the same 16S region by an Illumina amplicon approach (Vences et al. 2016) to confirm their identification (data not shown).

For an exploratory analysis, we aligned the new sequences with 16S sequences used by Kaffenberger et al. (2012) for all nominal species of *Gephyromantis*. Because the obtained tree (not shown) confirmed the new species to be related to the *Laurentomantis/Vatomantis* clade as also strongly suggested by morphology, we focused our analysis on this subgroup, i.e., all nominal species of the subgenera *Laurentomantis* and *Vatomantis*, *a*nd *G. klemmeri* which is known to be related to these subgenera (Kaffenberger et al. 2012), as well as *G. granulatus* (subgenus *Duboimantis*) as outgroup.

We aligned sequences in MEGA 7 (Kumar et al. 2016), yielding an alignment of 532 positions of the sequenced stretch of the 16S rRNA gene. As only a few indels were found in this alignment, we did not exclude any positions for further analysis. We used the Bayesian Information Criterion in jModelTest 2.1.4 (Darriba et al. 2012) to determine a SYM+G substitution model as best-fitting our data. We implemented this model in MrBayes 3.2 (Ronquist et al. 2012) and computed a Bayesian inference phylogenetic analysis, with two independent runs of 20 million generations, each comprising four Markov Chains (three heated and one cold), sampling every 1000 generations. Chain mixing and stationarity were assessed by examining the standard deviation of split frequencies and by plotting the -lnL per generation using Tracer 1.5 software (Rambaut and Drummond 2007). Results were combined to obtain a 50 %-majority rule consensus tree and the respective posterior probabilities of nodes, after discarding 25 % of the generations as burn-in (all compatible nodes with probabilities <0.5 kept). In addition, we computed a Maximum Likelihood (ML) tree in MEGA 7, with a GTR+G model (as the SYM model is not available

in this program), SPR level 5 branch swapping, and 500 nonparametric bootstrap replicates. Genetic distances (uncorrected pairwise p-distances) were also calculated in MEGA 7.

### Bioacoustic analyses

Recordings from Marojejy were made on a Marantz PMD661 MKII with a Sennheiser ME66/K6 supercardioid microphone, at a bandwidth of 44.1 kHz. Recordings from Sorata were made on an Edirol R-09 with its internal microphone. Call analysis was conducted in Cooledit 2.0 (Syntrillium Corp.). To obtain frequency information, the recording was transformed with Fast Fourier Transformation (FFT; width 1024 points). Spectrograms were created with a Hanning window of 512 or 256 bands. Measurements are given as mean ± one standard deviation, with range in parentheses. Terminology follows the recently-published recommendations of Köhler et al. (2017) with a note-centred approach. This definition is different from that of Vences et al. (2002) for *Laurentomantis* and Sabino-Pinto et al. (2014) for *Vatomantis*; the 'pulses' of those studies are here treated as notes, because each of these units in the new species described herein are distinctly pulsed, and therefore are treated as individual notes following Köhler et al. (2017). Recordings are deposited in the Animal Sound Archive of the Museum für Naturkunde, Berlin (DOI: 10.7479/nmx8-aq7v), and are available as Suppl. materials 1–2.

### Taxonomic work

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural act it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is urn:lsid:zoobank.org:pub:8A83DE58-A2EE-494F-A03C-820DC836CDDF. The online version of this work will be archived and made available from the following digital repositories: CLOCKSS and Zenodo.

## Results

Based on 16S sequences, the newly collected specimens represent an undescribed and hitherto unknown species of *Gephyromantis* that is highly distinct from all others (≥10 % p-distance). Exploratory phylogenetic analyses including all species of *Gephyromantis* clearly suggested their relationships with the subgenera *Laurentomantis* and *Vatomantis*, which also is strongly supported by morphological affinities, in particular by the greenish dorsal colour, granular skin, riparian habits, and paired subgular vocal sacs of partly white colour in males (see Diagnosis below for more details). A phylogenetic analysis of 16S sequences (total alignment length 532 bp) for all described species of *Laurentomantis* and *Vatomantis* as well as *G. klemmeri*, which was related to these subgenera in the multi-gene analysis of Kaffenberger et al. (2012), places the newly collected specimens sister to a clade with all described species of *Vatomantis*. *Gephyromantis klemmeri* is placed sister to *Laurentomantis*, although these basal nodes did not receive relevant support from ML bootstrap values or Bayesian posterior probabilities (Fig. 1). Genetic distances of the new specimens to all other species were high: 10.9–15.4 % to the three described species of *Vatomantis*, 10.0–13.2 % to species of *Laurentomantis*, and 12.2–12.5 % to *G. klemmeri*. The newly collected specimens from Sorata and Marojejy differed by 2.9 %, while no sequence differences were detected within each of these two localities, except for two mutations observed in one Marojejy specimen (ZCMV 15219).

Phenotypically the new specimens bear resemblance to both *Laurentomantis* and *Vatomantis*. Their advertisement call is more similar to *Laurentomantis*, but their morphological resemblance to *Vatomantis* is greater (see the diagnosis below). We here tentatively assign them to *Vatomantis* due to their morphological affinities and preliminary phylogenetic relationships. Given their very high genetic divergence to all other *Gephyromantis*, isolated phylogenetic position (not placed as close sister group to any other species), and morphological and bioacoustic differences, there is no doubt that these specimens belong to a new species, which we describe below.

### *Gephyromantis* (*Vatomantis*) *lomorina* sp. n.

http://zoobank.org/5D2109C8-AD0A-434D-816F-51722FE7DCD7
Figs 1–4, Table 1, Suppl. materials 1–2

**Holotype.** ZSM 419/2016 (ZCMV 15221), adult male, collected at 21h20 on 18 November 2016 near Camp Simpona (ca. 14.4366°S, ca. 49.7434°E, ca. 1325 m a.s.l.) in Marojejy National Park, Sava Region, northeastern Madagascar, by M. D. Scherz, J. H. Razafindraibe, M. C. Bletz, A. Rakotoarison, A. Razafimanantsoa, and M. Vences (Fig. 2).

**Paratypes.** ZSM 418/2016 (ZCMV 15220), female, and ZSM 420–421/2016 (ZCMV 15222 and 15271), two males, collected between 17 and 19 November 2016 from the same locality and by the same collectors as the holotype; UADBA-A 60294–60299 (ZCMV 15219, 15223, 15247, 15270, 15272, and 15273), one male, three females, a subadult and an unsexed adult, collected between 17 and 19 November 2016 from the same locality and by the same collectors as the holotype; ZSM 1549/2012 (FGZC 3714), adult male, collected on 30 November 2012 in a creek near the campsite on the Sorata massif (13.6829°S, 49.4403°E, 1325 m a.s.l.), Sava Region, northeastern Madagascar,

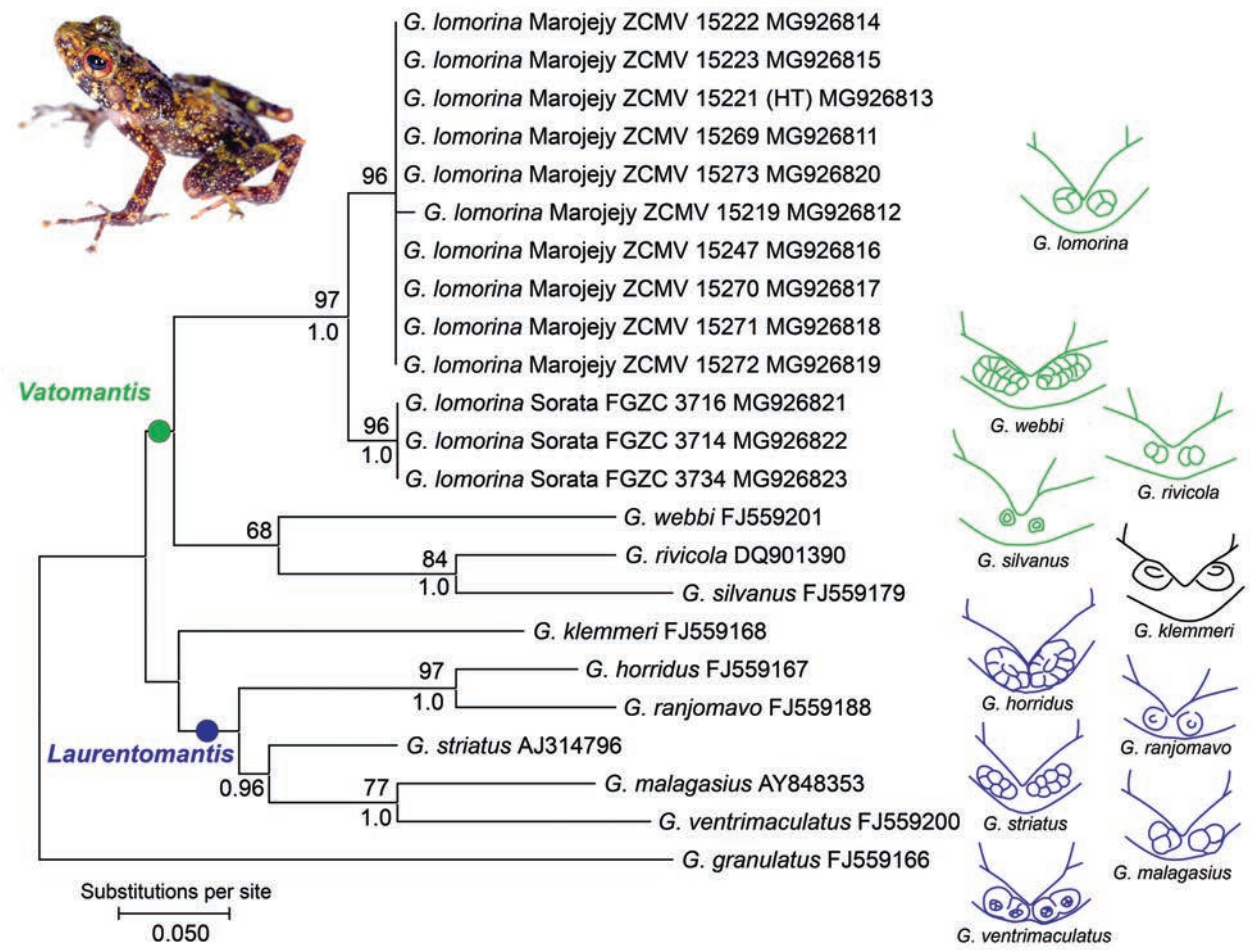


**Figure 1.** Preliminary phylogenetic tree of *Gephyromantis* (*Vatomantis*) *lomorina* sp. n., based on Maximum Likelihood analysis of a 532 bp fragment of the mitochondrial 16S rRNA gene. Numbers at nodes indicate bootstrap values in percent (500 replicates, above) and posterior probabilities from a Bayesian Inference analysis (20 million generations, below), shown only if >50 % (bootstrap) or >90 % (posterior probabilities). Each specimen/species is followed by the corresponding GenBank accession number used in the alignment. Schematic drawings of femoral glands of all species in the subgenera *Laurentomantis* and *Vatomantis* as well as of *G. klemmeri* are shown to the right of the phylogeny, and coloured according to the subgenus to which they are assigned.

by O. Hawlitschek, F. Glaw, A. Rakotoarison, F. M. Ratsoavina, T. Rajoafiarison, and A. Razafimanantsoa; ZSM 1545–1547/2012 (FGZC 3716, 3734, and 3664), adult males, and ZSM 1548/2012 (FGZC 3721), adult female, collected between 28 and 30 November 2012 from a creek below a bamboo forest on the Sorata massif (13.6772°S, 49.4413°E, 1394 m above sea level), Sava Region, northeastern Madagascar, by O. Hawlitschek, F. Glaw, A. Rakotoarison, F. M. Ratsoavina, T. Rajoafiarison, and A. Razafimanantsoa; ZSM 318/2016 (SM AEA 063), adult female, and UADBA-A uncatalogued (SM AEA 062), unsexed adult, collected between 18h45 and 18h50 on 30 May 2016 in Andravory (13.7385–13.7388°S, 49.5310°E, 1164–1179 m a.s.l.), Sava Region, Antsiranana Province, northeastern Madagascar, by S. Megson, R. Walker, W.-Y. Crawley, and T. H. Rafeliarisoa (Figs 3–4).

**Diagnosis.** A species assigned to the genus *Gephyromantis* on the basis of its granular skin, moderately enlarged finger tips, small femoral glands consisting of a small number of large granules and present in males only (thus of type 2 as defined by Glaw et al. 2000), and bifid tongue. Within the genus *Gephyromantis*, assigned to the subgenus *Vatomantis* on the basis of its small size, connected lateral metatarsalia, absence of an outer metatarsal tubercle, paired subgular vocal sacs of partly whitish colour, greenish skin colouration, and riparian ecology. *Gephyromantis lomorina* sp. n. is characterized by the possession of the following suite of morphological characters: (1) granular skin, (2) reddish eyes, (3) mottled green and black skin, (4) males with paired subgular vocal sacs of partly white colour, (5) males with bulbous type 2 femoral glands consisting of a small number (2–3) of large granules, (6) white spots on the venter, (7) SVL 20.2–25.5 mm, and (8) fourth finger much longer than second. Furthermore, the species is characterised by distinctive, 1681–1827 ms advertisement calls of relatively low intensity, consisting of 24–30 individual pulsed notes,

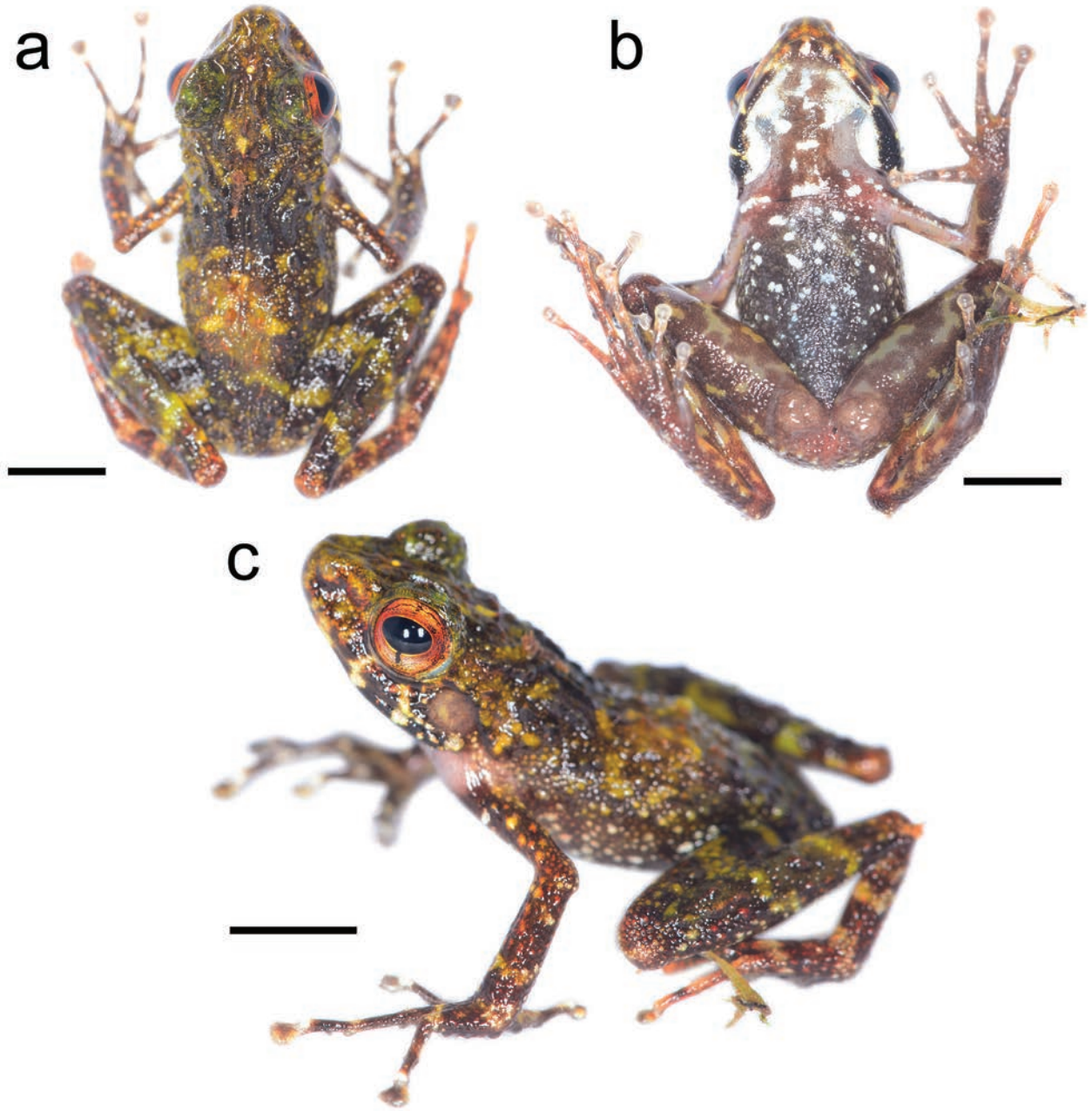


**Figure 2.** The holotype of *Gephyromantis lomorina* sp. n., ZSM 419/2016 (ZCMV 15221) in life. **(a)** Dorsal; **(b)** ventral; and **(c)** dorsolateral view. Scale bars indicate 5 mm.

with 2–4 pulses per note, an inter-note interval of 41–75 ms, and a dominant frequency of 5124–5555 Hz. DNA sequence data from the 16S gene fragment supports the high divergence of this taxon to all other *Gephyromantis*, and is in agreement with its subgeneric assignment, albeit without statistical support (Fig. 1).

Within the genus *Gephyromantis*, *G. lomorina* sp. n. can be distinguished from all subgenera except *Laurentomantis* and *Vatomantis* on the basis of the combination of femoral glands composed of few large granules (vs. composed of many, small granules; note that *G. klemmeri* is here treated separately from all other subgenera, below, due to its unclear assignment), SVL < 26 mm (vs. > 27 mm in all other subgenera except *Gephyromantis*), absence of a white stripe along the upper lip (vs. general presence in subgenus *Gephyromantis*), and absence of distinctly enlarged supraocular spines (vs. presence in *Asperomantis* and some *Duboimantis*). It may be distinguished from all members of the subgenus *Laurentomantis* (*G. ventrimaculatus* (Angel), *G. malagasius* (Methuen & Hewitt), *G. striatus* (Vences, Glaw, Andreone, Jesu & Schimmenti), *G. horridus* (Boettger), and *G. ranjomavo* Glaw & Vences) by paired subgular vocal sacs (vs. single), absence of outer metatarsal tubercles (vs. presence), and at least partly greenish dorsal skin (vs. mostly yellowish to brown to reddish), and from several of these by the

**Table 1.** Morphological data on specimens of *Gephyromantis lomorina* sp. n. Abbreviations: m = male, f = female, sa = subadult; for measurement abbreviations, see the Materials and methods. The holotype is bolded. Additive measurements (FARL, FORL, and HIL) are not explicitly shown but can be deduced from these data.

| Catalogue (field number) | Sex | SVL | HW | HL | TD | ED | END | NSD | NND | UAL | LAL | HAL | THIL | TIBL | TARL | FOL | FGL | FGW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ZSM 419/2016 (ZCMV 15221)** | **m** | **23.3** | **7.2** | **8.5** | **2.1** | **4.0** | **2.2** | **1.4** | **2.2** | **6.0** | **7.3** | **8.2** | **13.4** | **13.9** | **7.4** | **12.1** | **2.8** | **2.0** |
| ZSM 421/2016 (ZCMV 15271) | m | 22.2 | 6.6 | 8.2 | 1.9 | 3.4 | 2.1 | 1.5 | 2.2 | 4.8 | 6.0 | 8.0 | 12.2 | 12.8 | 7.0 | 10.6 | 2.2 | 1.8 |
| ZSM 420/2016 (ZCMV 15222) | m | 23.0 | 7.3 | 9.1 | 1.9 | 3.0 | 1.9 | 1.5 | 2.1 | 5.1 | 6.8 | 8.2 | 11.5 | 13.3 | 7.0 | 11.8 | 2.2 | 1.5 |
| ZSM 418/2016 (ZCMV 15220) | f | 25.5 | 7.7 | 9.1 | 2.0 | 4.1 | 2.4 | 1.4 | 2.1 | 5.2 | 7.7 | 8.4 | 13.6 | 14.8 | 6.7 | 12.6 | n/a | n/a |
| UADBA·A 60294 (ZCMV 15270) | m | 22.1 | 6.5 | 8.0 | 2.7 | 3.4 | 1.5 | 1.4 | 1.9 | 4.9 | 6.1 | 7.2 | 10.8 | 12.3 | 6.4 | 11.0 | 2.6 | 1.6 |
| UADBA·A 60298 (ZCMV 15273) | f | 24.6 | 7.6 | 9.0 | 2.0 | 3.8 | 2.2 | 1.4 | 2.1 | 6.4 | 6.4 | 8.3 | 12.1 | 13.5 | 7.3 | 12.4 | n/a | n/a |
| UADBA·A 60296 (ZCMV 15223) | sa | 20.2 | 5.7 | 7.8 | 1.6 | 3.1 | 1.9 | 1.6 | 1.9 | 4.8 | 5.9 | 7.6 | 11.1 | 12.0 | 6.6 | 9.5 | n/a | n/a |
| UADBA·A 60297 (ZCMV 15272) | f | 24.6 | 8.0 | 8.7 | 2.1 | 3.5 | 2.4 | 1.3 | 2.2 | 5.7 | 6.1 | 8.6 | 12.4 | 14.7 | 7.3 | 12.0 | n/a | n/a |
| UADBA·A 60295 (ZCMV 15219) | f | 23.2 | 6.8 | 8.5 | 2.0 | 3.6 | 2.0 | 1.4 | 2.0 | 5.6 | 6.9 | 8.1 | 12.6 | 14.9 | 7.6 | 11.6 | n/a | n/a |
| UADBA·A 60299 (ZCMV 15247) | f | 22.0 | 7.0 | 8.1 | 2.1 | 3.5 | 2.3 | 1.5 | 2.0 | 5.0 | 6.0 | 7.5 | 10.4 | 12.4 | 7.0 | 11.6 | n/a | n/a |
| ZSM 1549/2012 (FGZC 3714) | m | 23.3 | 8.0 | 8.3 | 2.3 | 3.3 | 2.6 | 1.5 | 1.9 | 5.8 | 6.7 | 8.8 | 11.1 | 13.2 | 6.7 | 12.6 | 3.0 | 2.1 |
| ZSM 1545/2012 (FGZC 3716) | m | 22.8 | 7.1 | 8.0 | 2.2 | 3.0 | 2.8 | 1.5 | 2.1 | 6.0 | 6.9 | 8.1 | 11.9 | 13.1 | 6.7 | 13.2 | 2.9 | 2.1 |
| ZSM 1546/2012 (FGZC 3734) | m | 24.6 | 8.0 | 9.7 | 2.2 | 3.3 | 2.8 | 1.6 | 2.3 | 7.0 | 7.5 | 9.3 | 13.1 | 14.5 | 6.6 | 13.4 | 3.4 | 2.5 |
| ZSM 1547/2012 (FGZC 3664) | m | 23.9 | 8.1 | 9.1 | 2.2 | 3.4 | 2.6 | 1.6 | 2.2 | 6.4 | 7.0 | 9.5 | 12.1 | 14.2 | 6.5 | 13.6 | 2.5 | 2.1 |
| ZSM 1548/2012 (FGZC 3721) | f | 24.1 | 7.7 | 9.2 | 2.2 | 2.8 | 2.8 | 1.4 | 2.2 | 5.5 | 6.6 | 9.3 | 12.5 | 14.0 | 6.5 | 13.8 | n/a | n/a |
| ZSM 318/2016 (SM AEA 063) | f | 25.2 | 7.9 | 9.4 | 2.1 | 2.6 | 3.0 | 2.0 | 2.4 | 7.1 | 6.4 | 8.9 | 13.6 | 14.2 | 7.2 | 13.6 | n/a | n/a |

absence of tibial glands in males (vs. typical presence). Within the subgenus *Vatomantis*, *G. lomorina* sp. n. may be distinguished from all species by its more granular dorsal skin (vs. granular but not rough) and venter spotted with white (vs. generally without whitish spotting except on the chin and over the sternum); from *G. rivicola* (Vences, Glaw & Andreone) and *G. webbi* (Grandison) by its reddish iris colouration (vs. copper and greenish, respectively); from *G. silvanus* (Vences, Glaw & Andreone) by its smaller size (SVL 20.5–25.5 mm vs. 31 mm) and partly whitish vocal sacs (vs. yellowish); from *G. webbi* by femoral glands composed of few large granules (vs. composed of many, small granules) and large inner metatarsal tubercle (vs. small). *Gephyromantis lomorina* sp. n. may be distinguished from *G. klemmeri* by its roughly granular dorsal skin (vs. smooth to shagreened), greenish skin colour (vs. brownish), reddish iris (vs. gold), and strongly protruding inner metatarsal tubercle (vs. small and not protruding).

The call of *G. lomorina* sp. n. may be distinguished from all *Vatomantis* and *Laurentomantis* species in having notes that are clearly pulsed (vs. unpulsed notes in all species except *G. ventrimaculatus*); *Gephyromantis ventrimaculatus* has a higher number of pulses per note notes than *G. lomorina* sp. n. (ca. 6 pulses per note vs. 2–4 in *G. lomorina* sp. n.). The call of *G. lomorina* sp. n. is somewhat similar to that of *G. klemmeri*, especially in having pulsed notes, but the call duration is much longer (1681–1827 ms vs. 626–982 ms), the call has a more distinct amplitude decay (vs. complex amplitude modulation, see Vences et al. 1997), the notes of the call are more homogeneous (vs. distinct components of the call), and it lacks frequency modulation (vs. frequency modulated toward the end of the call).

**Description of the holotype.** A specimen in a good state of preservation, a piece of tissue taken from the left thigh. SVL 23.3 mm; for other body measurements see Table 1. Body slender. Widest part of head marginally wider than widest part of body. Snout rounded in dorsal and lateral view, protruding slightly over upper jaw in lateral view. Nostrils not distinctly protruding, with lateral openings. Canthus rostralis distinct, concave. Loreal re-

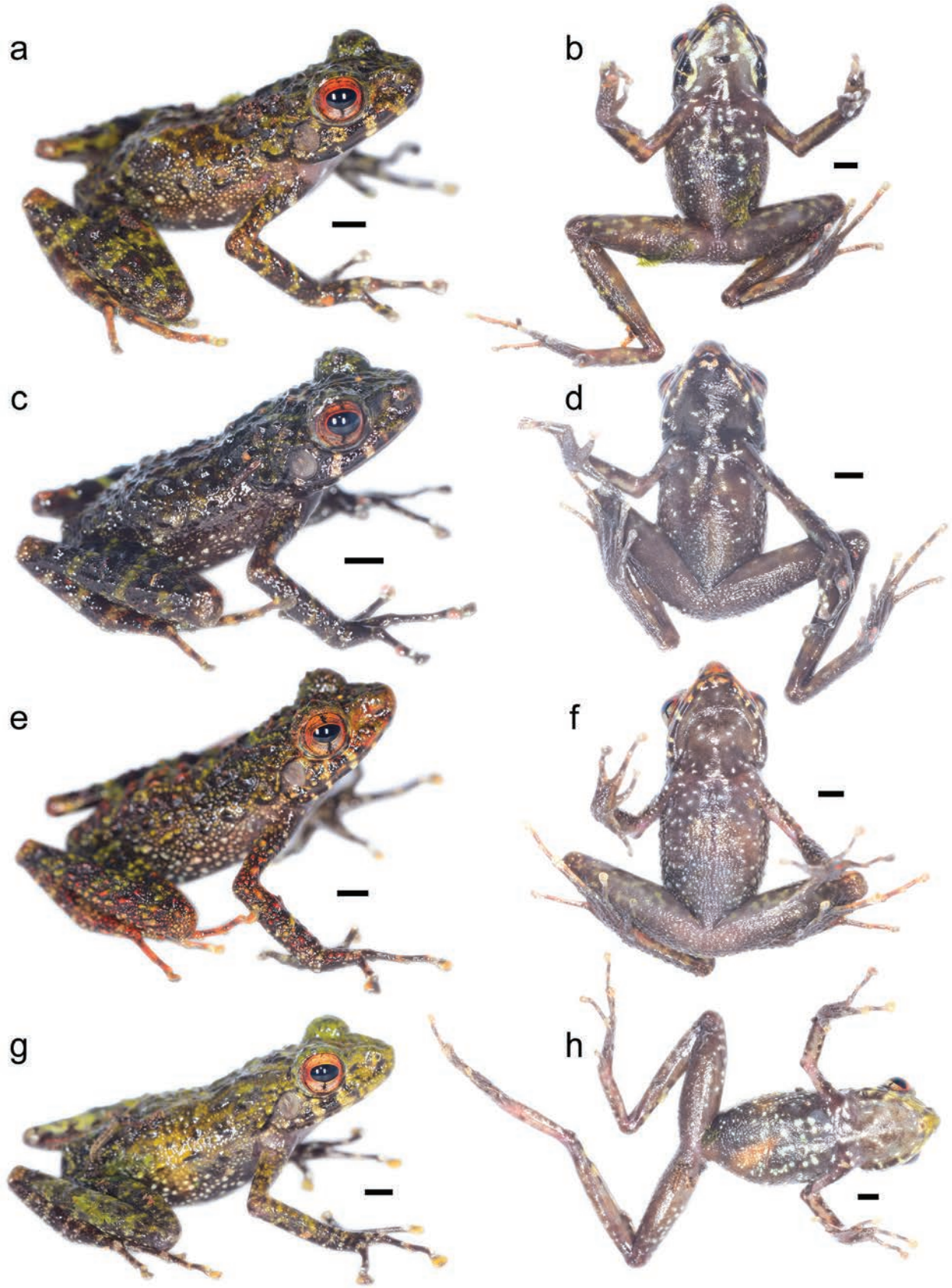


**Figure 3.** Morphological and chromatic variation among paratypes of *Gephyromantis* (*Vatomantis*) *lomorina* sp. n. from Marojejy in life. **(a–b)** ZSM 420/2016; **(c–d)** UADBA-A 60296; **(e–f)** UADBA-A 60295; and **(g–h)** ZSM 418/2016. Scale bars indicate 2 mm.

gion concave, vertical. Tympanum distinct, fairly small, 53% of eye diameter. Supraocular spines absent. Weakly distinct supratympanic fold running from the eye over the tympanum to above the insertion of the arm. Forelimbs and hindlimbs slender. Inner and outer metacarpal tubercle present, both indistinct. Finger discs enlarged, round. Subarticular tubercles distinct, dark in colour. No webbing between fingers. Comparative finger lengths

**Figure 4.** Photographs of *Gephyromantis* (*Vatomantis*) *lomorina* sp. n. and its habitat in Sorata. **(a,d)** ZSM 1545/2012; **(b,e)** ZSM 1547/2012; and **(c,f)** ZSM 1549/2012, not to scale; **(g)** habitat where several specimens were found in Sorata, showing **(h,i)** the appearance of the species in situ whilst calling at night.

1 < 2 < 4 < 3, fourth finger much longer than second finger. Toe discs slightly enlarged, smaller than finger discs. Traces of webbing between toes. Comparative toe length 1 < 2 < 3 = 5 < 4. Inner metatarsal tubercle rather large (length about 1.3 mm), protruding strongly distally to resemble a toe. Outer metatarsal tubercle absent. Lateral

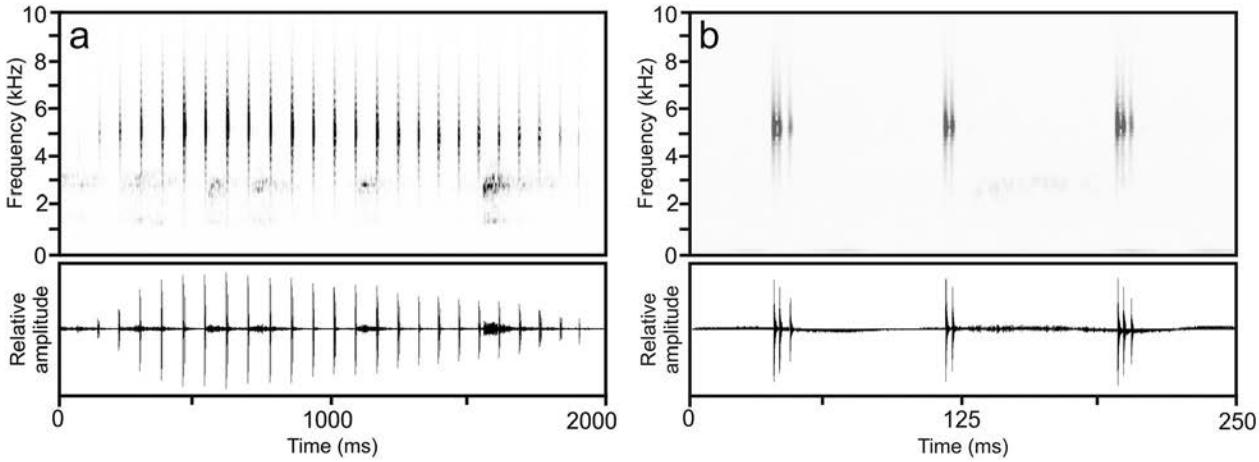


**Figure 5.** Spectrogram (above) and waveform (below) of a call of the holotype of *Gephyromantis* (*Vatomantis*) *lomorina* sp. n., ZSM 419/2016, from Marojejy. **(a)** A full call (spectrogram shown using FFT of 512 points to visualise call structure); and **(b)** a 250 ms section from the middle of a call, showing the degree of pulsation of each note (spectrogram shown using FFT of 128 points to visualise note structure).

metatarsalia connected. Dorsal skin granular, with numerous small tubercles arranged in mostly parallel lines running posteriorly over the dorsum, with convergent lines of tubercles on the posterior head, and weak rows of tubercles on the hindlimbs and forelimbs. Femoral glands round, consisting of three large granules with an indentation in their middle (similar to type 2 sensu Glaw et al. 2000). Vomerine teeth absent. Maxillary teeth present. Choanae small and lateral. Subgular vocal sacs whitish in distensible portion, blackish on the jaw, fairly small. Tongue bifid, free posteriorly.

Colouration in life (Fig. 2) dorsally mottled with greens, browns, blacks, and yellows. Particularly green over the eyes. Raised ridges on the back were mostly yellowish, but some also with an orange hint. Flanks and lateral head as dorsum. Legs dark brown with yellow-green cross-bands, three on the thigh, three on the shank, and two on the tarsus. The tarsus and dorsal foot were a more ruddy brown than the rest of the body, mottled with a tan orange on the toes and on the heel. A few tubercles on the legs were red. A whitish annulus was present before the terminal disc of each toe and finger. The forelimbs were as the shanks and foot, ruddy brown mottled with yellow-green and dark brown, with a few red tubercles. Whitish spots were present in the inguinal region and the ventral portion of the flank, and also two cream stripes were present below the eye that continued on the bottom lip. The tympanum was distinctly brownish. The venter was umber in base colour with more reddish portions of translucent skin on the ventral side of the arms. The chin had white portions along the lip and especially on the vocal sacs, but the jaw itself was blackish. The venter had distinct white spots. The ventral hindlimbs were umber with irregular pale olive and yellow patches on the ventral thigh and shank. The ventral tarsus, foot, and hand were umber. The femoral glands were fleshy in colour, and the area ventral to the cloaca was pinkish. The iris was copper above and below, and rusty anteriorly and posteriorly, with blackish reticulations and a blackish line above and below the centre of the pupil.

After six months in preservative, the colouration of the holotype has faded to become more uniformly brownish, and areas that were greenish in life have become cream. White areas of the venter are still immaculately white.

**Variation.** All paratypes resemble the holotype in gross morphology; see Table 1 for morphological variation. Tympanum diameter ranges from 47–79 % of eye, without strong sexual dimorphism in tympanum size. Females are marginally but not significantly larger than males (*t*-test, t = -1.9215, df = 13, $p$ = 0.07687). Several paratypes have smaller femoral glands than the holotype. Femoral glands are composed of 2 or 3 large granules (mean 2.875 ± 0.35, n = 8; all but one of eight examined specimens with 3 granules). Females have miniscule raised bumps in the femoral area. There is considerable variation in colouration of the specimens, with some individuals being much darker, and others being more green (Figs 3–4). The chin of females is more solidly dark than that of males, and they lack most white spots. A pair of cream stripes below the eye that continue on the lower lip is present in all specimens. Two specimens (UADBA-A 60299, and ZSM 1545/2012, Fig. 4) have a bright vertebral stripe.

**Bioacoustics.** Call recordings were made in Marojejy from the holotype ZSM 419/2016 at its collection locality at a distance of 0.5 m during light rain (Suppl. material 1, DOI: 10.7479/nmx8-aq7v). The call is interpreted as an advertisement call as it resembles the advertisement calls of the subgenus *Laurentomantis*, and was emitted without close proximity to other individuals, and while the frog was otherwise inactive (Köhler et al. 2017). Air temperature was not recorded. A strict FFT bandwidth filter was applied to the dataset to remove all sound below 400 Hz in order to remove wind artefacts. Two calls were recorded from the holotype, but numerous calls

were heard whilst searching for this species along the river where it was found. Calls consisted of a rapid series of 24–29 extremely short notes (note duration 6.3 ± 1.9 ms, range 2–10 ms, n = 53; Fig. 5a), each of which had 2.6 ± 0.6 pulses (2–4 pulses, n = 50), the peak amplitudes of which were separated by 2.7 ± 0.6 ms (1–4 ms, n = 53; Fig. 5b). Notes were separated by silent inter-note intervals of 64.6 ± 5.5 ms (47–75 ms, n = 51). The call was amplitude modulated, increasing in amplitude quickly and slowly decaying toward the end of the call. Call duration was 1769–1827 ms (n = 2), with one inter-call interval recorded of 2399 ms. Generally, however, the calls appeared to be emitted rather irregularly. Dominant frequency was 5124–5512 Hz, and the 90 % bandwidth was from 2723–2759 to 6391–6462 Hz.

Similar calls were recorded in Sorata from ZSM 1549/2012 at its collection locality (Suppl. material 2, DOI: 10.7479/nmx8-aq7v). Air temperature was not recorded. The calls strongly resembled those recorded from the holotype. Three calls were recorded, but one was cut off and another had loud calls of *Gephyromantis* (*Duboimantis*) sp. in the background, so only one was analysed. The call consisted of a rapid series of 31 extremely short notes (note duration 6.9 ± 0.8 ms, range 6–10 ms, n = 27 analysed), each of which had 2.0 ± 0.2 pulses (2–3 pulses, n = 27), the peak amplitudes of which were separated by 3.0 ± 0.4 ms (2–4 ms, n = 27). Notes were separated by silent inter-note intervals of 46.3 ± 3.8 ms (41–55 ms, n = 27). The call was amplitude modulated in the same way as that of ZSM 419/2016. Call duration was 1681 ms, and one inter-call interval was ca. 1900 ms. In general however calling was irregular. The dominant frequency was 5555 Hz, and the 90 % bandwidth was from 4979 to 6003 Hz. The call with a loud *Gephyromantis* (*Duboimantis*) sp. in the background was considerably shorter, and consisted of just 11 notes over a duration of 515 ms, but we suppose this call may have been disturbed as it lacked amplitude reduction toward its end.

**Distribution.** The new species is known from three localities in northeastern Madagascar: (1) Marojejy National Park (type locality), (2) Sorata massif, and (3) Andravory massif (Fig. 6). All specimens were collected between 1164 and 1394 m a.s.l.

**Natural history.** Specimens were collected near mountain streams in pristine montane riparian rainforest (Fig. 4g). In Marojejy National Park they were encountered during and after light rain, sitting in inconspicuous locations, especially on the fronds of tree ferns, but also on other low vegetation, between a few centimetres and up to 2 m above the ground. Specimens in Sorata were found in similar positions during dry weather, in the days just before the beginning of the rainy season. Males called irregularly and softly (see the call description above). Population density in Marojejy was remarkably high, with around three or four individuals being found along a 10 m stretch of stream. The observed density in Sorata was lower, possibly due to the absence of rain during the observation period. The species occurred in close sympatry with a number of other mantellids, but only few of these (especially *Mantidactylus* aff. *femoralis*) were found in the same microhabitat. Several specimens from Marojejy had pinkish mites (probably of the genus *Endotrombicula*; see Wohltmann et al. 2007) embedded within translucent whitish pustules on the skin of their fingers, toes, and bodies. Nothing is known about the reproduction of this species, but the calling sites suggest an association with lotic water.

**Available names.** There are no other, earlier names currently available (e.g., junior synonyms) that are assignable to the subgenera *Vatomantis* or *Laurentomantis* and that could apply to the new species.

**Etymology.** The specific epithet is the Malagasy word *lomorina*, meaning 'covered in moss', in reference to the green, mossy appearance of the species in life. It is used as an invariable noun in apposition to the genus name.

**Conservation.** The species occurs in two regions with very different conservation situations: the highly protected forests of Marojejy National Park, and the unprotected, isolated, and highly threatened forests of Sorata and Andravory. Maminirina et al. (2008) report a study site in the rainforest of Sorata at 970 m a.s.l., but in our surveys in 2012, we detected larger patches of forest only at elevations of ca. 1270 m and above. The new species was collected at lower elevation in Andravory (1164–1179 m a.s.l.), where forest persists. Higher elevation levels of Sorata are covered by high-elevation forests different to those where *G. lomorina* sp. n. was found, and these therefore may not support this species. In this area, the species is therefore directly threatened by the loss of the only forests in which it has been detected.

By contrast in Marojejy, forest extends down to roughly 200 m a.s.l., is highly protected, and the high elevation forest where this species occurs does not seem to be facing any immediate threats. Although the tourist load to Marojejy is relatively high, and the area upslope from the collection locality of the holotype and several paratypes is somewhat polluted with refuse from the nearby tourist camp, the species was abundant around this stream during our survey there in 2016, and presumably inhabits other streams around the same elevation across the massif.

Accommodating this spread of risk is a challenge for the IUCN Red List status. However, *G.* (*V.*) *lomorina* sp. n. is not the first species to have almost exactly this distribution. *Rhombophryne vaventy* Scherz, Ruthensteiner, Vences & Glaw was recently recovered from Sorata (Peloso et al. 2016, Scherz et al. 2016, Lambert et al. 2017) after initially having been described from the same type locality as *G. lomorina* sp. n. (Scherz et al. 2014). In the case of this species, Scherz et al. (2017a) argued for a classification of Endangered under IUCN criterion B1ab(iii), i.e. an extent of occurrence under 5000 km$^2$ (B1), known from fewer than five

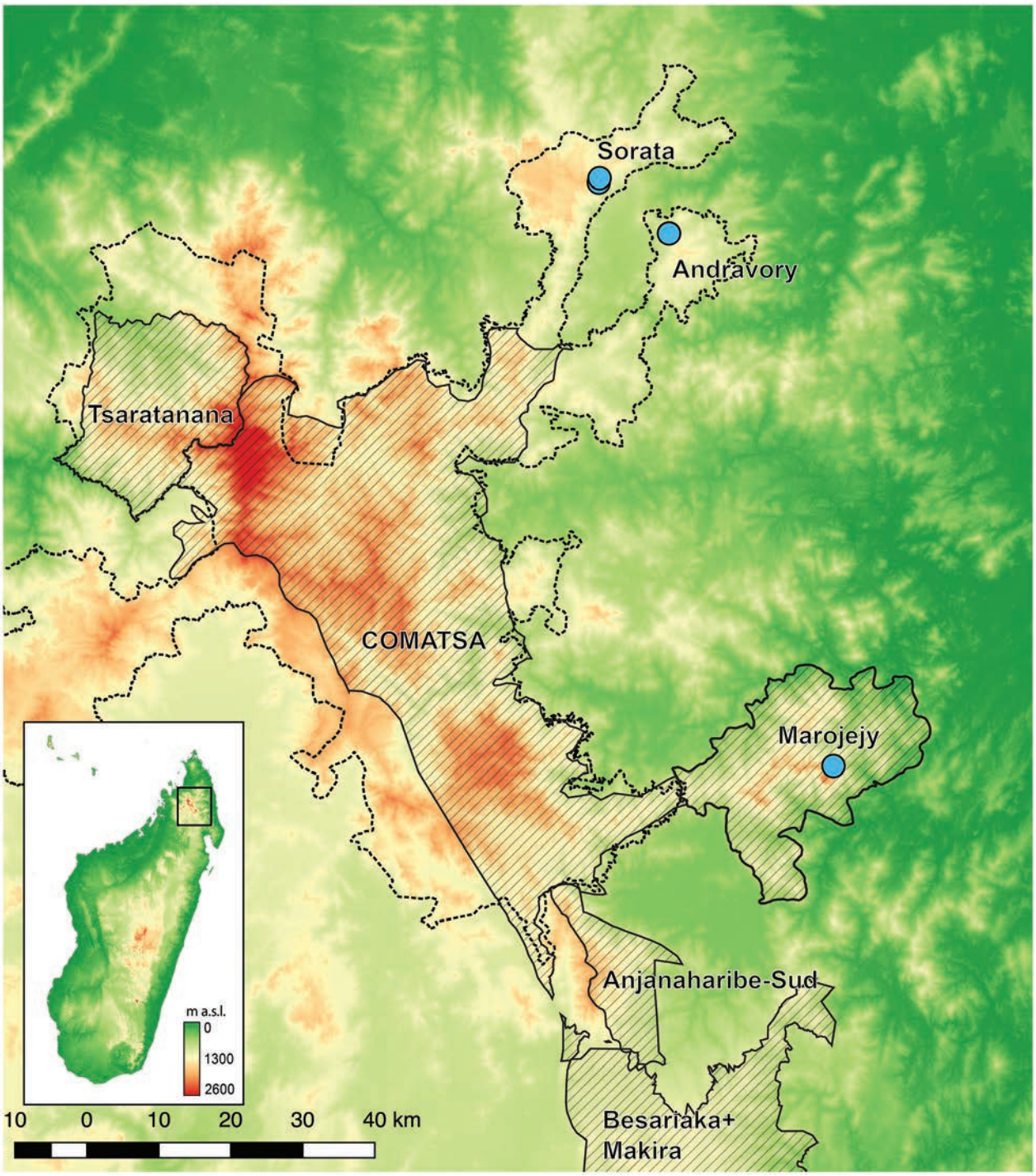


**Figure 6.** Distribution of *Gephyromantis* (*Vatomantis*) *lomorina* sp. n. in northern Madagascar. Areas with diagonal lines are official protected areas. The dotted outline indicates the proposed area with the scope of the WWF protection plan for this part of Madagascar (Biodev Madagascar Consulting 2014, WWF Madagascar 2015). Three arc second SRTM data from Jarvis et al. (2008).

threat-defined locations (a), and an observed, estimated, inferred, or projected decline (b) in the area, extent, and/or quality of habitat (iii). Given the similar situation in *G. lomorina* sp. n., i.e., very similar, limited distribution and ongoing reduction and threat to a substantial part of its habitat (i.e., the forests of Sorata and Andravory), we propose that the same threat status and justification be given for this species.

## Discussion

*Gephyromantis* (*Vatomantis*) *lomorina* sp. n. is a distinctive species, mostly due to its granular, greenish skin, which is rougher than in all other members of the subgenus *Vatomantis,* but not as rugose as in many species of the subgenus *Laurentomantis*. Indeed, it is in several aspects intermediate between these subgenera, having a

call that sounds similar to both (Vences et al. 2006). Its phylogenetic position is at present basically unresolved between these two subgenera. However, its morphology is clearly more similar to *Vatomantis* than to *Laurentomantis*, as it lacks an outer metatarsal tubercle (present in *Laurentomantis*), has a distinct brown tympanum (less distinct in *Laurentomantis*), lacks a broadened head (usually distinctly broadened in *Laurentomantis*) and has paired subgular vocal sacs (single in *Laurentomantis*) (Glaw and Vences 2006).

*Gephyromantis* (*Vatomantis*) *rivicola*, *G.* (*V.*) *silvanus*, *G.* (*V.*) *lomorina* sp. n., and most *Laurentomantis* species share a unique femoral gland morphology with glands being composed of a small number of large, round granules (each granule representing a single gland within the femoral macrogland; Vences et al. 2007; Fig. 1). Glaw et al. (2000) interpreted these unusual glands as possible intermediate steps between Type 2 glands (sharply delimited groups of numerous granules of up to 0.9 mm diameter) toward Type 3 and 4 glands (a rounded structure composed of few, large granules and an external central depression). The position of *G.* (*V.*) *lomorina* sp. n. appears to make this situation more complicated; formerly, it seemed that granule size had increased and number decreased in *G.* (*V.*) *rivicola* and *G.* (*V.*) *silvanus* while *G.* (*V.*) *webbi* had retained Type 2 glands typical of most other *Gephyromantis* species (Glaw et al. 2000, Vences et al. 2007). However, given the split of *G.* (*V.*) *lomorina* from a more basal node in that clade (Fig. 1), and given the ubiquity of these unusual glands in the sister subgenus *Laurentomantis* (Glaw et al. 2000, Kaffenberger et al. 2012), it seems that Type 2 femoral glands may have independently originated one or more times in this clade. A better resolved phylogeny of the clade will be necessary to better understand the evolution of their femoral gland morphology.

The apparently highly divergent *G.* (*V.*) *lomorina* sp. n. sheds some light on questions regarding the relationships of *G. klemmeri*. Formerly, *G. klemmeri* was considered a member of the subgenus *Gephyromantis*, but Kaffenberger et al. (2012) showed that it has affinities between *Laurentomantis* and *Vatomantis*. They forestalled action on transferring it to one of these subgenera until more data become available, as single genes disagreed as to its position. *Gephyromantis klemmeri* shares femoral gland morphology with both *Laurentomantis* and *Vatomantis*, having large glands with a small number of large granules. This lends credence both to its phylogenetic position being close to these subgenera, and also to the hypothesis that smaller numbers of larger granules in the femoral glands may be ancestral in this clade.

Kaffenberger et al. (2012) suggested three possible alternatives to dealing with the phylogenetic affinities of *G. klemmeri*: (a) including *G. klemmeri* in *Laurentomantis* (its position sister to *Laurentomantis* was supported with 94 % bootstrap support from maximum likelihood and >0.99 posterior probability, but was not supported in maximum parsimony analysis), (b) erecting a new monotypic subgenus, or (c) redefining a more inclusive subgenus *Laurentomantis* that besides *G. klemmeri* would also include *Vatomantis* as a junior synonym (the clade containing *Laurentomantis*, *Vatomantis*, and *G. klemmeri* was supported with 100 % bootstrap support from maximum likelihood, >0.99 posterior probability, and 86 % bootstrap support from maximum parsimony). Determining the best course of taxonomic action will in part depend on the resolution of the phylogenetic relationships of *G. klemmeri* and of *G.* (*V.*) *lomorina* sp. n., in the framework of a more comprehensive revision of *Laurentomantis* and *Vatomantis*, as these subgenera still contain further candidate species requiring in-depth analysis (Vieites et al. 2009).

*Gephyromantis* (*Vatomantis*) *lomorina* sp. n. also sheds light on the biogeography of northern Madagascar, providing yet more evidence for a strong link between Sorata and Marojejy. The environmental conditions of these two regions are similar (Brown et al. 2016), and various species originally described from one of the two areas have subsequently been discovered in the other, e.g. *Rhombophryne vaventy* (Peloso et al. 2016, Scherz et al. 2016, 2017a, Lambert et al. 2017), *Gephyromantis* (*Asperomantis*) *tahotra* (Glaw et al. 2011, Vences et al. 2017), and *G.* (*D.*) *schilfi* (Glaw and Vences 2000, Scherz et al. 2017b). These similarities are generally limited to species found above 1200 m, probably because forest below 1200 m in Sorata has been mostly eradicated.

We predict that similarities between faunal compositions of the mountainous massifs of northern Madagascar are limited by elevational connectivity. For instance, there is continued connectivity between regions of elevation up to 1400 m from Sorata to Marojejy and indeed roughly to the Manongarivo massif as well. There is no connectivity above this elevation however; areas of over 1400 m across the different massifs are separated by lower elevations, leading to island-like isolation of peak areas. Therefore, we predict that species occurring above 1400 m will show a greater degree of microendemism, and those below this elevation will have a greater probability of occurring more widely; the higher a species' centre of elevational distribution is located, the greater its chance of being microendemic. No absolute threshold of turnover is expected, because major climate fluctuations in the past will likely have blurred elevational boundaries over time.

So far, evidence appears to support this hypothesis; as already stated, several species from around 1300 m are shared between Marojejy and Sorata (and Andravory, though at present only limited and generally unpublished data are available from this forest), and some species known from higher elevations are so far thought to be microendemic to either region, e.g. *Rhombophryne longicrus* (Scherz et al. 2015), *Gephyromantis* (*Duboimantis*) *tohatra* (Scherz et al. 2017b), *Calumma jejy*, and *C. peyrierasi*. Assuming this hypothesis is correct, it raises questions about species that are microendemic at lower elevations, but opportunities to study and understand these taxa are increasingly limited by the fact that forest at lower elevations is disappearing outside of protected

areas. Conservation efforts must be redoubled to ensure that these study systems may remain long enough to be investigated and understood.

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## References

Biodev Madagascar Consulting (2014) Document de référence sur la NAP complexe Ambohimirahavavy Marivorahona en vue de l'evaluation environnementale par le CTE. Unpublished report. Accessed 14 June 2017. Available from: https://goo.gl/4KgD9W [Archived by WebCite® at http://www.webcitation.org/6saYQDhEv]

Brown JL, Sillero N, Glaw F, Bora P, Vieites DR, Vences M (2016) Spatial biodiversity patterns of Madagascar's amphibians and reptiles. PLoS One 11(1): e0144076. https://doi.org/10.1371/journal.pone.0144076

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Glaw F, Hoegg S, Vences M (2006) Discovery of a new basal relict lineage of Madagascan frogs and its implications for mantellid evolution. Zootaxa 1334: 27–43.

Glaw F, Köhler J, Vences M (2011) New species of *Gephyromantis* from Marojejy National Park, northeast Madagascar. Journal of Herpetology 45(2): 155–160. https://doi.org/10.1670/10-058.1

Glaw F, Vences M (2000) A new species of *Mantidactylus* from northeastern Madagascar (Amphibia, Anura, Ranidae) with resurrection of *Mantidactylus blanci* (Guibé, 1974). Spixiana 23(1): 71–83.

Glaw F, Vences M (2001) Two new sibling species of *Mantidactylus cornutus* from Madagascar. Spixiana 24(2): 177–190.

Glaw F, Vences M (2006) Phylogeny and genus-level classification of mantellid frogs (Amphibia, Anura). Organisms Diversity & Evolution 6(3): 236–253. https://doi.org/10.1016/j.ode.2005.12.001

Glaw F, Vences M, Gossmann V (2000) A new species of *Mantidactylus* (subgenus *Guibemantis*) from Madagascar, with a comparative survey of internal femoral gland structure in the genus (Amphibia: Ranidae: Mantellinae). Journal of Natural History 34: 1135–1154. https://doi.org/10.1080/00222930050020140

Jarvis A, Reuter HI, Nelson A, Guevara E (2008) Hole-filled seamless SRTM data V4, International Centre for Tropical Agriculture (CIAT). Available from: http://srtm.csi.cgiar.org.

Kaffenberger N, Wollenberg KC, Köhler J, Glaw F, Vieites DR, Vences M (2012) Molecular phylogeny and biogeography of Malagasy frogs of the genus *Gephyromantis*. Molecular Phylogenetics and Evolution 62(1): 555–560. https://doi.org/10.1016/j.ympev.2011.09.023

Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel M-O, Vences M (2017) The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. Zootaxa 4251(1): 1–124. https://doi.org/10.11646/zotaxa.4251.1.1

Köhler J, Vieites DR, Bonett RM, García FH, Glaw F, Steinke D, Vences M (2005) New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. BioScience 55(8): 693–696. https://doi.org/10.1641/0006-3568(2005)055[0693:NAAGCA]2.0.CO;2

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Lambert SM, Hutter CR, Scherz MD (2017) Diamond in the rough: a new species of fossorial diamond frog (*Rhombophryne*) from Ranomafana National Park, southeastern Madagascar. Zoosystematics and Evolution 93(1): 143–155. https://doi.org/10.3897/zse.93.10188

Maminirina CP, Goodman SM, Raxworthy CJ (2008) Les micro-mammifères (Mammalia, Rodentia, Afrosoricida et Soricomorpha) du massif du Tsaratanana et biogéographie des forêts de montagne de Madagascar. Zoosystema 30: 695–721.

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR, Version 2.0. Privately published, University of Hawaii.

Peloso PLV, Frost DR, Richards SJ, Rodrigues MT, Donnellan S, Matsui M, Raxworthy CJ, Biju SD, Lemmon EM, Lemmon AR, Wheeler WC (2016) The impact of anchored phylogenomics and taxon sampling on phylogenetic inference in narrow-mouthed frogs (Anura, Microhylidae). Cladistics 32(2): 113–140. https://doi.org/10.1111/cla.12118

Perl RGB, Nagy ZT, Sonet G, Glaw F, Wollenberg KC, Vences M (2014) DNA barcoding Madagascar's amphibian fauna. Amphibia-Reptilia 35: 197–206. https://doi.org/10.1163/15685381-00002942

Rambaut A, Drummond AJ (2007) Tracer v1.5. Available from: http://beast.bio.ed.ac.uk/Tracer.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Sabino-Pinto J, Mayer CJ, Meilink WRM, Grasso D, Raajmakers CCB, Russo VG, Segal M, Stegen G, Clegg J, Srikanthan AN, Glaw F, Vences M (2014) Descriptions of the advertisement calls of three sympatric frog species in the subgenus *Vatomantis* (genus *Gephyromantis*) from Madagascar. Herpetology Notes 7: 67–73.

Scherz MD, Hawlitschek O, Andreone F, Rakotoarison A, Vences M, Glaw F (2017a) A review of the taxonomy and osteology of the *Rhombophryne serratopalpebrosa* species group (Anura: Microhylidae) from Madagascar, with comments on the value of volume rendering of micro-CT data to taxonomists. Zootaxa 4273(3): 301–340. https://doi.org/10.11646/zootaxa.4273.3.1

Scherz MD, Rakotoarison A, Hawlitschek O, Vences M, Glaw F (2015) Leaping towards a saltatorial lifestyle? An unusually long-legged new species of *Rhombophryne* (Anura, Microhylidae) from the Sorata massif in northern Madagascar. Zoosystematics and Evolution 91(2): 105–114. https://doi.org/10.3897/zse.91.4979

Scherz MD, Razafindraibe JH, Rakotoarison A, Bletz MC, Glaw F, Vences M (2017b) Yet another small brown frog from high altitude on the Marojejy Massif, northeastern Madagascar. Zootaxa 4347(3): 572–582. https://doi.org/10.11646/zootaxa.4347.3.9

Scherz MD, Ruthensteiner B, Vences M, Glaw F (2014) A new microhylid frog, genus *Rhombophryne*, from northeastern Madagascar, and a re-description of *R. serratopalpebrosa* using micro-computed tomography. Zootaxa 3860(6): 547–560. https://doi.org/10.11646/zootaxa.3860.6.3

Scherz MD, Vences M, Rakotoarison A, Andreone F, Köhler J, Glaw F, Crottini A (2016) Reconciling molecular phylogeny, morphological divergence and classification of Madagascan narrow-mouthed frogs (Amphibia: Microhylidae). Molecular Phylogenetics and Evolution 100: 372–381. https://doi.org/10.1016/j.ympev.2016.04.019

Vallan D, Glaw F, Andreone F, Cadle JE (1998) A new treefrog species of the genus *Boophis* (Anura: Ranidae: Rhacophorinae) with dermal fringes from Madagascar. Amphibia-Reptilia 19(4): 357–368. https://doi.org/10.1163/156853898X00025

Vences M, Glaw F, Andreone F (1997) Description of two new frogs of the genus *Mantidactylus* from Madagascar, with notes on *Mantidactylus klemmeri* (Guibe, 1974) and *Mantidactylus webbi* (Grandison, 1953) (Amphibia, Ranidae, Mantellinae). Alytes 14(4): 130–146.

Vences M, Glaw F, Andreone F, Jesu R, Schimmenti G (2002) Systematic revision of the enigmatic Malagasy broad-headed frogs (*Laurentomantis* Dubois, 1980), and their phylogenetic position within the endemic mantellid radiation of Madagascar. Contributions to Zoology 70(4): 191–212.

Vences M, Glaw F, Marquez R (2006) The Calls of the Frogs of Madagascar. 3 Audio CD's and booklet. Fonoteca Zoológica, Madrid, Spain, 44 pp.

Vences M, Köhler J, Pabijan M, Bletz M, Gehring P-S, Hawlitschek O, Rakotoarison A, Ratsoavina FM, Andreone F, Crottini A, Glaw F (2017) Taxonomy and geographic distribution of Malagasy frogs of the *Gephyromantis asper* clade, with description of a new subgenus and revalidation of *Gephyromantis ceratophrys*. Salamandra 53(1): 77–98.

Vences M, Lyra ML, Perl BRG, Bletz MC, Stankovic D, Geffers R, Haddad CFB, Steinfartz S, Martins Lopes C, Jarek M, Bhuju S (2016) Freshwater vertebrate metabarcoding on Illumina platforms using double-indexed primers of the mitochondrial 16S rRNA gene. Conservation Genetics Resources 8(1): 1–5. https://doi.org/10.1007/s12686-016-0550-y

Vences M, Raxworthy CJ, Nussbaum RA, Glaw F (2003) A revision of the *Scaphiophryne marmorata* complex of marbled toads from Madagascar, including the description of a new species. Herpetological Journal 13: 69–79.

Vences M, Wahl-Boos G, Hoegg S, Glaw F, Oliveira ES, Meyer A, Perry S (2007) Molecular systematics of mantelline frogs from Madagascar and the evolution of their femoral glands. Biological Journal of the Linnean Society 92(2007): 529–539.

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences of the USA 106(20): 8267–8272. https://doi.org/10.1073/pnas.0810821106

Vieites DR, Wollenberg KC, Vences M (2012) Not all little brown frogs are the same: a new species of secretive and cryptic *Gephyromantis* (Anura: Mantellidae) from Madagascar. Zootaxa 3344: 34–46.

Wohltmann A, du Preez L, Rödel M-O, Köhler J, Vences M (2007) Endoparasitic mites of the genus *Endotrombicula* Ewing, 1931 (Acari: Prostigmata: Parasitengona: Trombiculidae) from African and Madagascan anurans, with description of a new species. 54: 225–235. https://doi.org/10.14411/fp.2007.031

WWF Madagascar (2015) Plan d'Aménagement et de gestion intégré du complexe d'aires protegées Ambohimirahavavy Marivorahona, Report Draft. Accessed 14 June 2017. Available from goo.gl/GGXP2D

## Supplementary material 1

### Advertisement call of *Gephyromantis lomorina* sp. n.

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Data type: WAV File (.wav)

Explanation note: Call recording of *Gephyromantis* (*Vatomantis*) *lomorina* sp. n. ZSM 419/2016 (ZCMV 15221). Calls recorded at 21h20 on 18 November 2016 near Camp Simpona (ca. 14.4366°S, ca. 49.7434°E, ca. 1325 m a.s.l.) in Marojejy National Park, Sava Region, Antsiranana Province, northeastern Madagascar, by M. D. Scherz. Frog was ca. 1 m above the ground on a fern near a small river, calling occasionally during light rain. Air temperature was not taken. Recording distance was 0.5 m. Animal Sound Archive: https://doi.org/10.7479/nmx8-aq7v.

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Link: https://doi.org/10.3897/zse.94.21037.suppl1

## Supplementary material 2

### Advertisement call of *Gephyromantis lomorina* sp. n.

Authors: Mark D. Scherz, Oliver Hawlitschek, Jary H. Razafindraibe, Steven Megson, Fanomezana Mihaja Ratsoavina, Andolalao Rakotoarison, Molly C. Bletz, Frank Glaw, Miguel Vences

Data type: WAV File (.wav)

Explanation note: Call recording of *Gephyromantis* (*Vatomantis*) *lomorina* sp. n. ZSM 1549/2012 (FGZC 3714). Calls recorded at night on 30 November 2012 on the Sorata massif (creek near campsite, 13.6829°S, 49.4403°E, 1325 m a.s.l.), Sava Region, Antsiranana Province, northeastern Madagascar, by O. Hawlitschek. Ecological data not available. Air temperature and recording distance were not noted. Animal Sound Archive: https://doi.org/10.7479/nmx8-aq7v.

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# Unrecognized biodiversity in a world's hotspot: three new species of *Melanorivulus* (Cyprinodontiformes: Rivulidae) from tributaries of the right bank of the Rio Paraná basin, Brazilian Cerrado

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## Abstract

The genus *Melanorivulus* presents a wide geographical distribution in the Neotropical region. Among *Melanorivulus*, the *M. pictus* species group has currently 18 species distributed in the Brazilian Cerrado, mainly in the upper Rio Paraná basin, with some species occurrences in the upper Rio Araguaia and Tocantins. In the present study, we describe three new *Melanorivulus* species, belonging to the *M. pictus* species group from different drainages of the right bank of the Rio Paraná basin in Mato Grosso do Sul state, Brazil. These new species are easily distinguished from the others by their unique colour patterns. *Melanorivulus interruptus* is distinguished from all species of the *M. pictus* group by the presence of oblique chevron-like red bars interrupted, mainly on the midline of the flank in males; while *M. ivinhemensis* by the yellow colouration of the caudal fin with thin red bars arranged only in the median region of the fin in males. *Melanorivulus amambaiensis* is distinguished from all species of the *M. pictus* species group by having an orange anal fin or sometimes more reddish-orange with distal margin grey or dark grey and chevron-like bars along the body, distinctly branched ventrally forming an inverted Y-shape in males. The high diversity of the *Melanorivulus* species with high levels of endemism demands the development of conservation strategies to avoid the loss of their vulnerable habitats in the Cerrado biome. We expect presence of more species of the *M. pictus* species group also along the lower reaches of the tributaries of the Rio Paraná. Additionally a dichotomic identification key of the *M. pictus* species group is provided.

## Introduction

Savanna biomes are distributed across tropical zones around the world. In South America, the Cerrado is the largest savanna biome, the second largest biome in Brazil (smaller only than the Amazon), and represents about 23 % of Brazilian territory (Ratter et al. 1997). The Brazilian Cerrado is included in the world's 25 main "hotspots", areas with many rare and endemic species and less than 30% remaining natural vegetation (Myers et al. 2000). It is one of the world's most threatened biomes, mainly because of the replacement of natural vegetation by pastures and row crops (Ratter et al. 1997, Myers et al. 2000).

The rivulid genus *Melanorivulus* is a typical component of the Cerrado fauna, but presents a wide geographical distribution in the Neotropical region, being also

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found in areas under the influence of the Chaco, Pantanal, Atlantic Forest and Amazon (e.g. Costa 2016, Costa et al. 2016, Nielsen 2017). However, most of the species are found in Brazil and occur in the Cerrado biome along several river basins drained mainly by the upper tributaries of the Rio Araguaia, Tocantins, Paraná and Paraguay basins and the adjacent Cerrado-Amazon ecotone (Costa et al. 2016). Recent killifish inventories of the Brazilian Cerrado have revealed a high diversity of *Melanorivulus* species (e.g., Costa 2012, 2017, 2018, Volcan et al. 2017), indicating that species inhabiting the region have small geographical ranges, often restricted to short segments of a single river drainage (Costa 2017, Volcan et al. 2017). Despite the wide geographical distribution and high diversity, little is known about the conservation, distribution, biology and ecology of most *Melanorivulus* species (Severo-Neto and Volcan 2018).

Among *Melanorivulus* species, the *M. pictus* species group is distinguished from all other congeners, by the presence of a vestigial ventral process of the angulo-articular (vs. process well-developed), curved first epibranchial (vs. approximately straight) and intense greenish blue or greenish golden to purplish blue flank above anal-fin base in males (vs. never similar colour pattern) (Costa 2007, 2008). The *Melanorivulus pictus* species group currently comprises 18 species (Volcan et al. 2017, Costa 2018), with 16 species occurring in the Rio Paraná basin: *M. apiamici* (Costa, 1989) in the Rio Paraná, *M. egens* (Costa, 2005), in the Rio São Domingos, *M. formosensis* (Costa, 2008) and *M. nigromarginatus* Costa, 2018 for the Rio Corrente, *M. giarettai* (Costa, 2008) for the Rio Araguari basin, *M. leali* Costa, 2013 for the Rio Grande basin, *M. nigropunctatus* Volcan, Klotzel & Lanés, 2017 and *M. ofaie* Volcan, Klotzel & Lanés, 2017 for the Rio Verde basin, *M. faucireticulatus* (Costa, 2008), *M. pictus* (Costa, 1989) and *M. vittatus* (Costa, 1989) for the Rio Claro basin, *M. polychromus* Nielsen, 2016 for the Rio São José dos Dourados basin, *M. rutilicaudus* (Costa, 2005) for the Rio Verde basin, *M. scalaris* (Costa, 2005) for the Rio Sucuruí basin, *M. proximus* Costa, 2018 for Rio Aporé and *M. linearis* Costa, 2018 for the upper Rio Pardo basin. Two other species occur exclusively in the upper Rio Tocantins and Araguaia basin: *M. planaltinus* (Costa & Brasil, 2008) for the Rio Cocal and *M. litteratus* (Costa, 2005) for the Salto stream, respectively.

A previous analysis indicates that the most recent common ancestor of *Melanorivulus* probably occupied a region comprising the eastern Amazon savanna and the ecotone Amazon-Cerrado, and the current distribution is the result of a series of dispersal and vicariance events during the evolutionary history of the genus (Costa et al. 2016). In the present study, we describe three new species of *Melanorivulus* belonging to the *M. pictus* species group that are endemic to different drainages of the right bank of the Rio Paraná basin in Mato Grosso do Sul state, Brazil. Additionally, we discuss the conservation, distribution patterns, and provide an identification key to the *M. pictus* species group.

## Material and methods

In December 2016, a 12-day collection campaign was conducted to sample potential habitats for the occurrence of killifish species in the Mato Grosso do Sul state, Brazil, which included the largest river basins associated with Rio Paraná. In order to define the sampling strategy, we analysed satellite images from Google Earth (earth.google.com) as well as locations of specimens vouched in the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul. Field works were undertaken travelling the main highways and roads to reach access to previously selected areas; at every site we performed an active search for fishes.

Fish samples were taken with a dip-net (D-shaped hand net, 60 cm × 40 cm, 2 mm mesh size), and then were euthanized with clove oil, fixed *in situ* with 4% formaldehyde, and later transferred into 70% ethanol. The material was collected under IBAMA/ICMBio authorization (process number 56894-1).

Morphological characters were obtained from specimens fixed in formalin after collection, and subsequently transferred to 70% ethanol. Fish measurements were taken point-to-point with digital calipers to the nearest 0.1 mm on the left side of the specimen following Costa (1995). Measurements are expressed as percentage of standard length (SL), except the head measurements, which are recorded as percentage of head length. Fin-ray counts include all elements. Scale count in the mid-longitudinal series includes all scales between the upper attachment of the opercular membrane and the base of the caudal fin, excluding small scales posterior to the hypural plate. Numbers of vertebrae were recorded only from cleared and stained specimens (C&S), prepared according to Taylor and Van Dyke (1985). Frontal squamation nomenclature follows that described by Hoedeman (1958), and for cephalic neuromasts series Costa (2001).

Descriptions of colour patterns were based on photographs of both sides of live specimens photographed in the field, which were fixed *in situ* after photos, and individuals maintained in aquaria (not preserved). Institutional abreviations are MCP (Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre) and ZUFMS (Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul, Campo Grande).

### *Melanorivulus interruptus* sp. n.

http://zoobank.org/2B106159-5398-44DC-B89C-7F8CB6CE676A

**Holotype.** MCP 53145, male, 32.3 mm SL, Brazil, Mato Grosso do Sul State, municipality of Campo Grande, first order stream, in the headwaters of Rio Pardo, 20°28'11"S, 54°29'25"W, altitude 589 m a.s.l.; M.V. Volcan & L.E.K Lanés, 13 Dec 2016.

**Paratypes.** MCP 53146, 4 males, 21.6–31.0 mm SL (1 C&S), 3 females, 22.5–29.0 mm SL, 1 juvenile, sex undetermined, 16.9 mm, all collected with the holotype.

ZUFMS 5409, 7 females, 20.2–26.0 mm SL (3 C&S), same locality as holotype; F. Severo-Neto, T.R.F. Sinani & S. Ichikawa, 5 Aug 2017.

**Diagnosis.** *Melanorivulus interruptus* is distinguished from all species of the *M. pictus* group, except *M. nigromarginatus*, by the presence of oblique chevron-like bars interrupted, mainly on the midline of the flank in males (vs. bars continuous, vestigial, irregular or presence of longitudinal rows of red dots on the side of the body; never bars interrupted on the midline of the body). It is distinguished from all other species in the *M. pictus* species group, except *M. planaltinus*, *M. leali* and *M. pictus*, by the presence of a rounded dorsal fin (vs. slight pointed or pointed dorsal fin). In addition, *M. interruptus* differs from *M. amambaiensis*, *M. apiamici*, *M. egens*, *M. faucireticulatus*, *M. ivinhemensis* and *M. leali* by a higher caudal fin ray count (32–33 vs. 28–31); from *M. egens*, *M. faucireticulatus*, *M. leali*, *M. litteratus*, *M. rutilicaudus* and *M. formosensis* by the position of the anal-fin origin between the pleural ribs of 15th and 16th vertebrae (vs. 13th and 15th vertebrae); from *M. apiamici*, *M. egens*, *M. faucireticulatus*, *M. pictus*, *M. polychromus* and *M. proximus* by the dorsal-fin origin at a vertical through the base of the 8th anal-fin ray (vs. vertical through base of 7th or 9th-10th anal-fin ray); from *M. amambaiensis*, *M. egens*, *M. faucireticulatus*, *M. ivinhemensis*, *M. planaltinus*, *M. polychromus*, *M. nigropunctatus*, *M. ofaie* and *M. formosensis* by the dorsal-fin origin between neural spines of 18th and 19th vertebrae (vs. 19th and 22th); from *M. egens*, *M. faucireticulatus*, *M. leali*, *M. litteratus*, *M. scalaris*, *M. vittatus*, *M. linearis* and *M. proximus* by the tip of pelvic fin reaching the urogenital papilla in males (vs. tip of pelvic fin reaching 1st to 4th anal fin ray). Another interesting diagnostic character is the presence of 8 rays in the pelvic fin in about 35% of the analysed specimens (vs. 5–7 rays, never 8 rays present in the pelvic fin).

**Description.** Morphometric data are presented in Table 1. Males larger than females. Largest male examined 32.3 mm SL, largest female 29.0 mm SL. Dorsal profile slightly convex from snout to end of dorsal-fin base. Ventral profile convex from lower jaw to origin of anal-fin. Dorsal and ventral profiles of caudal peduncle nearly straight. Body slender, approximately cylindrical and compressed. Greatest body depth at pelvic-fin base. Snout weakly pointed in lateral view. Jaws short.

Short dorsal and anal fins. Dorsal-fin rays 9–10. Dorsal fin rounded in males and females. Dorsal-fin origin on vertical through base of 8th anal-fin ray, and between neural spines of 18th and 19th vertebrae. Anal-fin rays 12–13. Anal fin slightly pointed in males and females. Origin of anal fin at vertical through pleural ribs of 15th-16th vertebrae. Caudal fin rounded 32–33 rays. Pectoral fin rays 14. Pectoral fins rounded, with posterior margin reaching vertical at about 60–90% of length between pectoral-fin and pelvic-fin bases. Pelvic-fin rays 7–8. Pelvic-fin posterior tip reaching vertical at anus to 3rd anal-fin ray. Pelvic-fin bases in close proximity.

**Table 1.** Morphometric data for the holotype and paratypes of *Melanorivulus interruptus* sp. n.

| | Holotype | Males (4) | Females (7) |
|---|---|---|---|
| Standard length | 32.3 | 21.6–31.0 | 22.3–29.0 |
| Percentages of standard length | | | |
| Body depth | 25.6 | 20.8–24.5 | 21.3–26.0 |
| Caudal peduncle depth | 14.4 | 13.3–14.8 | 11.4–14.8 |
| Predorsal length | 73.3 | 75.0–77.1 | 73.3–77.9 |
| Prepelvic length | 54.6 | 52.6–53.9 | 51.8–56.3 |
| Dorsal fin base length | 11.8 | 11.9–13.7 | 10.7–12.7 |
| Anal fin base length | 21.2 | 18.4–22.5 | 17.8–19.4 |
| Caudal fin length | 28.0 | 26.6–32.2 | 26.8–29.4 |
| Pectoral fin length | 18.3 | 19.0–20.6 | 17.7–20.2 |
| Pelvic fin length | 12.1 | 12.6–13.9 | 8.5–10.4 |
| Head length | 26.2 | 26.8–27.5 | 24.9–27.7 |
| Percentages of head length | | | |
| Head depth | 77.9 | 66.7–78.0 | 66.1–76.2 |
| Head width | 76.8 | 66.7–73.6 | 74.7–82.1 |
| Snout length | 18.5 | 12.6–17.7 | 15.1–19.2 |
| Lower jaw length | 19.0 | 16.4–21.7 | 18.7–21.6 |
| Eye diameter | 28.2 | 28.6–31.9 | 31.9–35.6 |

Scales cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 15–25% of caudal-fin base. No scales on dorsal and anal-fin bases. Frontal squamation E-patterned; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins; transverse row of scales anterior to H-scale. Longitudinal series of scales 30–32; transverse series of scales 8–9; scale rows around caudal peduncle 16. No contact organs on flank and fins.

Cephalic neuromasts: supraorbital 3+3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1+9-11+1, preorbital 2, otic 1, postotic 1-2, supratemporal 1, median opercular 1, ventral opercular 1, preopercular 2+4, mandibular 2-3+1, lateral mandibular 1-2, paramandibular 1. Two neuromasts on caudal-fin base.

Six branchiostegal rays. Gill rakers on first branchial arch 1+7. First epibranchial slightly curved. Total number of vertebrae 29–30, 13–14 precaudal vertebrae, 16–17 caudal vertebrae. Ventral process of angulo-articular short, pointed. Vomerine teeth 1–3. Dermosphenotic present. Basihyal sub-triangular, greatest width 45–50% of length; basihyal cartilage 20–25% of total basihyal length. Second pharyngobranchial teeth absent.

**Colouration in life.** Males (Figs 1 and 2). Flank light grey or light metallic blue; sometimes purple-blue close to anal fin; 7–9 oblique narrow red bars anteriorly directed, often forming chevron-like marks irregularly arranged and usually interrupted in the midline of the body; commonly, the chevron-like bars are vestigial in the anterior region and begin at the end of the pectoral fin; well-defined, but interrupted, bars begin behind insertion of the pelvic fins. Inconspicuous dark pigmentation between postorbital region and area above midlength of pectoral fin, forming an irregular mid-lateral dark stripe;

vertical rows of reddish dots on antero-ventral part of flank, between pectoral and pelvic fins. Dorsum yellowish-grey, venter whitish-grey. Dorsal portion of the head yellowish-grey; ventral portion yellowish-grey or pale golden without dots, marks or any pigmentation pattern; pale golden-greenish iridescence on opercular region. Jaws dark grey or brown. Iris pale yellow to pale brown, sometimes with dark brown bars on anterior and posterior portions. Dorsal fin yellow with four or five narrow red bars. Anal fin yellow, basal portion light blue-whitish, posterior portion pale blue with two or three faint red marks; distal region becoming gradually dark red-brown. Caudal fin yellow with five to seven red oblique parallel bars, covered generally 1/3–1/2 of caudal fin on mid-dorsal portion, sometimes red bars inconspicuous; ventral portion light yellow without bars, distal ventral region becoming gradually dark red-brown with a not well delineated margin of orange-brown colour. Pectoral fin yellowish-hyaline. Pelvic fin yellow with posterior margin orange-brown.

Females (Fig. 2). Generally flank similar to males, but with paler colours. Venter white. Dorsum and dorsal portion of head yellowish-grey; ventral portion of head yellowish-grey without spots and without any colouration pattern. Jaws yellowish-grey or brown; opercular region pale greenish-gold. Dorsal fin yellow, with three or four

**Figure 1.** *Melanorivulus interruptus* sp. n., MCP 53145, holotype, male, 32.3 mm SL, Campo Grande, Mato Grosso do Sul, Brazil.

**Figure 2.** *Melanorivulus interruptus* sp. n., MCP 53146, paratypes, living male (above) and female (below), Campo Grande city, Mato Grosso do Sul state, Brazil.

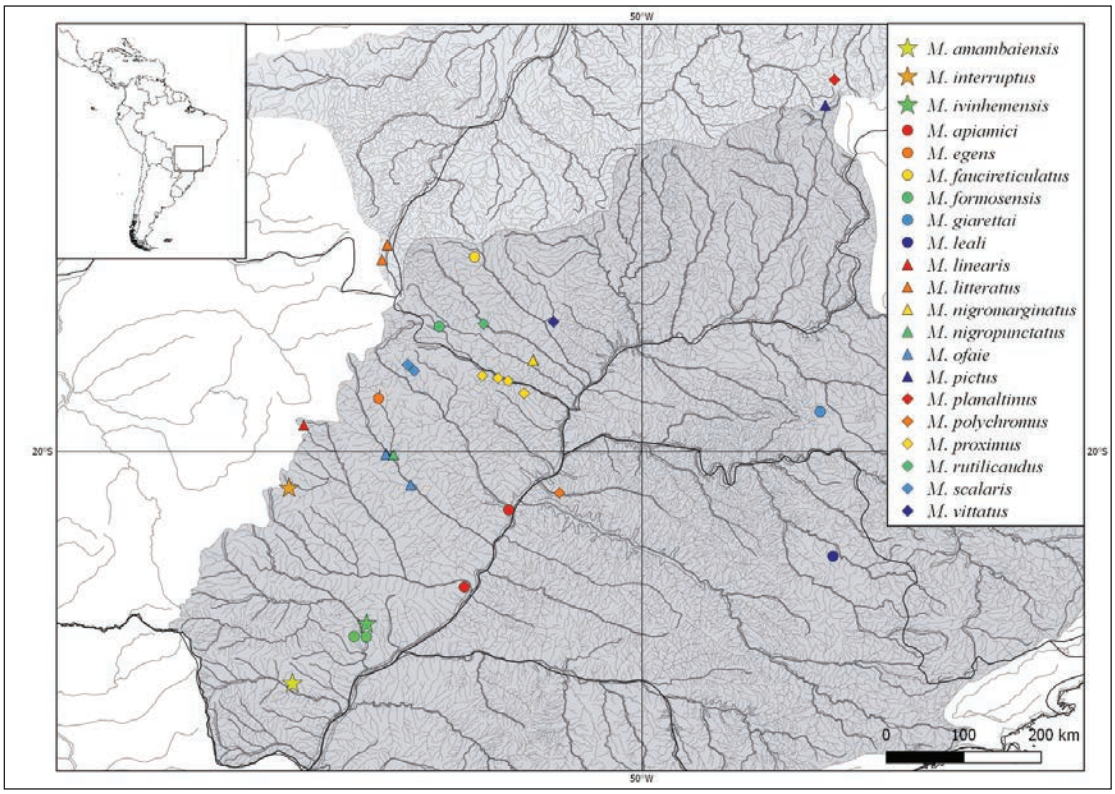


**Figure 3.** Distribution of species of the *Melanorivulus pictus* group in the Rio Paraná (grey) and Rio Araguaia and Tocantins (light grey) basins. Stars indicate the type localities of the new species.

faint grey bars on posterior region forming a reticulated pattern. Anal fin yellow; basal portion light blue; distal region becoming dark red-brown gradually; distal margin with high concentration of melanophores. Caudal fin pale yellow, with four or six grey bars usually interrupted in the inferior region (lower third of fin); a small irregularly shaped black spot on dorso-basal portion of fin, sometimes overlapping the first bar; faint grey margin. Pectoral fin hyaline or yellowish hyaline. Pelvic fin yellowish hyaline.

**Distribution.** *Melanorivulus interruptus* is only known so far from a small stream, in the headwaters of the Rio Pardo, a tributary of the right margin of Rio Paraná, state of Mato Grosso do Sul, Brazil (Fig. 3).

**Etymology.** The name *interruptus*, from the Latin adjective interrupted, referring to the presence of conspicuous oblique red bars forming chevron-like marks usually disrupted in the midline of the body in males.

**Habitat.** The species was recorded in a typical Cerrado area, inserted into the urban area of Campo Grande town at an altitude of 594 m a.s.l.. It was recorded in a small first order creek, but was only captured in border areas with dense aquatic vegetation, mainly grasses (Fig. 4). The biotope, which is locally called "veredas", a freshwater ecosystem comprising streams bordered by the buriti-palm, typically found in the Cerrado Biome, has moderate current speed. But, the place where the species was recorded is formed by small backwaters, with a clayey substrate and with a maximum depth of 10 cm. The species is encountered in areas exposed to the sun as well as in areas shaded by trees, mainly buritis-palm (*Mauritia flexuosa*). The region has a slightly undulating terrain and according to the classification of Koppen-Geiger has rainy summers and dry winters.

**Conservation.** There are imminent threats to the population of *M. interruptus*: the stream is located in the Campo Grande urban area, which is developing rapidly around the area of the new species; there is a large real estate development alongside the stream that can cause significant impacts on the environment quality; the stream is crossed by a high traffic road and it is subject to accidents with potentially dangerous loads; besides, there are agricultural activities over its complete extension. Although larger collection efforts are likely to be undertaken in the area to better understand the species distribution pattern, *M. interruptus* appears to have a very restricted distribution. In the adjacent watersheds distinct species occur (e.g. *M. apiamici* in the mouth of the Rio Pardo, *M. egens* in the upper Rio São Domingos and *M. nigropunctatus* and *M. ofaie* in the Rio Verde – see Figure 4 with the distribution map of the *M. pictus* species group). Thus, according to the previously mentioned threats, a high degree of endemism and the observations made in the field, *M. interruptus* was considered "Vulnerable", following the criteria of IUCN (2012). The species has an area of occupation of less than 2000 km², it occurs in only one locality and it suffers from a reduction in its occupation area and in its habitat quality (VU B2abii,iii).

**Figure 4.** First order stream (typical "veredas" habitat), tributary of headwaters of Rio Pardo, *M. interruptus* type locality.

***Melanorivulus ivinhemensis* sp. n.**

http://zoobank.org/2531E407-3D5B-428D-9CD3-37AB7A13600C

**Holotype.** MCP 53147, male, 30.1 mm SL, Brazil, Mato Grosso do Sul State, municipality of Nova Andradina, first order stream of Rio Ivinhema, 22°11'53"S, 53°20'12"W, altitude 300 m a.s.l.; M.V. Volcan & L.E.K Lanés, 09 Dec 2016.

**Paratypes.** MCP 53153, 6 males, 20.3–27.7–31.0 mm SL, 7 females, 18.5–29.7 mm SL (1 C&S), 2 juvenile, sex undetermined, 14.0–15.4 mm, all collected with the holotype. MCP 53148, 2 juvenile, sex undetermined, 14.3–16.9 mm, wetland in the boarder of BR 376 highway, 22°21'22"S, 53°30'32"W, altitude 252 m a.s.l.; M.V. Volcan & L.E.K Lanés, 08 Dec 2016. MCP 53149, 1 male, 18.6 mm SL, 2 females, 25.7–30.4 mm SL, first order stream of Rio Ivinhema, 22°21'51"S, 53°39'42"W, altitude 310 m a.s.l.; M.V. Volcan & L.E.K Lanés, 08 Dec 2016. ZUFMS 5365, 5 males, 21.8–29.5 mm SL (3 C&S), 3 females, 18.1–20.4 mm SL, first order stream of Rio Ivinhema, 22°12'17"S, 53°27'17"W, altitude 317 m a.s.l.; T.R.F. Sinani & M.O. Bordignon, 17 Sep 2016.

**Diagnosis.** *Melanorivulus ivinhemensis* is distinguished from all other species of the *M. pictus* species group by having a yellow caudal fin with thin red bars arranged only in the median region of the fin in males (vs. red bars absent or red bars extending from the ventral or median region to the dorsal region of the caudal fin), except in *M. rutilicaudus*. Besides, it is distinguished from all *M. pictus* species by the origin of the anal fin at a vertical through pleural ribs of 15th-18th vertebrae (vs. 13–15 vertebrae), except in *M. amambaiensis*, *M. planaltinus*, *M. scalaris*, *M. polychromus*, *M. ofaie*, *M. interruptus* and *M. nigropunctatus*; and by the greater snout length in males (16.1–21.3 vs. 11.4–16.3), except in *M. amambaiensis*, *M. apiamici*, *M. interruptus*, *M. vittatus*, *M. polychromus* and *M. nigropunctatus*. Distinguished from *M. planaltinus*, *M. rutilicaudus*, *M. interruptus* by a lower caudal fin ray count (29–31 vs. 32–34) and from *M. amambaiensis* by a higher caudal fin ray count (29–31 vs. 25–28). Distinguished from *M. faucireticulatus*, *M. giarettai*, *M. interruptus*, *M. leali*, *M. vittatus* and *M. formosensis* by a short basihyal cartilage 10–15% of total basihyal length (vs. 20–25%). From *M. interruptus*, *M. vittatus*, *M. ofaie*, *M. nigropunctatus*, *M. proximus*, *M. linearis* and *M. nigromarginatus* it is distinguished by a smaller dorsal fin base length in females (8.9–10.4% vs. 10.5–14.8%). Distinguished from *M. giarettai*, *M. proximus*, *M. linearis*, *M. nigromarginatus* and *M. planaltinus* by lower number of gill rakers on the first branchial arch (1+7 vs 1-2+8), and from the *M. pictus* by the dorsal-fin origin on a vertical through base of 8th or 9th anal-fin ray (vs. on vertical through base of 7th anal-fin ray).

**Description.** Morphometric data are presented in Table 2. Females usually larger than males, largest female 30.4 mm SL, largest male examined 30.1 mm SL. Dorsal profile slightly convex from snout to end of dorsal-fin base, straight on caudal peduncle. Ventral profile convex from lower jaw to origin of anal-fin, approximately straight to end of caudal peduncle. Body slender, approximately cylindrical and compressed, greatest body depth anterior of

**Table 2.** Morphometric data for the holotype and paratypes of *Melanorivulus ivinhemensis* sp. n.

| | Holotype | Males (n=9) | Females (n=9) |
|---|---|---|---|
| Standard length | 30.1 | 18.6–29.5 | 18.5–30.4 |
| Percentages of standard length | | | |
| Body depth | 23.4 | 19.3–23.5 | 19.1–23.1 |
| Caudal peduncle depth | 14.3 | 12.4–14.3 | 11.7–14.0 |
| Predorsal length | 75.6 | 74.0–79.0 | 75.1–79.4 |
| Prepelvic length | 54.9 | 51.8–55.5 | 53.3–58.5 |
| Dorsal fin base length | 9.4 | 9.3–12.4 | 8.9–10.4 |
| Anal fin base length | 19.1 | 17.2–23.3 | 16.3–20.9 |
| Caudal fin length | 29.2 | 24.9–31.0 | 26.6–31.6 |
| Pectoral fin length | 17.1 | 18.4–20.8 | 17.5–20.7 |
| Pelvic fin length | 11.4 | 9.1–12.2 | 8.5–10.4 |
| Head length | 25.0 | 25.1–28.0 | 23.8–28.2 |
| Percentages of head length | | | |
| Head depth | 71.6 | 60.5–73.6 | 61.7–70.8 |
| Head width | 81.1 | 70.5–76.2 | 72.3–82.0 |
| Snout length | 17.3 | 16.1–21.3 | 12.9–20.6 |
| Lower jaw length | 20.6 | 18.3–22.0 | 16.8–21.6 |
| Eye diameter | 33.0 | 29.6–37.0 | 29.1–34.4 |

pelvic fin base, in the pelvic fin base or at the anus. Snout slightly pointed. Jaws short.

Short dorsal and anal fins. Dorsal-fin rays 7–9. Dorsal fin rounded or slightly pointed in males, rounded in females. Dorsal-fin origin on a vertical through base of 8th or 9th anal-fin ray, and between neural spines of 20th and 22th vertebrae. Anal-fin rays 12–14. Anal fin slightly pointed in males and females. Origin of anal fin at a vertical through pleural ribs of 15th-18th vertebrae. Caudal fin oval shaped, deeper than long, 29–31 rays. Pectoral fin rays 11–13. Pectoral fins rounded, with posterior margin reaching vertical at about 60–90% of the length between pectoral-fin and pelvic-fin bases. Pelvic-fin rays 6–7, one individual with one single pelvic fin. Posterior tip of pelvic fin reaching vertical between anus to 2nd anal-fin ray. Position of pelvic-fin bases variable, in close proximity, or with bases separated by a great distance, similar to the size of the base of the pelvic fin.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 15–25% of caudal-fin base. No scales on dorsal and anal-fin bases. Frontal squamation E and F-patterned or with two patterns, one on each side in one specimen. E-scales generally not overlapping medially. In one specimen E-scales marginally overlapped. Transverse row of scales anterior to H-scale; scales arranged in regular circular pattern around A-scale. A-scale usually without exposed margins, four specimens with posterior margin exposed, overlapping the B-scale. Longitudinal series of scales 29–31; transverse series of scales 8–9; scale rows around caudal peduncle 16. No contact organs on flank and fins.

Cephalic neuromasts: supraorbital 3+3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1+11–12+1, preorbital 2, otic 1, postotic 1–2, supratemporal 1, median opercular 1, ventral opercular 1–2, preopercular 2+4–6, mandibular 3–4+1, lateral mandibular 1–2, paramandibular 1. Two neuromasts on caudal-fin base.

Six branchiostegal rays. Gill rakers on first branchial arch 1+7. First epibranchial slightly curved. Total number of vertebrae 29–31, 14 precaudal vertebrae, 15–17 caudal vertebrae. Ventral process of angulo-articular short, pointed. Vomerine teeth 2–5. Dermosphenotic present. Basihyal sub-triangular, greatest width 45–50% of length; basihyal cartilage 10–15% of total basihyal length. Second pharyngobranchial teeth absent.

**Colouration in life.** Males (Fig. 5). Metallic greenish-gray flank, purple-blue close to anal fin; red dots on anterior flank area, sometimes few red dots on the anteroventral region; one to three oblique narrow bars between the medial pectoral fin portion and close to the pelvic fin base, sometimes one or two of these bars forming chevron-like marks with an angle on the ventral region of the flank; 7–9 oblique narrow red bars anteriorly directed, forming chevron-like marks with an angle on midline of flank, and generally positioned posteriorly to the pelvic fin base on the caudal peduncle. Two parallel and oblique dark brown bars often interconnected by a brown-dark horizontal line between postorbital and posterior opercular region, sometimes forming a broad and well-defined mid-lateral dark stripe between postorbital region and area above the pectoral fin at mid length. Yellowish-grey dorsum with small black dots, venter white. Dorsal portion of the head yellowish-grey sometimes with small black dots; ventral portion white; golden iridescence on opercular region. Dark grey jaws varying to pale yellowish-grey. Iris pale yellow. Dorsal fin yellow with three or five oblique narrow red bars on posterior portion of fin. Anal fin yellow or orange-yellow, sometimes with a distal dark margin, basal portion light metallic blue-whitish, posterior portion pale blue with two or three faint reddish oblique bars. Caudal fin yellow or hyaline-yellowish, presenting three to six vertical or slightly oblique parallel red or red brownish bars in the central region. Pectoral fin yellowish hyaline. Pelvic fin yellowish hyaline or orange and hyaline, sometimes with a distal dark margin.

Females (Fig. 6). Flank similar to males. Dorsum and dorsal portion of head greenish-gray with small black dots; ventral portion of head white, with black marks, sometimes inconspicuous; pale golden iridescence on opercular region. Jaws pale yellowish-grey. Iris pale yellow, sometimes with dark brown bars on anterior and posterior portions. Dorsal fin yellowish-orange, with horizontally elongated dark brown to black spots or bars in the medial region; dark grey narrow margin delineating entire dorsal fin. Anal fin yellowish-orange, basal portion pale blue with two interrupted reddish bars, posterior portion pale blue with two or three reddish oblique bars, distal region becoming gradually dark reddish brown on marginal border, distal margin with high concentration of melanophores. Caudal fin orange, sometimes orange and hyaline in central portion, with three to six dark brown to black bars, sometimes bars formed by dense, vertically elongated spots, the rays and

**Figure 5.** Colour pattern variation of male paratypes of *M. ivinhemensis* sp. n., MCP 53153, Nova Andradina municipality, Rio Ivinhema, Mato Grosso do Sul state.

inter-spaces between them pigmented; dark brown to black circular or sub triangular spot on dorso-basal portion of the caudal fin; dark grey margin. Pectoral fins hyaline. Pelvic fins orange-yellow, with a reddish-brown anterior margin.

**Distribution.** *Melanorivulus ivinhemensis* is so far only known from first order streams and small wetlands associated with both margins of the lower course of the Rio Ivinhema, Rio Paraná basin (Figure 3).

**Etymology.** The name *ivinhemensis* is a reference to the occurrence of the new species in the Rio Ivinhema basin.

**Habitat notes.** *Melanorivulus ivinhemensis* was recorded in marginal areas of small first order streams and in wetlands completely exposed to the sun on a slightly undulating terrain at altitudes ranging from 267 to 315 m a.s.l. (Fig. 7). One wetland was deep (about 70 cm of maximum depth), with muddy substratum and turbid water (Fig. 7b),

**Figure 6.** Female paratypes of *M. ivinhemensis* sp. n., MCP 53153, Nova Andradina municipality, Rio Ivinhema, Mato Grosso do Sul state.

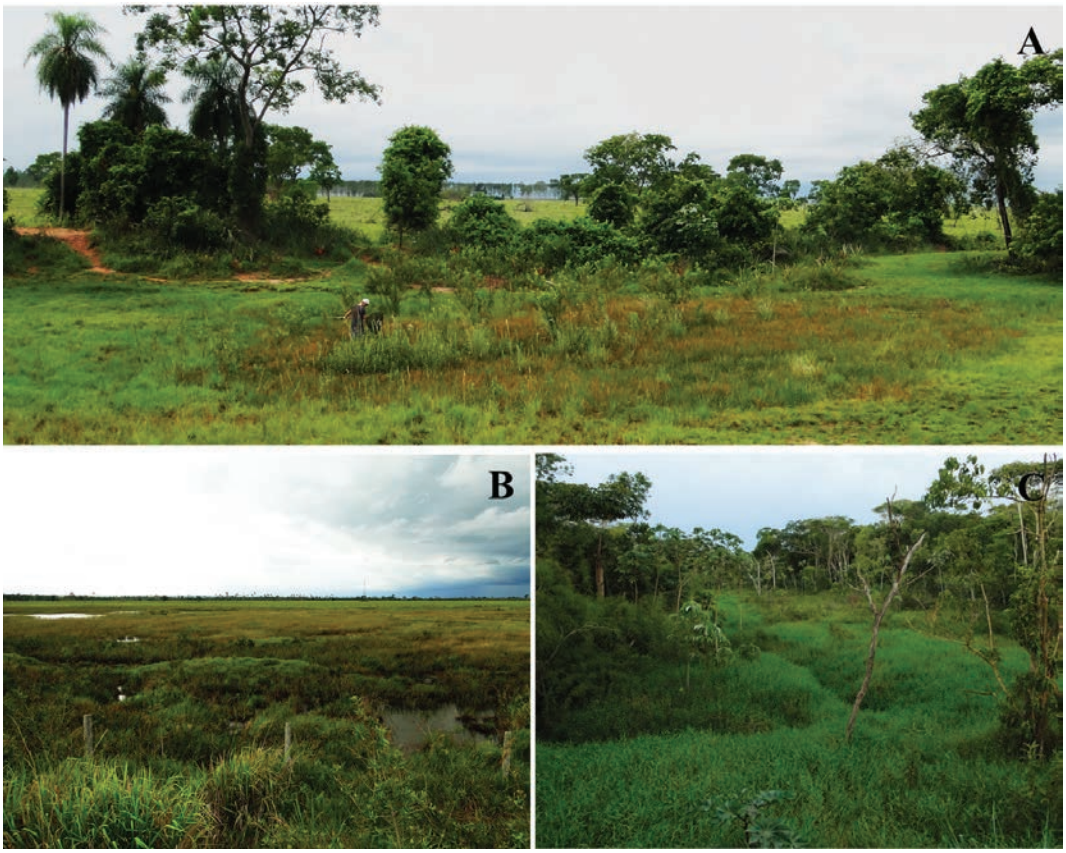


**Figure 7.** Sampling sites of *Melanorivulus ivinhemensis* sp. n. (**a**) Wetland associated with a small drainage, direct tributary of Rio Ivinhema, type locality, (**b**) wetland along BR 376 highway, (**c**) small drainage, direct tributary of Rio Ivinhema.

while in the other localities the species was recorded in shallow areas, not exceeding 20 cm depth, in crystalline waters and with a clayey substrate (Fig 7a, c). Eight fish species were recorded co-occurring with *M. ivinhemensis*: *Aphyocharax dentatus* Eigenmann & Kennedy, 1903, *Pyrrhulina australis* Eigenmann & Kennedy, 1903, *Hyphessobrycon anisitsi* (Eigenmann, 1907), *Hyphessobrycon eques* (Steindachner, 1882), *Serrapinnus kriegi* (Schindler, 1937), *Cichlasoma dimerus* (Heckel, 1840), *Hoplerythrinus unitaeniatus* (Spix & Agassiz, 1829) and *Moenkhausia sanctaefilomenae* (Steindachner, 1907).

**Conservation status.** The new species is endemic of Rio Ivinhema and was recorded in four different areas, in a range of about 25 km along both sides of the river and there is a large density of potential environments for its occurrence along this stretch. It has been recorded in areas with no major impacts – although under intense agricultural activity – and therefore there is no evidence that the species is threatened with extinction.

**Table 3.** Morphometric data for the holotype and paratypes of *Melanorivulus amambaiensis* sp. n.

| | Holotype | Males (n=5) | Females (n=11) |
|---|---|---|---|
| Standard length | 27.9 | 20.9–25.9 | 24.0–28.7 |
| Percentages of standard length | | | |
| Body depth | 21.6 | 20.4–20.9 | 19.1–21.5 |
| Caudal peduncle depth | 12.9 | 12.4–13.5 | 11.7–13.7 |
| Predorsal length | 76.1 | 74.9–79.1 | 74.5–76.4 |
| Prepelvic length | 56.8 | 53.6–55.3 | 52.0–54.5 |
| Dorsal fin base length | 12.3 | 10.1–12.0 | 9.3–11.4 |
| Anal fin base length | 21.4 | 19.1–21.4 | 18.5–20.7 |
| Caudal fin length | 27.5 | 28.1–30.7 | 26.5–28.6 |
| Pectoral fin length | 17.1 | 17.9–21.0 | 17.3–20.1 |
| Pelvic fin length | 11.3 | 9.4–12.0 | 8.5–11.6 |
| Head length | 26.5 | 26.2–26.9 | 24.6–27.6 |
| Percentages of head length | | | |
| Head depth | 69.9 | 61.6–69.5 | 62.4–71.4 |
| Head width | 75.7 | 70.5–72.4 | 70.1–79.3 |
| Snout length | 15.2 | 16.7–17.8 | 15.2–20.6 |
| Lower jaw length | 20.6 | 18.3–21.3 | 18.0–21.0 |
| Eye diameter | 28.7 | 28.5–32.1 | 28.7–33.1 |

### *Melanorivulus amambaiensis* sp. n.

http://zoobank.org/6E2F9D08-66CB-4B41-866E-3AB0F878EFAB

**Holotype.** MCP 53150, male, 27.9 mm SL, Brazil, Mato Grosso do Sul State, near of municipality of Naviraí, first order stream of Rio Amambai, margin of BR 487 highway, 22°57'11"S, 54°26'52"W, altitude 332 m a.s.l.; M.V. Volcan & L.E.K Lanés, 14 Dec 2016.

**Paratypes.** MCP 53151, 4 males, 20.9–25.9.0 mm SL (1 C&S), 10 females, 22.8–28.7 mm SL (3 C&S), all collected with the holotype. ZUFMS 5497, 1 male, 21.6 mm SL, 4 females, 22.2–22.9 mm SL, all collected with the holotype.

**Diagnosis.** The presence of an orange or orange-red anal fin with grey or dark grey distal margin (vs. no similar colour pattern) and chevron-like red bars in inverted Y-shape in the flank of males (vs. no similar pattern) distinguishes the *M. amambaiensis* from all other species of the *M. pictus* species group. Additionally, the new species is distinguished by the lower caudal fin ray count (25–28 vs. 29–34), except *M. faucireticulatus*; by the lower body depth in males (29.4–21.6 mm SL vs. 21.8–26.5), except from *M. vittatus*, *M. polychromus*, *M. nigropunctatus*, *M. interruptus* and *M. ivinhemensis*; by a lower caudal peduncle depth in males (12.4–13.5 mm SL vs. 13.5–16.8 mm SL), except in *M. polychromus*, *M. nigropunctatus*, *M. interruptus* and *M. ivinhemensis*. Females are distinguished from *M. apiamici*, *M. faucireticulatus*, *M. giarettai*, *M. planaltinus*, *M. rutilicaudus*, *M. scalaris*, *M. nigropunctatus*, *M. proximus*, *M. linearis*, *M. nigromarginatus* and *M. formosensis* by the lower body depth (19.1–21.5 mm SL vs. 21.6–26.0 mm SL); and from *M. apiamici*, *M. egens*, *M. faucireticulatus*, *M. giarettai*, *M. planaltinus*, *M. nigropunctatus*, *M. linearis*, *M. nigromarginatus* and *M. ofaie* by a shorter pre-dorsal length (74.5–76.6 mm SL vs. 76.4–83.3 mm SL).

**Description.** Morphometric data are presented in Table 3. Females larger than males, largest female examined 28.7 mm SL, largest male 27.9 mm SL. Dorsal profile slightly convex from snout to end of dorsal-fin base, straight on caudal peduncle. Ventral profile weakly convex from lower jaw to operculum. Straight from operculum to origin of pelvic-fin and in the caudal peduncle. Body slender, cylindrical and compressed, greatest body depth at origin of pelvic-fin base. Snout blunt. Jaws short.

Short dorsal and anal fins. Dorsal-fin rays 8–9. Dorsal fin slightly pointed in males, rounded in females. Dorsal-fin origin on a vertical through base of 8th or 9th anal-fin ray, and between neural spines of the 20th and 21th vertebrae. Anal-fin rays 12–14. Anal fin slightly pointed in males and females. Origin of anal fin at a vertical through pleural ribs of 15th–16th vertebrae. Caudal fin oval shaped, longer than deep, 25–28 rays. Pectoral fin rays 11–13. Pectoral fins rounded, with posterior margin reaching vertical at about 70–90% of length between pectoral-fin and pelvic-fin bases. Pelvic-fin rays 6–7. Posterior tip of pelvic fin reaching a vertical at slightly anterior to the anus to 1st anal-fin ray. Pelvic-fin bases in close proximity.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of the head. Body squamation extending over anterior 15–20% of caudal-fin base. No scales on dorsal and anal-fin bases. Frontal squamation E and F-patterned or with two patterns, one on each side (present in one specimen). E-scales not overlapping medially. Transverse row of scales anterior to H-scale; scales arranged in regular circular pattern around A-scale. A-scale without exposed margins. Longitudinal series of scales 27–31; transverse series of scales 8–9; scale rows around caudal peduncle 16. No contact organs on flank and fins.

Cephalic neuromasts: supraorbital 3+3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1+9–11+1–2, preorbital 2, otic 1, postotic 1–2, supratemporal 1,

**Figure 8.** Colour pattern variation of male paratypes of *M. amambaiensis* sp. n., MCP 53151, small stream tributary of Rio Amambaí, Mato Grosso do Sul state.

median opercular 1, ventral opercular 1–2, preopercular 2+4–5, mandibular 2–3+1, lateral mandibular 1–2, paramandibular 1. Two neuromasts on caudal-fin base.

Six branchiostegal rays. Gill rakers on first branchial arch 1+7–8. First epibranchial slightly curved. Total number of vertebrae 30–31, 13–14 precaudal vertebrae, 17 caudal vertebrae. Ventral process of angulo-articular short, pointed. Vomerine teeth 2–3. Dermosphenotic present. Second pharyngobranchial teeth absent.

**Colouration in life.** Males (Fig. 8). Flank light metallic blue-whitish, sometimes purplish above anal fin; numerous oblique narrow red bars irregularly arranged, forming reticulated chevron-like marks anteriorly directed with a branching on the lower portion in inverted Y-shape, sometimes with a branching on the upper and lower portions in X-shape; between these bars one to four red dots obliquely distributed mostly on dorsal portion of flank; reddish dots on anteroventral part of flank, sometimes forming rows, mostly between bases of the pectoral and the pelvic fin; one to three oblique reddish-brown bars between the postorbital region and the pectoral fin; reddish-brown spots on the humeral region. Dorsum metallic grey or yellowish grey, venter white. Dorsal portion of head yellowish-grey; ventral portion white, without marks or any pigmentation pattern; golden iridescence on opercular region. Jaws grey to pale yellowish-grey. Iris pale yellow. Dorsal fin yellowish-hyaline with three or four oblique narrow brownish-red or red bars; basal portion pale yellow. Anal fin orange or orange-red, basal portion pale blue with reddish dots forming reticulated pattern, posterior portion pale blue sometimes with two or three reddish oblique bars, distal region grey or dark grey on the marginal border. Caudal fin hyaline or yellowish-hy-

**Figure 9.** Colour pattern variation of females of *M. amambaiensis* (not preserved), sp. n., small stream tributary of Rio Amambaí, Mato Grosso do Sul state.

aline, yellowish-orange on ventral portion; four to seven narrow oblique brownish-red or red bars cover 2/3 of caudal fin, absent in ventral portion. Pectoral fin hyaline, or yellowish-hyaline. Pelvic fin orange or yellowish-orange, sometimes with orange-brown anterior margin.

Females (Fig. 9). Flank similar to males, but more evident reddish-brown or brown spots between pectoral and pelvic fins. Numerous oblique narrow red bars irregularly arranged, forming reticulated chevron-like marks anteriorly directed, sometimes with a branching on the lower portion in inverted Y-shape. Dorsum and dorsal portion of head greenish-gray with small black dots; ventral portion of head white, with dark grey spots; golden iridescence on opercular region. Jaws greyish-brown. Iris pale yellow, sometimes bordered with grey. Dorsal fin yellowish-orange or yellowish hyaline, with three reddish-brown narrow horizontal bars forming a reticulated pattern. Anal fin orange-yellow, basal portion pale blue with reddish dots forming a reticulated pattern, posterior portion pale blue with two or three reddish oblique bars, distal region becoming gradually dark reddish brown on the marginal border. Caudal fin yellowish-orange, with three to seven grey or dark grey bars; small elongated black spot on dorso-basal portion of the fin, sometimes overlapping the most anterior bar. Pectoral fins hyaline. Pelvic fins yellowish-orange, sometimes with reddish-brown anterior margin.

**Distribution.** The species is only known from its type locality, a small first order drainage and direct tributary of Rio Amambaí, state of Mato Grosso do Sul, Brazil (Fig. 3).

**Etymology.** The name *amambaiensis* is a reference to the occurrence of the new species in the Rio Amambaí basin.

**Habitat notes.** *Melanorivulus amambaiensis* was recorded in a small natural drainage, direct tributary of the Rio Amambaí, with depth not exceeding 50 cm and with low flow in an area totally exposed to the sun, parallel to BR 487 highway (Fig. 10). The region has a smoothly undulating terrain and the area of occurrence is at an altitude of 327 m a.s.l.. The drainage has a clayey substrate, which gives an orange colour to the water when disturbed. Only *Gymnotus* aff. *carapo* Linnaeus, 1758 was registered co-occurring with *M. amambaiensis*.

**Conservation status.** The species is known only from its type locality. It seems to be a micro endemic of the Rio Amambaí basin, since other species occur in the adjacent basins. Part of its original area was fragmented by BR 487 and there is high frequency traffic of vehicles on this highway. Consequently, the species is subject to accidents with potentially dangerous loads, which present a threat to the population of *M. amambaiensis*. In addition, the surrounding region is largely degraded by agricultural activities that also threaten its habitat quality. Thus, *M. amambaiensis* was accounted as "Vulnerable" according to the IUCN (2012) standards. The species has an area of occurrence of less than 2000 km², occurs in only one locality and suffers from a reduction in its area of occupation and in the quality of its habitat (VU B2abii, iii).

**Key to *Melanorivulus pictus* species group from the Rio Paraná, Tocantins and Araguaia basin.**

1 Red pigmentation forming chevron-like bars in the body of males ..... 2
– Red pigmentation in the flank arranged in irregular lines to form vermiculate color pattern in males ..... *Melanorivulus giarettai*
2 Pelvic fin present, 1–5 vomerine teeth ..... 3
– Absence or extreme reduction of pelvic fin, 5–7 vomerine teeth ..... *Melanorivulus planaltinus*
3 5 rays in the pelvic fin, 10 rays in the pectoral fin, 27 vertebrae ..... *Melanorivulus apiamici*
– 6–8 rays in the pelvic fin, 11–14 rays in the pectoral fin, 29–32 vertebrae ..... 4
4 Red bars present in the caudal fin ..... 5
– Red bars absence in the caudal fin ..... *Melanorivulus egens*
5 25–28 caudal fin ray, anal fin orange or orange-red in males ..... *Melanorivulus amambaiensis*
– 28–34 caudal fin ray, anal fin yellow, light yellow to orange or yellowish-hyaline in males ..... 6
6 7 scales in transversal series, oblique rows of red dots on flank, more concentrated on caudal peduncle, forming chevron-like marks ..... *Melanorivulus leali*
– 8–10 scales in transversal series, oblique red bars forming chevron-like bars along the body, with forward-pointing vertex, generally in the posterior portion of the body ..... 7
7 Chevron-like red bars interrupted on midline of flank, fragmented or vestigial in the body of adult males ..... 8
– Chevron-like red bars well defined, generally not interrupted in the body of males ..... 10
8 Dorsal fin rounded in males, 14 pectoral fin rays ..... *Melanorivulus interruptus*
– Dorsal fin pointed or slightly pointed in males, 12–13 pectoral fin rays ..... 9
9 Frontal squamation E-patterned, pectoral fin hyaline and pelvic fin light yellow in males... *Melanorivulus nigromarginatus*
– Frontal squamation F-patterned, pectoral and pelvic fin orange in males ..... *Melanorivulus nigropunctatus*
10 Red bars present only on middle of caudal fin in males ..... 11
– Caudal fin with dorsal and middle portions presenting vertical red bars ..... 12
11 10–12 dorsal fin rays, 32–34 caudal fin rays, 34–35 scales in longitudinal series, 10 scales in transversal series ..... *Melanorivulus rutilicaudus*
– 7–9 dorsal fin rays, 29–31 caudal fin rays, 29–31 scales in longitudinal series, 8–9 scales in transversal series ..... *Melanorivulus ivinhemensis*
12 Dorsal fin origin on vertical of 7th ray of anal fin, 11–12 anal fin ray, dorsal fin rounded ..... *Melanorivulus pictus*
– Dorsal fin origin on vertical of 7–10th ray of anal fin, 12–14 anal fin ray, dorsal fin pointed or slightly pointed ..... 13
13 6 pelvic fin rays, dorsal fin origin between neural spines of vertebrae 21th-22th, absence of red color in dorsal fin ..... *Melanorivulus polychromus*
– 6–7 pelvic fin rays, dorsal fin origin between neural spines of vertebrae 18th-21th, red pigmentation present in dorsal fin ..... 14
14 Frontal squamation E-patterned ..... 15
– Frontal squamation F-patterned ..... *Melanorivulus litteratus*
15 Broad sub-basal red strip on dorsal fin in males, black reticulate pattern in ventral portion of the head of females, 28–30 caudal fin rays ..... *Melanorivulus faucireticulatus*
– No distinctive broad sub-basal red strip on dorsal fin in males, sometimes black pigmentation present in ventral portion of the head of females never forming an reticulate pattern, 30–34 caudal fin rays ..... 16
16 One gill raker on upper limb of first branchial arch ..... 17
– Two gill rakers on upper limb of first branchial arch ..... *Melanorivulus proximus*
17 Red dots present on the anteroventral portion of flank, distinctive dark marks on humeral region in males ..... 18
– Red dots absent on the anteroventral portion of flank, absence of dark marks on humeral region in males ..... *Melanorivulus linearis*
18 Flank greenish blue to metallic blue, with oblique red bars forming narrow chevron-like bars, posterior tip of pelvic-fin reaching vertical at anus to 1st anal-fin ray ..... 19
– Flank intense metallic blue with broad red chevron-like oblique bars, posterior tip of pelvic-fin reaching vertical at 1st to 4th anal-fin ray ..... 20
19 8 scales in transversal series, origin of anal fin at vertical through pleural ribs of 14th–15th vertebrae, chevron-like red bars with a branching on the upper portion forming Y-shaped red marks in males ..... *Melanorivulus formosensis*
– 9–10 scales in transversal series, origin of anal fin at vertical through pleural ribs of 15th-16th vertebrae, chevron-like red bars in males never forming Y-shaped red marks ..... *Melanorivulus ofaie*
20 Small red squares in the flank aligned to form broad red chevron-like oblique bars in males, 12–13 pectoral fin rays, 2 vomerine teeth ..... *Melanorivulus scalaris*
– Broad chevron-like oblique red bars in males not forming squares, 14 pectoral fin rays, 3–4 vomerine teeth ..... *Melanorivulus vittatus*

**Figure 10.** Water source of a first-order stream on the left bank of the Rio Amambaí, marginally situated along the BR 487 highway, type locality of *M. amambaiensis*.

## Discussion

Relationships among species belonging to the *M. pictus* group are not clear. Usually there is a high overlap in meristic, morphometric and osteological characters among congeners, however with well defined, homogeneous and structured colour patterns in each species (Fig. 11). The relatively low variability of character states among species of the *M. pictus* group makes colour pattern characters an essential source to diagnose species and to es-

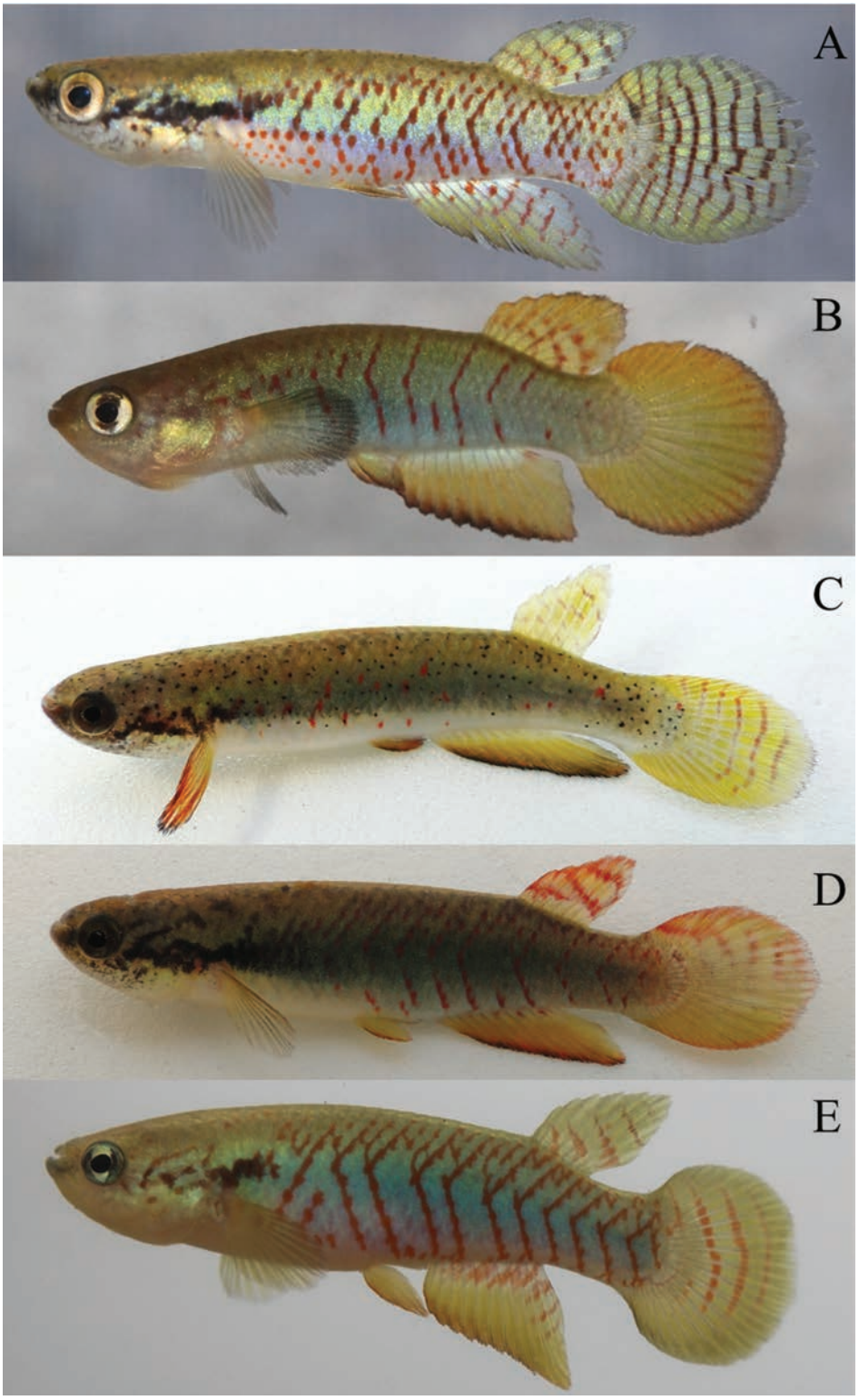


**Figure 11.** Males of different species of the *M. pictus* group recorded in the region of the right bank of Rio Paraná. **A)** *M. apiamici*; **B)** *M. egens*; **C)** *M. nigropunctatus*; **D)** *M. ofaie*; **E)** *M. scalaris*. Photos by Matheus Volcan.

timate their relationships, since molecular data are still not available for most species (Costa, 2018). The only molecular study corroborates species and groups delimitations according to colour patterns (Costa et al. 2016).

Costa (2016) discussed the importance of using live colour pattern characters to diagnose species and species groups of *Melanorivulus*. According to Costa (2016, 2017) patterns involving the caudal fin contain a high

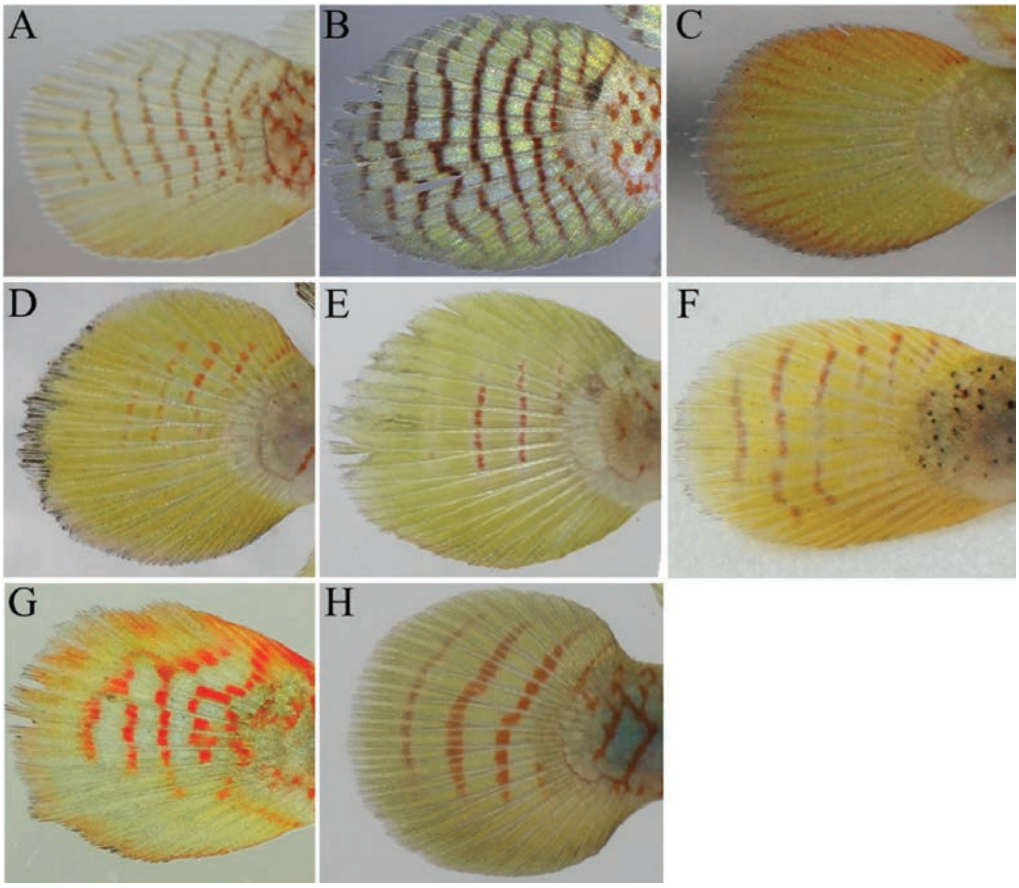


**Figure 12.** Variation of colour pattern of the caudal fin of males of different species of the *Melanorivulus pictus* group from Rio Paraná, Brazil. **A)** *M. amambaiensis*; **B)** *M. apiamici*; **C)** *M. egens*; **D)** *M. interruptus*; **E)** *M. ivinhemensis*; **F)** *M. nigropunctatus*; **G)** *M. ofaie*; **H)** *M. scalaris*.

concentration of phylogenetically informative characters, useful to delimit most species of the *M. zygonectes*, *M. pinima* and *M. dapazi* group. The same is also valid to distinguish species of the *M. pictus* group (Costa, 2018).

The three new species present distinct and unique colour patterns, mainly in relation to the pigment and arrangement of the chevron-like bars along the body, the colour pattern of the fins, as well as the position and shape of the bars in the caudal fin. Males of *M. ivinhemensis*, for example, have a yellow caudal fin, with vertical bars arranged only in the median portion of the fin, *M. interruptus* has a yellow caudal fin with diagonal red bars, sometimes vestigial and inconspicuous, extending from the median to the dorsal region, while males of *M. amambaiensis* have a hyaline or yellowish-hyaline caudal fin with yellowish-orange ventral portion and irregular brownish-red bars. This is always combined with an orange or orange-red anal fin with a dark grey margin (Fig. 12). Likewise, the colour and the arrangement pattern of vertical bars on the caudal fin, as well as the black spot in the caudal peduncle are highly variable in shape and size among females of the different species of the *M. pictus* species group (Fig. 13) and also are informative for species identification.

It is hypothesized that *M. interruptus* is closely related to *M. linearis* as judged by the absence of distinctive dark marks on the humeral region, and the absence of red dots on the anteroventral portion of the flank. With *M. egens* it shares the E-pattern of the frontal squamation, the yellow unpaired fins and the absence or reduction of red bars or red pigmentation at the base of the anal fin in males. Females of these three species also present all unpaired fins yellow with a dark grey distal margin as well as hyaline pectoral fins.

*Melanorivulus ivinhemensis* shares some characteristics with *M. rutilicaudus* and seems to be more closely related to this species. Mainly this is because both have the unpaired fins coloured yellow and red bars are present only in the middle of the caudal fin in males, a unique pattern among the species of the *M. pictus* species group. On the other hand, relationships between *M. amambaiensis* and the other species of the *M. pictus* species group are not clear because of the unique colour pattern. Chevron-like red bars with inverted Y-shaped red marks and an orange or orange-red anal fin with a dark grey distal margin in males are not encountered in any other species of the group.

Species of the *M. pictus* group generally present a high degree of endemism, occurring in small stretches of basins that drain the upper Rio Paraná and the upper Rio Araguaia and Tocantins (See Figure 3 distribution map of *M. pictus* species group) and are known for a few sites only (e.g. Costa 2005, 2007, 2008, 2018, Volcan et al.

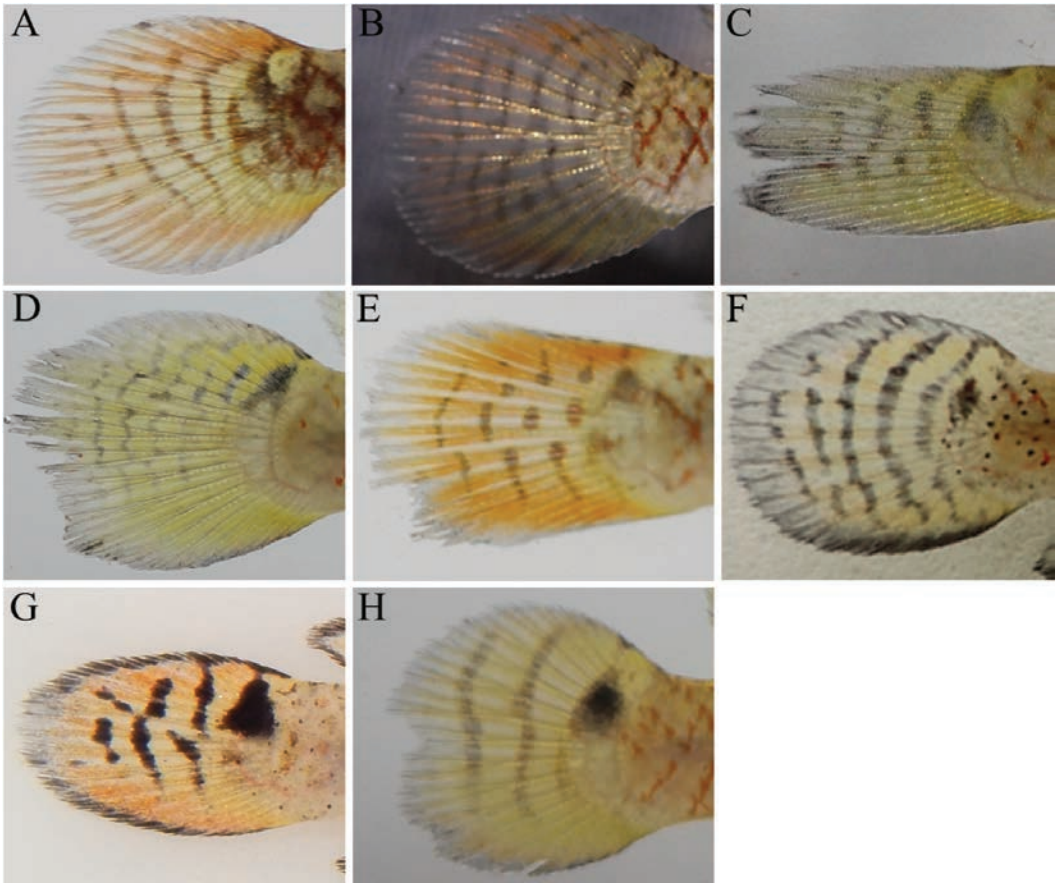


**Figure 13.** Variation of colour pattern of the caudal fin of females of different species of the *Melanorivulus pictus* specis group from Rio Paraná, Brazil. **A)** *M. amambaiensis*; **B)** *M. apiamici*; **C)** *M. egens;* **D)** *M. interruptus*; **E)** *M. ivinhemensis*; **F)** *M. nigropunctatus*; **G)** *M. ofaie*; **H)** *M. scalaris*.

2017). This pattern is observed for different *Melanorivulus* species groups (e.g. clade *M. zygonectes* and *M. dapazi*), where species are restricted to small portions of hydrographic basins in a similar way (Costa 2016, 2017). This micro endemism is extremely concerning from the conservation perspective. The loss and/or degradation of a small stretch of a river basin may result in the extinction of a species, as in the case of the vulnerable populations of *M. amambaiensis* and *M. interruptus,* which are known only from their type localities.

Most species of the *M. pictus* group are reported for highlands, mainly in the headwaters of the main tributary drainage on the right bank of the Rio Paraná (Costa 2012, 2018, Volcan et al. 2017). On the other hand, perhaps caused by insufficient sampling, there is a large gap of records in lower areas, downstream the headwaters (Figure 3). Here, only *M. apiamici* is recorded, in lower areas associated to the main channel of the Rio Paraná. From the availability of potentially suitable habitats, we would expect the presence of representatives of the *Melanorivulus pictus* species group also along the lower reaches of most of the tributaries of the Rio Paraná.

Costa (2017) suggests that the high species diversity in the Cerrado is correlated with the topography, which generated geographical isolation of populations and may explain the present distribution of distinct species of *Melanorivulus* along different altitudinal zones of river drainages. However, in addition to topography, it is observed that many of the species are restricted to small watersheds, sometimes in similar ranges of altitude, as in the case of *M. linearis* and *M. interruptus* that both are recorded in the headwaters of the Rio Pardo or *M. nigropunctatus* and *M. ofaie*, both occur on opposite sides of the Rio Verde. Thus, besides the topography, micro watersheds can also constitute barriers, suggesting that their restricted areas of distribution are a consequence of the low vagility of these species. We can assume a similar situation of high diversity and putative undiscovered species in other areas and basin tributaries of the upper Rio Paraná. These drain large areas of the Cerrado, mainly downstream from the headwaters of the main drainage formations of the upper Rio Paraná, where there is insufficient sampling of rivulid fish.

## Comparative material

All from Brazil, Mato Grosso do Sul state. *Melanorivulus nigropunctatus*: Holotype. MCP 50017, male, 24.1 mm SL, stream tributary of left margin of Rio Verde,

20°02'29"S, 53°9'42"W; B. Klotzel, 29 Dec 2013. Paratypes. MCP 50018, 5 males, 17.9–23.6 mm SL (1 C&S), 2 females, 20.7–22.7 mm SL (1 C&S), all collected with the holotype. *Melanorivulus ofaie*: Holotype. MCP 50019, male, 30.5 mm SL, stream tributary of right margin of Rio Verde, 20°25'27"S, 52°56'34"W; B. Klotzel, 29 Dec 2013. Paratypes. MCP 50022, 5 male, 19.9 mm SL (1 C&S), 6 females, 17.3–24.3 mm SL (2 C&S), collected with the holotype. MCP 50020, 3 males, 17.2–23.3 mm SL, 4 females, 16.2–19.3 mm SL, stream tributary of right margin of Rio Verde, 20°01'50"S, 53°15'14"W.; B. Klotzel, 11 Jul 2013. MCP 50021, 4 juveniles, sex undetermined, 12.8–15.6 mm SL, stream tributary of right margin of Rio Verde, 20°02'11"S, 53°15'26"W; B. Klotzel, 11 Jul 2013. ZUFMS-PIS 4736, 4 male, 21.5–23.3 mm SL, 3 females, 17.3–20.1 mm SL. same locality as holotype; B. Klotzel, 11 Jul 2013. *Melanorivulus apiamici*: ZUFMS 5500, 4 males 20.2–24.2 mm SL, 1 female, 20.8 mm SL, topotypes, Bataguassu, 21°43'32"S, 52°15'41"W; M.V. Volcan & L.E.K. Lanés, 12 Dec 2016. *Melanorivulus egens*: MCP 53152 3 males 17.8–20.6 mm SL, 1 female, 17.5 mm SL, topotypes, Camapuã, 19°19'25"S, 53°21'06"W; M.V. Volcan & L.E.K. Lanés, 12 Dec 2016. *Melanorivulus rossoi*: ZUFMS PIS 5431, 5 males 18.1–21.0 mm SL, 4 females, 18.5–19.8 mm SL, Campo Grande, 20°40'08"S, 54°45'20"W; F. Severo-Neto, 18 Jun 2015. *Melanorivulus scalaris*: ZUFMS 2114, 6 males 14.1–23.6 mm SL, 3 females, 17.7–22.1 mm SL, topotypes, Costa Rica, 18°53'57"S, 52°58'04'W; O. Froehlich, M. Casaro, N.C Penatti & L.S. Inocêncio, 31 Mar 2004. *Melanorivulus punctatus*: ZUFMS 5254, 5 males, 17.5–18.9 mm SL, 5 females, 16.1–17.8 mm SL, Corumbá, 19°34'36"S, 57°01'10"W; F. Severo-Neto, 17 Dec 2015.

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## References

Costa WJEM (1995) Pearl killifishes: The Cynolebiatinae. Systematics and Biogeography of a Neotropical annual fish subfamily (Cyprinodontiformes: Rivulidae). TFH Publications, Neptune City.

Costa WJEM (2001) The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. Ichthyological Exploration of Freshwaters 12: 333–383.

Costa WJEM (2005) Seven new species of the killifish genus *Rivulus* (Cyprinodontiformes: Rivulidae) from the Paraná, Paraguay and upper Araguaia river basins, central Brazil. Neotropical Ichthyology 3(1): 69–82. http://dx.doi.org/10.1590/S1679-62252005000100003

Costa WJEM (2007) *Rivulus illuminatus*, a new Killifish from the Serra dos Caiapós, upper rio Paraná basin, Brazil (Teleostei: Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 18(3): 193–198.

Costa WJEM (2008) *Rivulus formosensis*, a new aplocheiloid Killifish from the upper Corrente River drainage, upper rio Paraná basin, central Brazil. (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 19(1): 85–89.

Costa WJEM (2011) Phylogenetic position and taxonomic status of *Anablepsoides, Atlantirivulus, Cynodonichthys, Laimosemion* and *Melanorivulus* (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 22(3): 233-249.

Costa WJEM (2012) Two new species of *Melanorivulus* from the Caiapós hill, upper Araguaia river basin, Brazil (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 23: 211–218.

Costa WJEM (2016) Comparative morphology, phylogenetic relationships, and taxonomic revision of South American killifishes of the *Melanorivulus zygonectes* species group (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 27(2): 107–152.

Costa WJEM, Amorim PF, Rizzieri RC (2016) Molecular phylogeny and biogeography of the South American savanna killifish genus *Melanorivulus* (Teleostei: Rivulidae). Vertebrate Zoology 66(3): 267–273.

Costa WJEM (2017) Three new species of the killifish genus *Melanorivulus* from the central Brazilian Cerrado savanna (Cyprinodontiformes, Aplocheilidae). ZooKeys 645:51–70. https://doi.org/10.3897/zookeys.645.10920

Costa WJEM (2018) Three new species of the killifish genus *Melanorivulus* from the Rio Paraná Basin, central Brazilian Cerrado (Cyprinodontiformes, Aplocheilidae). Zoosystematics and Evolution 94(1): 17–27. https://doi.org/10.3897/zse.94.21321

Hoedeman JJ (1958) The frontal scalation pattern in some groups of tooth carps. Bulletin of Aquatic Biology 1: 23–28.

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, 32 pp.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501

Nielsen DTB (2017) Description of two new species of the *Melanorivulus zygonectes* species group (Cyprinodontiformes: Cynolebiidae) from Rio Xingu and Rio Tapajós basins, Brazil. Aqua 23(2): 55–67.

Ratter J, Ribeiro J, Bridgewater S (1997) The Brazilian Cerrado vegetation and threats to its biodiversity. Annals of Botany 80: 223–230. https://doi.org/10.1006/anbo.1997.0469

Severo-Neto F, Volcan MV (2018) Population dynamics of *Melanorivulus rossoi*, a restricted geographic distribution killifish species. Environmental Biology of Fishes 101(2) 245–255. https://doi.org/10.1007/s1064

Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–119.

Volcan MV, Klotzel B, Lanés LEK (2017) Two new species of *Melanorivulus* (Cyprinodontiformes: Cynolebiidae) from Rio Verde drainage, upper Rio Paraná basin, Brazil. Zootaxa 4236(1): 82–94. https://doi.org/10.11646/zootaxa.4236.1.4

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# Additional type and other notable specimens of Mollusca from the Montagu Collection in the Royal Albert Memorial Museum & Art Gallery, Exeter

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## Abstract

This paper completes the review of the Col. George Montagu collection of shells in the Royal Albert Memorial Museum & Art Gallery, Exeter. A further twenty-one lots of type material were discovered bringing the total number of type lots to ninety-four. A number of other taxa that are of historic and potential nomenclatural significance are discussed. Specimens that relate to taxa of authors other than Montagu were isolated and consideration was given to the many non-British taxa that Montagu included in his works. To complete the study a list of all species represented in this collection is given.

## Introduction

In 2017 (Oliver et al. 2017) published a catalogue of the extant type material available for molluscan species described by George Montagu in 1803, 1804, 1808, 1813 and 1816. Following on from this further curatorial work was carried out in the Royal Albert Memorial Museum & Art Gallery, primarily to investigate specimens that were described by contemporaries of Montagu notably Richard Pulteney, Edward Donovan, William Turton, Thomas Rackett and E. Mendes da Costa. Of special interest here were the many non-British species included by Montagu. During this work a number of type specimens reported as missing by Oliver et al. in 2017 were located. Given that the Montagu collection had been used as a source of display material, at least twice, and remounted during the process, since its acquisition it is not surprising that the original data had become obscured. The first part of this paper reports on these newly discovered type specimens.

The remainder of the paper analyses some notable, but not type specimens and the non-British shells extant in the Montagu collection. Many species that Montagu included had been noted by previous British authors such as E. M. da Costa (1778), Pulteney, (1799) and Donovan (1804). Our aim was to compare Montagu's specimens with the published accounts to ascertain how accurately this material had been identified and could it be used as a proxy for type material for which little now remains.

Finally we will give an overview of the entire collection as it now exists in RAMM by presenting a list comparing the names used by Montagu and their current identifications. This will give further insight into how accurate Montagu's works were.

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## Additional type specimens

The format of this section follows that adopted by Oliver et al. 2017.

Line 1 gives the original name as given by Montagu.

Line 2 gives the current name, superfamily and family placement. The synonymy of taxa generally follows MolluscaBase but not all taxa are yet included and other sources are quoted where appropriate.

Line 3 gives the full original reference

Line 4 gives the recorded localities as given by Montagu, therefore type localities. None of the original labels indicate the locality; all are subsequently inferred from literature.

Following this gives the data on available specimens, any confirmed designations and our suggested type status are given, Confirmed type material in bold font.

Wherever possible we have tried to give accurate determinations to the type material but we are not expert across the range of taxa included here. Anyone wishing to make lectotype selections is strongly advised to research the original descriptions, figures and specimens and not to rely unquestioning on this catalogue. The primary aim here is to make the collection available to research and is not definitive.

Abbreviations used in the catalogue are as follows: **EXEMS**, accepted international acronym for the Royal Albert Memorial Museum and Art Gallery in Exeter, England; **RAMM**, abbreviation of Royal Albert Memorial Museum & Art Gallery; **sh.**, a complete shell, if a bivalve then both valves present; **v.**, a single valve of a bivalve; **frag.** an incomplete and broken shell.

***arcuatum*** *Cardium* Montagu, 1803. Figure 1.
*Lucinella divaricata* (Linnaeus, 1758) [Lucinoidea, Lucinidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 85, Tab. 3 fig. 2.
Dredged in Falmouth Harbour, Cornwall.
**EXEMS Moll3744, 1v. Syntype**. Seen in RAMM by Jeffreys (1879: p.3) {"*C(ardium) arcuatum*" *Loripes divaricatus* L.}.

***avonensis*** *Mytilus* Montagu, 1803. Figure 2.
*Anodonta anatina* (Linnaeus, 1758) [Unionoidea, Unionidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 172–173, not figured.
River Avon, Wiltshire, England.
**EXEMS Moll4037-8, 2sh. Syntypes.**

***boisyi*** *Mactra* Montagu, 1803. Figure 3.
*Abra alba* (Wood, 1802) [Tellinoidea, Semelidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 98–99, Tab. 3 fig. 7.
Sandwich in Kent (from Boys), Salcombe; Biddeford; Studland.
**EXEMS Moll4287, 2sh. +3v. Syntypes.** No original labels but entered in register as "*Ligula Boysii*".

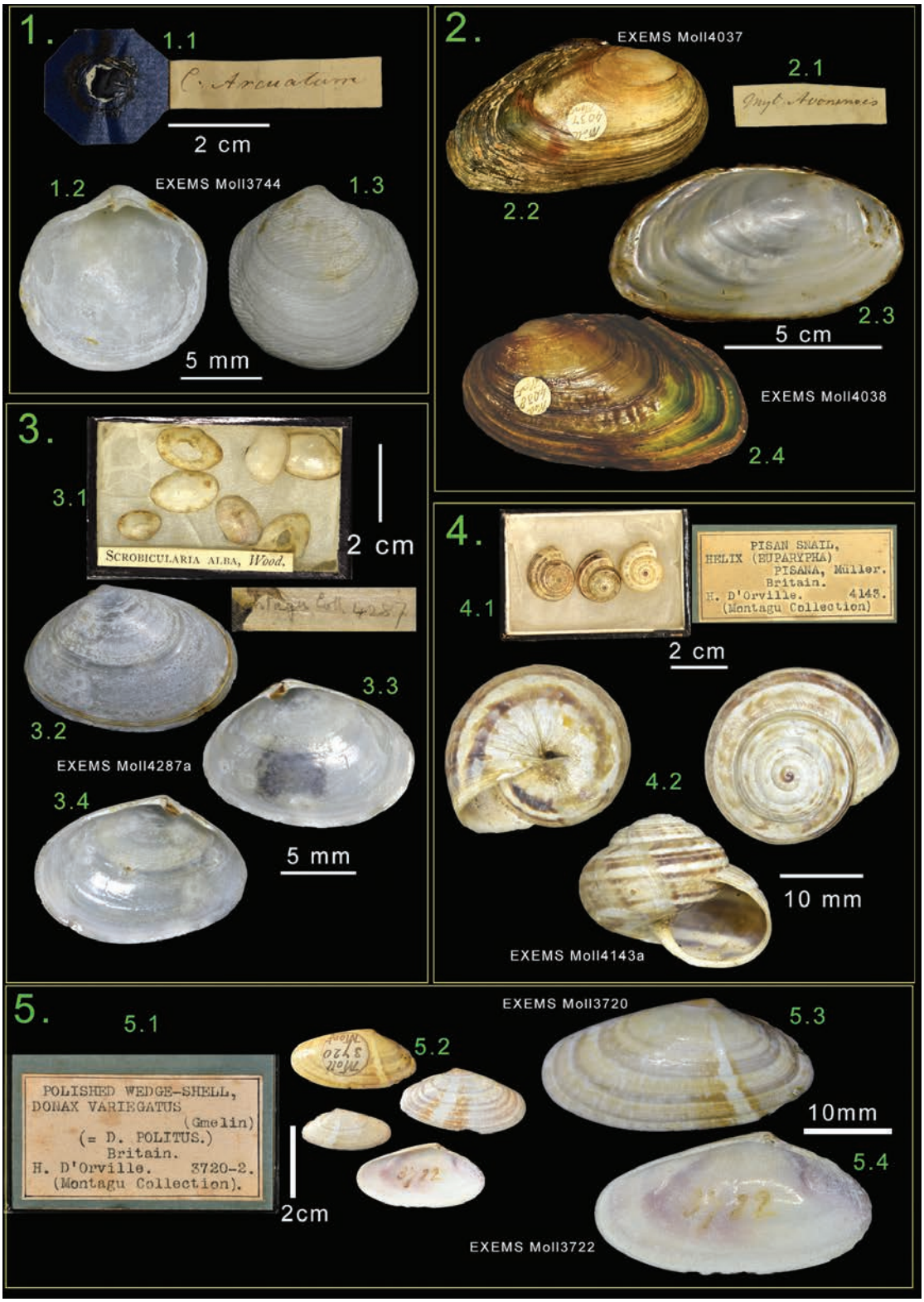
***cingenda*** *Helix* Montagu, 1803.Figure 4.
*Theba pisana* (Müller, 1774) [Helicoidea, Helicidae].
Montagu G 1803. *Test. Brit. Part 2*. p. 418–420, not figured.
Tenby.
**EXEMS Moll4143, 3sh. Syntypes**. No original labels but entered in register as "*Helix pisana, H. cingenda*".

***complanatus*** *Donax* Montagu, 1803. Figure 5.
*Donax variegatus* (Gmelin, 1791) [Tellinoidea, Donacidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 106–107, Tab. 5, fig. 4.
Milton Sands, Falmouth.
**EXEMS Moll3720-22, 2sh + 1v, Syntypes.** No original label but listed as "*D. complanatus*" in register.

***crenella*** *Helix* Montagu, 1803. Figure 6.
*Vallonia costata* (Müller, 1774) [Pupilloidea, Valoniidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 441–443, Tab. 13, fig. 3.
From Boys said to be found at Bullstrode (Lightfoot).
EXEMS Moll4149, blue card with 4 scars no shells attached.
At some time three lots of *Vallonia* were put together, two from the Montagu collection (lots 4148 and 4149) and one from the Malan collection (lot 4892). Lot 4148 carries a label '*crenella*' while lot 4149 is labelled '*paludosa*'; lot 4892 is labelled '*pulchella*'. The three cards bear 16 scars in total but no shells are attached, seven shells are present but of these only one can be associated with a card. One shell has the remains of blue paper and glue corresponding exactly with a scar on the blue '*paludosa*' card. Consequently we cannot associate any shells with the '*crenella*' card and none of the shells can be regarded as type material of that taxon.
The shell from the *paludosa* card and two others are *Vallonia excentrica* Sterki, 1893 while the other four are probably all *V. costata* (Müller, 1774) (All identified by Ben Rowson).

***diaphana*** *Bulla* Montagu 1803. Figure 7.
*Trivia arctica* (Pulteney, 1799) [Velutinoidea, Trividae].
Montagu G 1803. *Test. Brit. Part 1*. p. 225–226, Tab. 7 fig. 8.
Salcombe Bay; Falmouth.
**EXEMS Moll4311, 2 shells (1 damaged), Syntypes.** Attached to an original hexagonal mounting card. Wrongly identified as *Diaphana hyalina* (Turton) = *Diaphana minuta* Brown, 1827.

***lacuna*** *Helix* Montagu, 1803. Figure 8.
*Lacuna parva* (da Costa, 1778) [Littorinoidea, Littorinidae].




**Figures 1–5. 1.** *Cardium arcuatum* Montagu, 1803 **[***Lucinella divaricata* (Linnaeus, 1758)] **1.1** Original hexagonal, blue, card mount and label. **1.2/1.3** Internal and external views of single right valve, Syntype, EXEMS Moll3744. **2.** *Mytilus avonensis* Montagu, 1803 [*Anodonta anatina* (Linnaeus, 1758)] **2.1** Original label. **2.2** external of EXEMS Moll4037, Syntype. 2.3/2.4 external and internal of left valve of EXEMS Moll4038, Syntype. **3**. *Mactra boysii* Montagu, 1803 [*Abra alba* (Wood, 1802)] **3.1** Modern box with label indicating Montagu collection. **3.2** external of right valve and **3.3/3.4** internals of left and right valves of EXEMS Moll4287a, Syntype. **4.** *Helix cingenda* Montagu, 1803 [*Theba pisana* (Müller, 1774)]. **4.1** "Rowley" box and label containing 3 syntypes numbered 4143. **4.2** basal, apical and lateral views of EXEMS Moll4143a, Syntype. **5.** *Donax complanatus* Montagu, 1803. [*Donax variegatus* (Gmelin, 1791)]. **5.1** "Rowley" label for lot 3720-2. **5.2** 4 single valves EXEMS Moll3720–2, Syntypes. **5.3** External view of left valve, EXEMS Moll3720. **5.4** Internal view of right valve, EXEMS Moll3722

**Figures 6–10. 6.** *Vallonia* spp. **6.1** Mount with two Montagu blue hexagonal cards for *Helix crenella* Montagu, 1803 (EXEMS Moll4149) and *Helix paludosa* da Costa, 1778 (EXEMS Moll4148), no shells attached. **6.2** 3 shells identified as *Vallonia excentrica* Sterki, 1893, one can be associated with *paludosa* card (arrowed). **6.3** 4 shells tentatively identified as *Vallonia costata* (Müller, 1774). **7.** *Bulla diaphana* Montagu, 1803 [*Trivia arctica* (Pulteney, 1799)]. **7.1** "Rowley" box for lot 4311 with 2 shells, Syntypes. **7.2** Montagu hexagonal blue card mount with 1 shell attached. **7.3** Apertural and abapertural views of largest but damaged syntype, EXEMS Moll4311a. **8.** *Helix lacuna* Montagu, 1803 [*Lacuna parva* (da Costa, 1778)]. **8.1** Montagu black hexagonal card mount with 5 shells, bottom right (arrowed) is *Lacuna vincta*. **8.2** basal, apertural and abapertural view of 1 of 4 syntypes, EXEMS Moll4189. **9.** *Nautilus lacustris* Montagu, 1803 [*Segmentina nitida* (Müller, 1774)]. 9.1 "Rowley" box with blue card and 4 shells, Syntypes, EXEMS Moll4106. 9.2 Montagu blue hexagonal card mount and printed label. 9.3 apical, basal and lateral views of syntype EXEMS Moll4106a. **10.** *Helix lutea* Montagu, 1803 [*Radix balthica* (Linnaeus, 1758)]. **10.1** Old box and original hand-written label for lot 4117. **10.2** Apertural and abapertural views of unique syntype EXEMS Moll4117.

Montagu G 1803. *Test. Brit. Part 2*. p. 428–429, Tab. 13 fig. 6.
Folkstone in Kent.
**EXEMS Moll4189, 4sh. Syntypes.** Attached to a black octagonal card, 5th shell is *Lacuna vincta*.

***lacustris*** *Nautilus* Montagu, 1803. Figure 9.
*Segmentina nitida* (Müller, 1774) [Planorboidea, Planorbidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 191–195, Tab. 6 fig. 3.
Sandwich from Boys.
**EXEMS Moll4146, 4 sh. Syntypes.** Attached to an original Montagu hexagonal card mount.

***lutea*** *Helix* Montagu, 1803. Figure 10.
*Radix balthica* (Linnaeus, 1758) [Lymnaeoidea, Lymnaeidae].
Montagu G 1803. *Test. Brit. Part 2*. p. 380–381, Tab. 16 fig. 6.
South Devon; Salcombe Bay.
**EXEMS Moll4117, 1sh. Syntype.** Originally glued to a scrap of paper labelled "*lutea*" in Montagu's hand.

***novacula*** *Solen* Montagu, 1803. Figure 11
*Ensis siliqua* (Linnaeus, 1758) [Solenoidea, Pharidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 47–48, not figured.
Laugharne, Carmarthenshire.
**EXEMS Moll3706-7, 2sh. Syntypes**. 3707 with original label "*novacula*" attached. Seen by Jeffreys (1879, p.3) {"*Solen novacula*" *S. siliqua*, having the cardinal teeth broken off}.

***octanfracta*** *Helix* Montagu, 1803. Figure 12.
*Omphiscola glaber* (Müller, 1774) [Lymnaeoidea, Lymnaeidae].
Montagu G 1803. *Test. Brit. Part 2*. p. 396–398, Tab. 11 fig. 8.
Between Fowey and Looe in Cornwall.
**EXEMS Moll4121, 11sh. Syntypes.** No original label, entered in register as "*Limnaea glaber, L. octanfracta*".

***petraea*** *Helix* Montagu, 1803. Figure 13.
*Melarhaphe neritoides* (Linnaeus, 1758) [Littorinoidea, Littorinidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 403–404, not figured.
Mouth of R. Aun (Avon) at Bantum (Bantham) in Devon; Swanage.
**EXEMS Moll4195, 6sh. Syntypes.** Once attached to a Montagu hexagonal blue mounting card.

***pinna*** *Solen* Montagu, 1803. Figure 14.
*Pandora pinna* (Montagu, 1803) [Pandoroidea, Pandoridae].
Montagu G 1803. *Test. Brit. Part 2*. p. 566–567, Tab. 15 fig. 3.
Torcross.
**EXEMS Moll3703, 1sh. Possible syntype.** This shell has been remounted on a wooden block and although it carries a number from the Montagu collection there are no original labels. In the register it is listed as "*Pandora inaequivalvis* Linn" with a subscript of "*Pandora obtusa*". As the name *pinna* is not present on the labels or register we are reluctant to give type status to this shell.

***plebeia*** *Donax* Pulteney sensu Montagu, 1803. Figure 15.
*Donacilla cornea* (Poli, 1791) [Mactroidea, Mesodesmatidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 107–108, Tab. 5 fig 2.
Weymouth (from Bryer).
**EXEMS Moll4277, 7v. Syntypes.** Loose in box with original Montagu label "*plebeia*".
Although attributed to Montagu in MolluscaBase and CLEMAM this name is first used by Pulteney (1799) as stated by Montagu.

***rufa*** *Nerita* Montagu, 1803. Figure 16.
*Euspira* sp. juveniles [Naticoidea, Naticidae].
Montagu, G. 1808. *Suppl. Test. Brit.* p. 150–152, Tab. 30, fig. 3.
EXEMS Moll4190. 3sh. Attached to octagonal blue card.
Montagu described this species from a shell given to him or observed from the Laskey cabinet but the size of the shell far exceeds that of the present lot. These may be the small shells collected from Devon and mentioned by Montagu on p. 152. We would not consider these to be of type status.

***sexdentatus*** *Turbo* Montagu, 1803. Figure 17.
*Vertigo antivertigo* (Draparnaud, 1801) [Pupilloidea, Vertiginidae].
Montagu G 1803. *Test. Brit. Part 2*. p. 337–338, Tab. 12 fig. 8.
Cornwall; Devon; Lackham in Wiltshire.
EXEMS Moll4129, 8 sh. Attached to an original blue hexagonal mounting card.
The type status of these shells cannot be confirmed because they are not *Vertigo antivertigo*. All eight shells were removed from the card and their apertures cleaned and none have six apertural teeth. They are all *Vertigo pygmaea* (Draparnaud, 1801) [confirmed by Beata Pokryszko and Robert Cameron] and as such cast doubt about Montagu's ability to discriminate these small species.

***subtruncatus*** *Turbo* Montagu, 1803. Figure 18.
*Truncatella subcylindrica* (Linnaeus, 1767) [Truncatelloidea, Truncatellidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 300, Tab. 10, fig 1.

**Figures 11–15. 11.** *Solen novacula* Montagu, 1803 [*Ensis siliqua* (Linnaeus, 1758)] **11.1** External of right valve of syntype EXEMS Moll3707 with original label. **11.2** Internals of left and right valves of syntype EXEMS Moll3707. **12.** *Helix octanfracta* Montagu, 1803 [*Omphiscola glaber* (Müller, 1774)]. **12.1** "Rowley" box with 11 shells, Syntypes, EXEMS Moll4121. **12.2** Apertural and abapertural views of a single syntype, EXEMS Moll4121a. **13.** *Helix patraea* Montagu, 1803 [*Melarhaphe neritoides* (Linnaeus, 1758)]. **13.1** "Rowley" box with card and 6 shells, Syntypes, EXEMS Moll4185. **13.2** Montagu hexagonal blue card with printed label. **13.3** Apertural and abapertural views of syntype EXEMS Moll4195a. **14.** *Solen pinna* Montagu, 1803 [*Pandora pinna* (Montagu, 1803)]. **14.1** Green coloured wooden mount with single Montagu specimens attached. **14.2** Exterior of right and left valves of EXEMS Moll3703, possible syntype. **15.** *Donax plebeia* Montagu, 1803 [*Donacilla cornea* (Poli, 1791)]. **15.1** Original label and 7 shells all syntypes EXEMS Moll4277. **15.2** Internal and external views of a single right valve, EXEMS Moll4277a.

**Figures 16–21. 16.** *Nerita rufa* Montagu, 1803 [*Euspira* sp. juveniles]. **16.1** Montagu hexagonal blue card mount and hand written label with 3 shells all EXEMS Moll4190. **16.2** Abapertural, apertural and basal views of a single shell, EXEMS Moll4190a. **17.** *Turbo sexdentatus* Montagu, 1803 [*Vertigo antivertigo* (Draparnaud, 1801)]. **17.1** "Rowley" box with blue card mount and 8 shells, EXEMS Moll4129. **17.2** Apertural view of a worn shell. **17.3** Apertural and abapertural views of a fresh shell. All are *Vertigo pygmaea* (Draparnaud, 1801). **18.** *Turbo subtruncatus* Montagu, 1803 [*Truncatella subcylindrica* (Linnaeus, 1767)**].** **18.1** Montagu blue hexagonal card mount with printed label. **18.2** Larger, but broken, syntype EXEMS Moll4255b. **18.3** Smaller syntype, mostly apex EXEMS Moll5255b. **19.** *Trochus tenuis* Montagu, 1803 [*Calliostoma granulatum* (Born, 1778)]. **19.1** "Rowley" box with 3 shells, syntypes, EXEMS Moll4184. **19.2** Apertural and basal views of a single syntype, EXEMS Moll4184a. **20.** *Helix trochiformis* Montagu, 1803 [*Euconulus fulvus* (Müller, 1774)]. **20.1** Montagu blue hexagonal card and printed label with 4 shells, all syntypes, EXEMS Moll4313. **20.2** Basal and apertural views of a single syntype, EXEMS Moll4313a. **20.3** Scanning electron micrograph of the apex. **21.** *Helix umbilicata* Montagu, 1803 [*Pyramidula umbilicata* (Montagu, 1803)]. **21.1** "Rowley" box with card mount and 7 shells, all syntypes EXEMS Moll4147. **21.2** apertural, dasal and apical views of a single syntype, EXEMS Moll4147a.

Southampton; Salcombe.
**EXEMS Moll4255b, 1sh. + 1 broken sh. Syntypes.** In tube, once attached to Montagu blue hexagonal mounting card.

***tenuis*** *Trochus* Montagu, 1803. Figure 19.
*Calliostoma granulatum* (Born, 1778) [Trochoidea, Trochidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 275–277, Tab. 10 fig 3.
Poole, Weymouth from Pulteney.
**EXEMS Moll4184, 3sh. Syntypes.** No original labels but listed as *T. tenuis* in register.

***trochiformis*** *Helix* Montagu, 1803. Figure 20.
*Euconulus fulvus* (Müller, 1774) [Gastrodontoidea, Euconulidae].
Montagu G 1803. *Test. Brit. Part 2*. p. 427–428 Tab. 11 fig. 9.
By R. Avon; Lackham in Wiltshire.
**EXEMS Moll4313, 4 sh. Syntypes.** Attached to Montagu blue hexagonal mounting card.

***umbilicata*** *Helix* Montagu, 1803. Figure 21.
*Pyramidula umbilicata* (Montagu, 1803) [Pupilloidea, Pyramidulidae].
Montagu G 1803. *Test. Brit. Part 2*. p. 434–435 Tab. 13 fig. 2.
Tenby.
**EXEMS Moll4147, 7sh. Syntypes.** Attached to Montagu blue hexagonal mounting card but no original label. Register entry as "*Helix rupestris, H. umbilicata*".

## Notable specimens

***ambiguus*** *Turbo*, sensu Montagu ms. Figure 22.
EXEMS Moll4274, 1sh. With original Montagu label.
*Turbo ambiguus* is now considered to be a *Fossarus* and does not resemble the present shell in any way, which is an *Epitonium* and was not included in any of Montagu's publications. The origin of the view that *T. ambiguus* was an *Epitonium* is unclear although Dillwyn (1817) was of the opinion that it was similar to *E. clathrus*. In a manuscript by William Lyons of Tenby he noted that Montagu identified a shell sent by him as this species. Montagu seemed to be aware that it is a different species from the similar *Epitonium clathrus* but failed to include it. It was subsequently described as *Epitonium turtonis* (Turton, 1819).

***costatus*** *Turbo* J.Adams, 1797. Figure 25.
*Manzonia crassa* (Kanmacher, 1798) [Trochoidea, Turbinidae].
Montagu G 1803. Test. Brit. Part 2. p. 311–312, Tab. 10, fig. 3
EXEMS Moll4212, 6sh (+ 1sh *Rissoa parva*).
The descriptions and figures given by Adams in his papers (Adams 1797a,b, 1800a, b) are scant and poor. Adams' shells cannot be located and there is no evidence that Montagu ever saw them, deriving all his information from the papers. The identity of Adams' species is not always clear but Montagu's interpretations of them are among the first and may well have influenced later interpretations such as those by Dillwyn (1817) and Turton (1819).
Here we illustrate *Turbo costatus* sensu Montagu and see that it conforms to the current concept as *Manzonia crassa.*

***discrepans*** *Mytilus* Montagu, 1803 (1808) in part. Figure 23.
*Musculus niger* (Gray, 1824) [Mytiloidea, Mytilidae].
Montagu, G. 1808. *Suppl. Test. Brit*. p. 65, Tab. 26, fig. 4.
EXEMS Moll3938, 1sh.
The shells originally described by Montagu in 1803 as *M. discrepans* are *Musculus discors* but in 1808 he noted a very large shell with a dark periostracum coming from Scotland and figured this on plate 26. This shell is probably the one present here and is *Musculus niger.*

***distorta*** Ligula Montagu, 1808. Figure 24.
*Thracia convexa* (Wood, 1815) [Thracioidea, Thraciidae].
Montagu, G. 1808. *Suppl. Test. Brit.* p. 166, not figured.
Locality uncertain, may be Devon.
EXEMS Moll4288, 1v. Hinge mostly lost and valve broken in two. Original Montagu label present.
Montagu appears to have had some confusion with both *Thracia pubescens* and *Thracia distorta.* He described *Thracia distorta* in 1803 under *Mya* and considered his larger thin shells as *Ligula distorta*.
The one existing shell is *Thracia convexa* described and named by Wood some seven years later, but there is no mention of Montagu's shells by Wood (1815).

***hians*** *Mactra* Pulteney, 1799. Figure 26.
*Lutraria oblonga* (Gmelin, 1791) [Mactroidea, Mactridae].
Montagu G 1803. *Test. Brit. Part 1*. p. 101–102, not figured.
Between Truro and Falmouth.
EXEMS Moll3770-1, 2sh. (1 with original Montagu label).
Montagu (1803) considered that this species was the *magna* of da Costa and *oblonga* of Gmelin, a situation that continued until Lucas (1985) argued that *magna* of da Costa was a synonym of *lutraria* Linnaeus, a position adopted by Huber (2010). Mollus-

**Figures 22–31. 22.** *Turbo ambiguus* sensu Montagu ms [*Epitonium turtonis* (Turton, 1819)] Label, abapertural and apertural views of EXEMS Moll4274. **23.** *Mytilus discrepans* sensu Montagu 1808 [*Musculus niger* (Gray, 1824) Exterior of left valve in box with original label EXEMS Moll3938. **24.** *Ligula distorta* Montagu, 1808 [*Thracia convexa* (Wood, 1815)], Label, interior and exterior of single right valve, EXEMS Moll4288. **25.** *Turbo costatus* Adams, 1797 [*Manzonia crassa* (Kanmacher, 1798)] Montagu hexagonal blue card mount with 7 shells, furthest right is *Rissoa parva*; one enlarged, apertural view, EXEMS Moll4212. **26.** *Mactra hians* Pulteney, 1799 [*Lutraria oblonga* (Gmelin, 1791)], external and internal views of a left valve, EXEMS Moll3771. **27.** *Turbo interruptus* Adams, 1800 [*Rissoa parva* (da Costa, 1778)], Montagu black card mount with 3 shells, one enlarged abapertural view, EXEMS Moll4224. **28.** Montagu's specimens of *Pecten jacobaeus* (Linnaeus, 1758) EXEMS Moll4000. **29.** *Venus laminosa* Montagu, 1808 [*Chamelea striatula* (da Costata, 1778)], EXEMS Moll4290. **30.** *Otina ovata* (Brown, 1827) Montagu's mount and 1 remaining shells of his "new No 3". **31.** *Bulla punctalineata* Montagu ms [*Roxania utriculus* (Brocchi, 1814)], EXEMS Moll4302.

caBase, which appears to follow Huber, considers *hians* of Pulteney to be a synonym of *oblonga* but *magna* of da Costa a synonym of *lutraria*. However the figures in da Costa and Pulteney are identical, those of Pulteney being based on da Costa. This would appear to challenge both Lucas and Huber and necessitates a new look at this synonymy.

***interruptus*** *Turbo* J. Adams, 1800. Figure 27.
*Rissoa parva* (da Costa, 1778) [Rissooidea, Rissoidae].
Montagu G 1803. Test. Brit. Part 2. p 329–330, not figured.
EXEMS Moll4224, 3sh.
This is another example of an Adams species represented in the Montagu collection and as with *Turbo costatus* (above) these shells conform with current taxonomy as a smooth form *of Rissoa parva* (da Costa).

***jacobaeus*** *Pecten* Linnaeus. Figure 28.
*Pecten jacobaeus* (Linnaeus, 1758) [Pectinoidea, Pectinidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 144–145, not figured.
EXEMS Moll4000, 1sh.
Montagu included this species with little doubt although he accepted that it was rarely collected off the south of the British Isles. The single shell in RAMM is *P. jacobaeus* but this Mediterranean species is not known to live around the British Isles. The specimen is entire and in good condition and would appear to have been live collected.

***laminosa*** Venus Montagu, 1808. Figure 29.
*Chamelea striatula* (da Costa, 1778) [Veneroidea, Veneridae].
Off May Island, Firth of Forth (from Laskey)….
EXEMS Moll4290 1sh.; 4291 1sh.; 4293 2v.
Montagu in describing *Venus laminosa* noted that he found the group of *Venus cancellata* difficult. He described *Venus laminosa* in 1808 (pp. 38–40) linking it with *Venus cancellata* and using a shell from the Laskey cabinet from the Firth of Forth as the basis. Jeffreys (1863, p. 346) gives it as a var. of *Venus gallina* and from then on the name disappears from the literature and is not in MolluscaBase or CLEMAM.
In RAMM there are 3 lots marked as new on the labels but in the register two of these lots are identified as *Venus gallina* var *laminosa*.
These shells are certainly *Chamelea gallina (striatula)* but their status as types is doubtful as the type was in the Laskey cabinet. The description is of a wholly white shell with four teeth in each valve and this does not agree with the RAMM shells. Montagu stated that he had a single shell from Devonshire but was about half the size.

***Otina ovata*** (Brown, 1827). Figure 30.
EXEMS Moll4244, 1sh.
This species was described by Turton in 1819 as *Helix otis* but is present in the Montagu collection labelled “New No 3”.

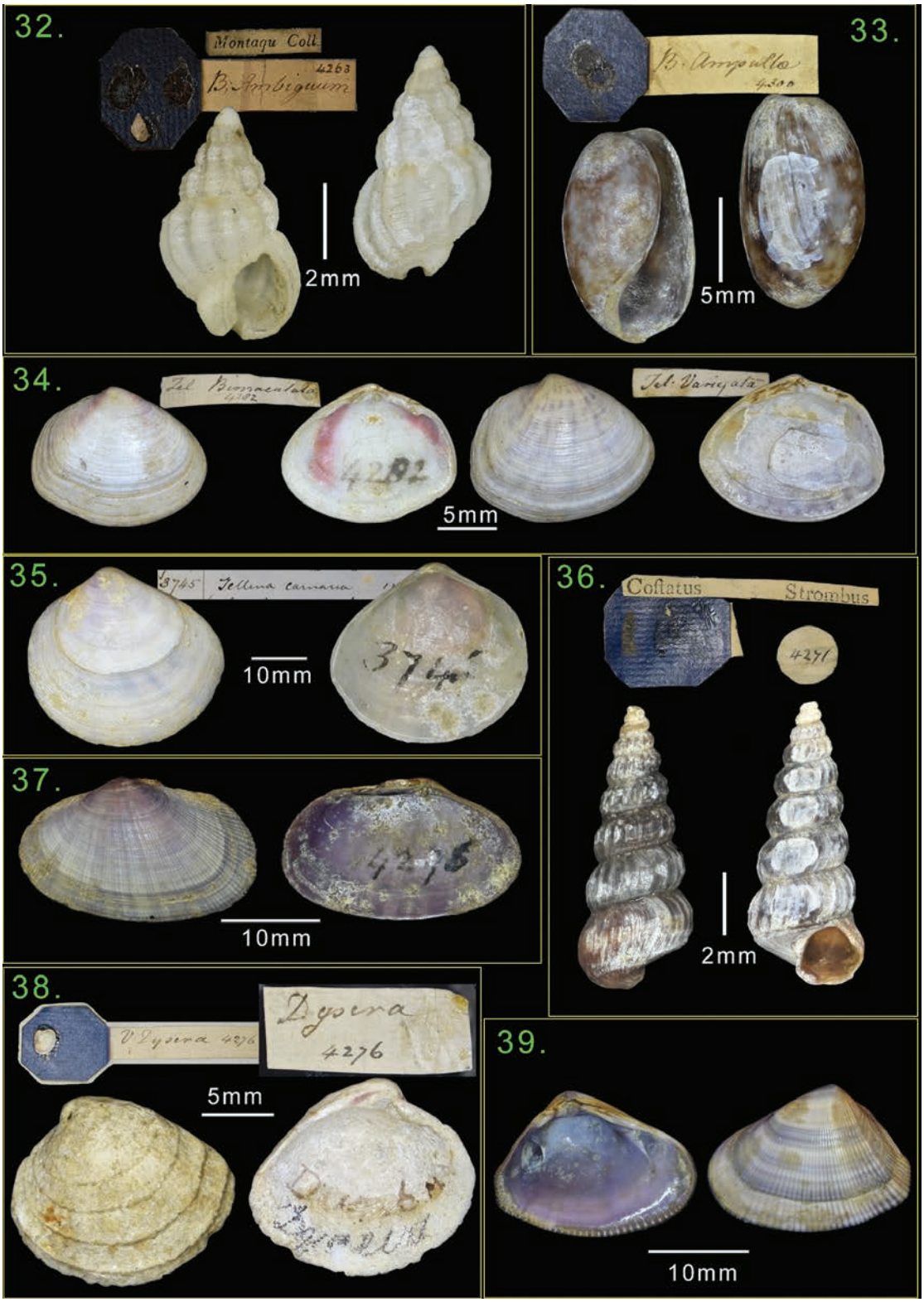
***punctalineata*** *Bulla* Montagu ms. Figure 31.
EXEMS Moll4302, 1sh.
The single shell is *Roxania utriculus* (Brocchi, 1814) but Montagu’s name was never published; if it had been it would have take priority over that of Brocchi. This is a British species.

## Exotic species

***ambiguum*** *Buccinum* Pulteney, 1799. Figure 32.
Montagu G 1803. *Test. Brit. Part 1*. p. 242–243, pl. 9 fig 7.
EXEMS Moll4263, 1sh.
This species is included and figured by Montagu and is currently regarded a synonym of the Caribbean nassariid *Phrontis antillarum* (D’Orbigny, 1847) by MolluscaBase. Jeffreys (1867, p. 353) regarded it as a synonym of the common British *Tritia incrassata* (Strøm, 1768).
There are three scars on the mounting card but only one shell is present and this is about half the size of that given by Montagu. Given the small size of the RAMM shell there is uncertainty about its identity with a consensus that it may well be a worn and juvenile *Tritia incrassata* and that it is not *Phrontis antillarum*. Montagu stated that he had shells from Pulteney but also from Mr Bryer, all from Dorset.

***ampulla*** *Bulla* Linnaeus / *Bulla striata*. Figure 33.
Montagu G 1803. *Test. Brit. Part 1*. p. 206, pl. 7 fig 1.
EXEMSMoll4300, 1sh.
This single shell is probably that described as *Bulla ampulla*; the label *ampulla* is hand written and attached to a blue card but that for *striata* is printed and probably later in date. Both names are entered in the register. *Bulla ampulla* is Indo-Pacific and *B. striata* is not British. Given the number of West Indian exotics included by Montagu it is more likely that this is *Bulla occidentalis* A. Adams, 1850, the western Atlantic sister species to *B. striata*. (Manuel Malaquias pers. comm.)

***bimaculata*** *Tellina* Linnaeus, 1758. Figure 34.
*Heterodonax bimaculatus* (Linnaeus, 1758) [Tellinoidea, Psammobiidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 69–70.
EXEMS Moll4280 (as *variegata*) 2sh. & EXEMS Moll4282 1sh.
Montagu described both under *Tellina bimaculata* and stated that he got shells from Mr Bryer collected




**Figures 32–39. 32.** *Buccinum ambiguum* Pulteney, 1799, Single remaining shell from Montagu mount is *Tritia incrassata* (Strøm, 1768), EXEMS Moll4263. **33.** *Bulla ampulla* Linnaeus, 1758 Montagu mount and original label probably now *Bulla occidentalis* A. Adams. 1850. **34.** Valves and original Montagu labels of *Tellina bimaculata* and *Tellina variegata*, both are *Heterodonax bimacu*lata (Linnaeus, 1758), EXEMS Moll4280 & 4282. **35.** *Tellina carnaria* Linnaeus, 1758 [*Strigilla carnaria* (Linnaeus, 1758)], register entry and single valve for EXEMS Moll3745. **36.** *Strombiformis costatus* da Costa, 1778 [*Cerithideopsis costata* (da Costa, 1778)], Montagu mount and single remaining shell. EXEMS Moll4271. **37.** *Venus deflorata* Linnaeus, 1758 [*Asaphis deflorata* (Linnaeus, 1758)], EXEMS Moll4296. **38.** *Venus dysera* Gmelin, 1791, Montagu mount with 1 valve and 1 loose valve marked "Dunbar" EXEMS Moll4276, probably *Chione cf. elevata* (Say, 1822). **39.** *Donax denticulatus* Linnaeus 1758, one of 2 valves from the Montagu collection EXEMS Moll3728.

between Weymouth and Portland. Both EXEMS-Moll4280 & 4282 are *Heterodonax bimaculata* (Linnaeus, 1758) a Caribbean shell.

***carnaria*** *Tellina* Linnaeus, 1758. Figure 35.
*Strigilla carnaria* (Linnaeus, 1758) [Tellinoidea, Tellinidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 73–74.
EXEMS Moll3745, 1v.
Montagu included this species but was sceptical of the reports of da Costa and Pulteney that it was common, Montagu himself having never found it. Montagu did not state where the shell he described came from. The RAMM valve is *Strigilla carnaria*, a widespread Caribbean species.

***costatus*** *Strombiformis* da Costa, 1778. Figure 36.
*Cerithideopsis costata* (da Costa, 1778) [Cerithioidea, Planaxiidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 255.
EXEMS Moll4271, 1sh.
This species is included by Montagu under *Strombus costatus* Pult. and stated that he collected it from Milton Sands in South Devon; da Costa stated that it was found in Cornwall. Montagu appears to accept that this was an English shell. Jeffreys also stated that this is the *Strombus turboformis* Montagu, 1808 but this taxon is not mentioned in MolluscaBase.
In the USNM, the Jeffreys collection contains 13 shells as from the Republic of Ireland but Jeffreys (1867) himself refuted this species as British. One of the USNM lots is labelled as from ballast. These shells may actually be from Dillwyn who went to Ireland to collect on spoil heaps but from the description in Dillwyn (1817, p. 678–9) and as Jeffreys states these are *Cerithium reticulatum*.
The shell in RAMM is the Caribbean *Cerithideopsis costata* (da Costa, 1778) and is labelled as *costatus* so cannot be taken for *turboformis*, the type of which remains unfound.

***deflorata*** *Venus* Linnaeus, 1758. Figure 37.
*Asaphis deflorata* (Linnaeus, 1758) [Tellinoidea, Psammobiidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 123, pl. 3 fig 4
EXEMS Moll4296, 1sh. + 1v.
Although numbered in the Montagu register these shells are not named. They agree entirely with Montagu's description and the smaller complete shell may be the one mentioned by Montagu as coming from Falmouth. Pennant (1777) was the first to say this was a British shell but he gave no locality.

***denticulatus*** *Donax* Linnaeus, 1758. Figure 39.
*Donax denticulatus* Linnaeus , 1758 [Tellinoidea, Donacidae].
Montagu G 1803. *Test. Brit. Part 1*. p. 104–5.
EXEMS Moll3728, 2v.
The two valves present in RAMM carry the label "*Do trunculus*" but this was changed in the register to *Donax denticulatus*. Montagu describes *D. denticulatus* and his description fits with these shells. He stated that he was given shells by Mr Bryer presumably from Weymouth. Montagu doubted it to be British, it is a West Indian species currently accepted as *Donax denticulatus* Linnaeus, 1758.

***dysera*** *Venus* Gmelin, 1791. Figure 38.
*Chione cf. elevata* (Say, 1822) [Veneroidea, Veneridae].
Montagu, G. 1808. Suppl. Test. Brit. p. 42 and p.174.
EXEMS Moll4276, 2v worn.
Two valves labelled as *dysera* are present, one labelled from Dunbar in Scotland. The Dunbar locality agrees with the locality of Firth of Forth given by Montagu and as received from Laskey (Laskey 1811). This species is included in the Supplement (1808, p. 42 and in the list of North British on p. 174). Both valves are worn and belong to the genus *Chione* but their identity is not certain although most probably *Chione elevata* Say, 1822 rather than *C. dysera* or *C. cancellata* (Peter Roopnarine pers. comm.).

***fausta*** *Tellina* Pulteney, 1799. Figure 42.
*Arcopagia fausta* (Pulteney, 1799) [Tellinoidea, Tellinidae].
Montagu G 1803. Test. Brit. Part 1. p. 64–5.
EXEMS Moll4278, 1v.
Montagu included this species without questioning its origin. Pulteney is said to have found it at Weymouth. Montagu quotes a size of 1.75 inches but the RAMM shell is very large, 2.75 inches (70 mm). It is Arcopagia (Johnsonella) fausta (Pulteney, 1799), which is Caribbean in distribution.

***fragilis*** *Solen* Pulteney, 1799. Figure 40.
*Tagelus divisus* (Spengler, 1794) [Tellinoidea, Solecurtidae].
Montagu G 1803. Test. Brit. Part 1. p. 51–2. & 1808 Suppl. Test. Brit. p.26
EXEMS Moll3716, 1v.
Considerable confusion surrounded this taxon in early years through its likeness to Solen antiquatus =Azorinus chamasolen resulting in both Maton & Rackett and Turton making incorrect references to other authors such as Pennant. The name is Pulteney's and was described by Montagu (1803, p. 51–52) who states he received a shell from Rackett collected on the Dorset coast. In 1808 (p. 26) Montagu refered to the confusion made by Rackett establishing that *fragilis* is not *antiquatus*.
The single valve in RAMM is *Tagelus divisus* (Spengler, 1794) a species from the NE coast of the USA.

**Figures 40–46. 40.** *Solen fragilis* Pulteney, 1799 [*Tagelus divisus* (Spengler, 1794)], single valve and Montagu label, EXEMS Moll3716. **41.** *Venus granulata* Gmelin 1791 [*Leukoma granulata* (Gmelin, 1791)], Single valve with Montagu label, EXEMS Moll4286. **42.** *Tellina fausta* Puteney, 1799 [*Arcopagia fausta* (Pulteney, 1799)] Montagu label and single valve, EXEMS Moll4278. **43.** *Tellina laeta* Pulteney, 1799, 2 valves with a Montagu label, EXEMS Moll4279; upper, white valve is *Eurytellina alternata* (Say, 1822); lower, pink valve is *Eurytellina cf. angulosa* (Gmelin, 1791). **44.** *Venus guineensis* Gmelin, 1791 [*Lamelliconcha circinata* (Born, 1778)], Single valve and Montagu label marked "Weymouth", EXEMS Moll4295. **45.** *Helix octona* Linnaeus, 1758 [*Subulina octona* (Bruguière, 1789)], Single shell from Montagu blue card mount attached to wooden plinth; label reads "Mr Dillwin, Bantry Bay", EXEMS Moll4275. **46**. *Buccinum lineatum* da Costa, 1778 [*Angiola lineata* (da Costa, 1778)], Montagu card mount with 2 remaining shells, one enlarged.

***granulata*** *Venus* Gmelin, 1791. Figure 41.
*Leukoma granulata* (Gmelin, 1791) [Veneroidea, Veneridae].
Montagu G 1803. Test. Brit. Part 1. p. 122–3.
EXEMS Moll4286, 1v.
This species is described by Montagu and he stated that he took it from sand in Falmouth harbour. The RAMM shell is Leukoma granulata (Gmelin, 1791) a Western Atlantic species.

***guineensis*** Venus Gmelin, 1791. Figure 44.
*Lamelliconcha circinata* (Born, 1778) [Veneroidea, Veneridae].
Montagu, G. 1808. Suppl. Test. Brit. p. 48 and p.168.
EXEMS Moll4295, 1v.
Venus guineensis was included in the Supplement (1808, p. 48) on the basis of a shell from Scotland collected by Laskey. The shell in RAMM is labelled as from Weymouth and is probably the shell mentioned on page 168 where Montagu noted a small shell from the Bryeran cabinet collected from Weymouth. The RAMM shell is Lamelliconcha circinata (Born, 1778), Venus guineensis is a synonym, a species from the West Indies..

***laeta*** *Tellina* Pulteney, 1799. Figure 43.
Montagu G 1803. *Test. Brit*. Part 1. p. 57–8.
EXEMS Moll4279, 2v.
Montagu included this species in Testacea Britannica and again had specimens from Mr Bryer collected between Poole and Weymouth. Current synonymy places this species as a junior synonym of Tellina angulosa Gmelin (1791) but we are doubtful of this for one of the valves here. The larger white specimen has the pallial sinus attached to the adductor by a thin line; following Boss (1968) this would place it as Eurytellina alternata (Say, 1822).

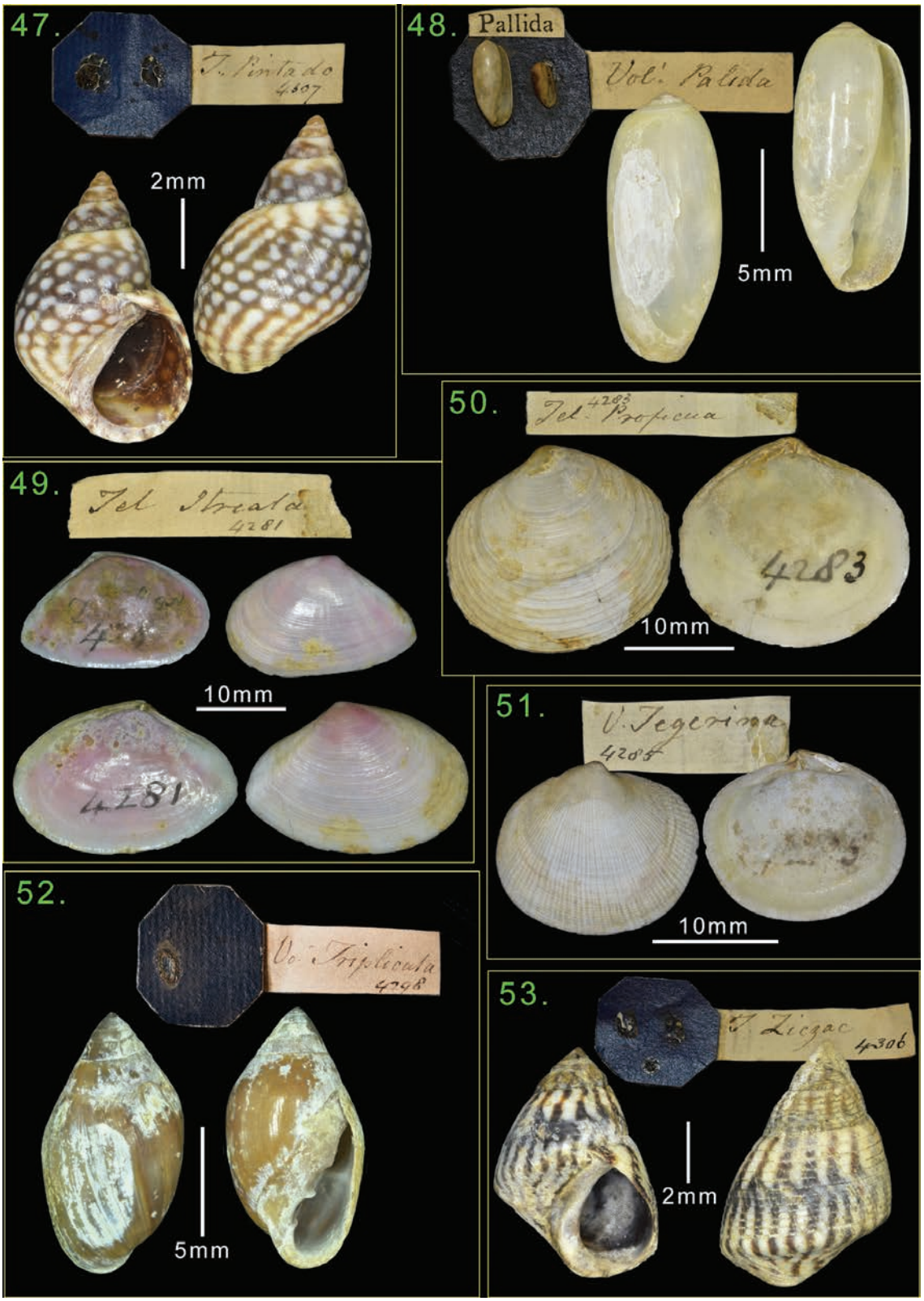
***lineatum*** *Buccinum* da Costa, 1778. Figure 46.
*Angiola lineata* (da Costa, 1778) [Cerithioidea, Planaxidae].
Montagu G 1803. Test. Brit. Part 1. p. 245–6.
EXEMS Moll4264, 2sh.
Buccinum lineatum da Costa is included in the Testacea Britannica but Montagu was initially doubtful about the inclusion of this species as British but he then stated that they collected it between Weymouth and Portland and that Mr Bryer also collected it from Weymouth. The two shells (remaining of four) now present in the RAMM collection are undoubtedly *Angiola lineata* da Costa, 1778 as currently understood. It is very doubtful that they were collected alive in the British Isles.

***octona*** *Helix* Linnaeus, 1767. Figure 45.
*Subulina octona* (Bruguière, 1789) [Achatinoidea, Achatinidae].
Montagu, G. 1808. Suppl. Test. Brit. p. 144.
EXEMS Moll4275, 1sh.
This species was introduced as British by Pulteney (1799) and restated by Maton & Rackett (1807) and then by Montagu, all referrring this taxon to Linnaeus. Montagu (1808) was reluctant to accept this species as British but it would appear that specimens were in circulation in the early 19th century. The Lyons collection, (dating from around 1810) in Tenby contains two lots both with Weymouth (from Miss Pocock) as their origin, and in keeping with the original record of Pulteney. The RAMM label gives the source as "Mr Dillwin, Bantry Bay" and this would coincide with Dillwyn's visit to Ellen Hutchins the Irish botanist in 1809. The RAMM and Tenby shells are in good condition but it is not known if they were collected from sites in the UK or from collections brought back from its native Caribbean.
Jeffreys (1864, p. 299) concluded that Pulteney had mistakenly used the name for *Omphiscola glaber* by adopting the name of *Helix octona* Pennant, 1777.

***pallida*** *Voluta or Bulla* Linnaeus, 1758. Figure 48.
*Hyalina pallida* (Linnaeus, 1758) [Marginellidae].
Montagu G 1803. Test. Brit. Part 1. p. 232–233.
EXEMS Moll4301, 2 sh.
Montagu stated that da Costa must have been mistaken in regarding it as common on west coasts and goes on to say that neither he nor Pulteney ever found it. The provenance of the two shells in RAMM is not known, the species is widespread in the Caribbean

***pintado*** Turbo Figure 47.
*Littoraria pintado* (Wood, 1828) [Littorinoidea, Littornidae].
EXEMS Moll4307, 2sh.
This species is not included in any of Montagu's publications and it is puzzling, as it was not described until 1828 by Wood. The mount and label are typical of all of the Montagu mounts in Exeter. This is the only original label in the Montagu collection that bears a species name erected after the death of Montagu. There are a number of unpublished names on similar labels and one can only surmise that this is a manuscript name adopted at a later date by Wood.
These shells have the operculum in place suggesting they were collected alive, which is unlike the majority of the exotic shells in the collection as most are worn and have some degree of incrustation upon them. It is noteworthy that the specimens of *Echinolittorina ziczac* (Gmelin, 1791) in the Montagu collections also have the operculum in place and appear live collected.

***proficua*** *Tellina* Pulteney, 1799. Figure 50.
*Semele proficua* (Pulteney, 1799) [Tellinoidea, Semelidae].




**Figures 47–53. 47.** *Turbo pintado* Wood, 1828. [*Littoraria pintado* (Wood, 1828)], Montagu card mount and label with 1 of 2 shells, EXEMS Moll4307. **48.** *Voluta pallida* Linnaeus, 1758 [*Hyalina pallida* (Linnaeus, 1758), Montagu card and label with 2 shells, larger shell cleaned, EXEMS Moll4301. **49.** *Tellina striata* Gmelin, 1791 [*Eurytellina lineata* (Turton, 1819)], Montagu label and 2 valves, upper valve marked "Dunbar", EXEMS Moll4281. **50.** *Tellina proficua* Pulteney, 1799 [*Semele proficua* (Pulteney, 1799)], Montagu label and single valve, EXEMS Moll4283. **51.** *Venus tigerina* Linnaeus, 1753, Montagu label and single valve, EXEMS Moll4285, is *Codakia orbicularis* (Linnaeus, 1785). **52.** *Voluta triplicata* Donovan, 1802 [*Tralia ovula* (Bruguière, 1789)], Montagu mount, label and single shell, EXEMS Moll4298. **53.** *Turbo ziczac* Gmelin, 1791 [*Echinolittorina ziczac* (Gmelin, 1791)], Montagu mount, label and 1 of 2 shells, EXEMS Moll4306.

Montagu G 1803. Test. Brit. Part 1. p. 66–7.
EXEMS Moll4283, 1v.
Tellina proficua Pulteney is described by Montagu and he stated that he received his shell from Rackett and that it came from Dorsetshire. Montagu's shell consists of a single left valve and is rather worn, it does agree with Semele proficua (Pulteney, 1799) as currently understood. Pulteney stated he also got his shells from Dorsetshire between Poole and Weymouth. This a Caribbean species.

***striata*** *Tellina* Gmelin. Figure 49.
*Eurytellina lineata* (Turton, 1819) [Tellinoidea. Tellinidae].
EXEMS Moll4281, 2v.
Montagu described this species in 1803, figured it in 1808 and stated he got his shells from Mr Bryer from between Poole and Weymouth. One valve however is marked "Dunbar" and this species is noted in 1808, List of North British Shells.
Boss (1966) placed Tellina striata sensu Montagu into synonymy of Tellina lineata Turton, 1819, now Eurytellina lineata (Turton, 1819) a widespread Caribbean species.

***tigerina*** *Venus* Linnaeus, 1758. Figure 51.
*Codakia orbicularis* (Linnaeus, 1785) [Lucinoidea, Lucinidae].
EXEMS Moll4285, 1v.
Montagu described and figured Venus tigerina indicating that it is a West Indian shell. The RAMM shell is Codakia orbicularis (Linnaeus, 1758) [confirmed by John Taylor pers. comm.] from the West Indies and agrees approximately in size but not in shape, so doubtfully the illustrated shell.

***triplicata*** *Voluta* Donovan, 1802. Figure 52.
*Tralia ovula* (Bruguière, 1789) [Ellobioidea, Ellobiidae].
Montagu, G. 1808. Suppl. Test. Brit. p. 99.
EXEMS Moll4298, 1sh.
Montagu included this species in the Supplement and stated that he had a shell of this species from Guernsey. A single shell is present in RAMM with an original Montagu label and card.
Martins (1996) noted that the type of triplicata could not be found and cited Montagu as the next British author to include the species in the British fauna. Dillwyn (1817) quotes directly from Montagu. Frias Martins (1996) illustrated a shell from the Turton collection identified a *triplicata* but coming from the West Indies.
The Montagu shell is *Tralia ovula* Bruguière, 1789 as confirmed by Tony Frias Martins (pers. comm.).

***ziczac*** *Turbo* Gmelin, 1791. Figure 53.
*Echinolittorina ziczac* (Gmelin, 1791) [Littorinoidea, Littorinidae].
Montagu, G. 1808. Suppl. Test. Brit. p. 135.
EXEMS Moll4306, 2sh.
The two shells in RAMM have intact operculums indicating they were live collected. Montagu included this species in his Supplement but said nothing of its provenance. It is a common Caribbean shell.

## List of taxa present in the Montagu collection in RAMM Exeter

The following is a summary of the entire Montagu collection in RAMM. The collection contains few duplicate lots and was perhaps a voucher series retained by Montagu's wife Eliza, following the sale of the major part of the collection to the then British Museum. All lots are listed including duplicates. The first line in bold text is the name as used by Montagu in his publications and in these he gave the authority but not the date, the second in normal text is our current identification using nomenclature from MolluscaBase. A number of specimens bear label names that are not present in any of Montagu's publications or only bear labels that are more recent. Type material is denoted by the prefix superscript T.

***Anomia ephippium*** **Linnaeus-**
*Anomia ephippium* Linnaeus, 1758
***Arca fusca*** **Donovan-**
*Tetracra tetragona* (Poli, 1791)
***Arca lactea*** **Linnaeus-**
*Striarca lactea* (Linnaeus, 1758)
***Arca minuta*** **Gmelin-**
*Nuculana minuta* (Müller, 1776)
***Arca nucleus*** **Linnaeus-**
*Nucula nucleus* (Linnaeus, 1758)
***Arca pilosa*** **Linnaeus-**
*Glycymeris glycymeris (*Linnaeus, 1758)
***Buccinum ambiguum*** **Pulteney-**
*Tritia incrassata* (Strøm, 1768)
***Buccinum lapillus*** **Linnaeus-**
*Nucella lapillus* (Linnaeus, 1758)
***Buccinum lineatum*** **da Costa-**
*Angiola lineata* (da Costa, 1778)
**T*Buccinum minimum* Montagu**
*Chauvetia brunnea* (Donovan, 1804)
***Buccinum reticulatum*** **Linnaeus-**
*Tritia reticulata* (Linnaeus, 1758)
**T*Buccinum terrestre* Montagu**
*Cecilioidea acicula* (Müller, 1774)
***Buccinum undatum*** **Linnaeus-**
*Buccinum undatum* Linnaeus, 1758
***Bulla akera*** **Gmelin-**
*Akera bullata* (Müller, 1776)
***Bulla ampulla*** **Linnaeus-**
*Bulla occidentalis* A.Adams, 1850
***Bulla aperta*** **Linnaeus-**
*Philine aperta* (Linnaeus, 1767)
***Bulla cylindaecea*** **Pennant-**
*Cylichna cylindracea* (Pennant, 1777)

[T]***Bulla diaphana*** **Montagu**
*Trivia arctica* (Pulteney, 1799)
***Bulla fontinalis*** **Linnaeus-**
*Physa fontinalis* (Linnaeus, 1758)
[T]***Bulla haliotoidea*** **Montagu**
*Lamellaria perspicua* (Linnaeus, 1758)
***Bulla hydatis*** **Linnaeus-**
*Haminoea hydatis* (Linnaeus, 1758)
***Bulla hypnorum*** **Linnaeus-**
*Aplexa hypnorum* (Linnaeus, 1758)
***Bulla lignaria*** **Linnaeus-**
*Scaphander lignarius* (Linnaeus, 1758)
[T]***Bulla obtusa*** **Montagu**
*Retusa obtusa* (Montagu, 1803)
[T]***Bulla plumula*** **Montagu**
*Berthella plumula* (Montagu, 1803)
***Cardium amnicum*** **Gmelin-**
*Pisidium amnicum (*Müller, 1774)
[T]***Cardium arcuatum*** **Montagu-**
*Lucinella divaricata* (Linnaeus, 1758)
***Cardium corneum*** **Linnaeus-**
*Sphaerium corneum* (Linnaeus, 1758)
***Cardium echinatum*** **Linnaeus-**
*Acanthocardia echinata* (Linnaeus, 1758)
***Cardium edule*** **Linnaeus-**
*Cerastoderma edule* (Linnaeus, 1758)
***Cardium edule*** **Linnaeus-**
*Cerastoderma edule* (Linnaeus, 1758)
[T]***Cardium elongatum*** **Montagu-**
*Cerastoderma edule* (Linnaeus, 1758)
***Cardium exiguum*** **Gmelin-**
*Parvicardium exiguum* (Gmelin, 1791)
***Cardium fasciatum*** **Montagu-**
*Parvicardium exiguum* (Gmelin, 1791)
***Cardium lacustre*** **Gmelin-**
*Musculium lacustre* (Müller, 1774)
***Cardium laevigatum*** **Linnaeus-**
*Laevicardium crassum* (Gmelin, 1791)
[T]***Cardium rubrum*** **Montagu**
*Lasaea rubra* (Montagu, 1803)
***Cardium rusticum*** **Donovan-**
*Cerastoderma edule* (Linnaeus, 1758)
***Chiton albus*** **Linnaeus-**
*Stenosemus albus* (Linnaeus, 1767)
***Chiton fascicularis*** **Linnaeus-**
*Acanthochitona fascicularis* (Linnaeus, 1767)
[T]***Cypraea bullata*** **Montagu-**
*Trivia arctica* (Pulteney, 1799)
***Cypraea europaea*** **Montagu-**
*Trivia arctica* (Pulteney, 1799)
[T]***Cypraea europaea*** **Montagu-**
*Trivia monacha* (da Costa, 1778)
[T]***Cypraea voluta*** **Montagu-**
*Erato voluta* (Montagu, 1803)
[T]***Donax castaneus*** **Montagu-**
*Ervilia castanea* (Montagu, 1803)
[T]***Donax complanatus*** **Montagu-**
*Donax variegatus* Gmelin, 1791)
***Donax denticulatus*** **Linnaeus-**
*Donax denticulatus* Linnaeus, 1758
***Donax irus*** **Linnaeus-**
*Irus irus* (Linnaeus, 1758)
[T]***Donax plebeia*** **Montagu-**
*Donacilla cornea* (Poli, 1791)
***Donax trunculus*** **Linnaeus-**
*Donax vittatus* da Costa,1778
***Haliotis tuberulata*** **Linnaeus-**
*Haliotis tuberculata* Linnaeus, 1758
***Helix alba*** **Gmelin-**
*Gyraulus albus* (Müller, 1774)
***Helix aspersa*** **Gmelin-**
*Cornu aspersum* (Müller, 1774)
***Helix auricularia*** **Linnaeus-**
*Radix auricularia* (Linnaeus, 1758)
[T]***Helix cantiana*** **Montagu-**
*Monacha cantiana* (Montagu, 1803)
[T]***Helix caperata*** **Montagu-**
*Candidula intersecta* (Poiret, 1801)
[T]***Helix cingenda*** **Montagu**
*Theba pisana* (Müller, 1774)
***Helix contorta*** **Linnaeus-**
*Bathyomphalus contortus* (Linnaeus, 1758)
***Helix cornea*** **Linnaeus-**
*Planorbarius corneus* (Linnaeus, 1758)
***Helix cristata*** **Müller-**
*Valvata cristata* (Müller, 1774)
[T]***Helix decussata*** **Montagu-**
*Zebinella decussata* (Montagu, 1803)
***Helix detrita*** **Montagu non Müller-**
*Drymaeus elongatus* (Röding, 1798)
***Helix fossaria*** **Montagu?-**
*Galba truncatula* (Müller, 1774)
***Helix gibbsii*** **ms in Leach**
*Monacha cartusiana* (Müller, 1774)
***Helix hortensis*** **Gmelin-**
*Cepaea hortensis* (Müller, 1774)
[T]***Helix labiosa*** **Montagu, 1803-**
*Rissoa membranacea* (Adams, 1800)
[T]***Helix lackhamensis*** **Montagu-**
*Ena montana* (Draparnaud, 1801)
***Helix laevigata*** **Linnaeus-**
*Velutina velutina* (Müller, 1776)
***Helix lapicida*** **Linnaeus-**
*Helicigona lapicida* (Linnaeus, 1758)
***Helix limosa*** **Linnaeus and** ***Helix peregra*** **Linnaeus –**
*Radix labiata* (Rossmässler, 1835)
***Helix lubrica*** **Gmelin-**
*Cochlicopa lubrica* (Müller, 1774)
[T]***Helix lutea*** **Montagu, 1803-**
*Radix balthica* (Linnaeus, 1758)
***Helix nautileus*** **Linnaeus-**
*Gyraulus crista* (Linnaeus, 1758)
***Helix obscura*** **Gmelin-**
*Merdigera obscura* (Müller, 1774)
[T]***Helix octanfracta*** **Montagu-**
*Omphiscola glabra* (Müller, 1774)

***Helix octona* Linn.-**
*Subulina octona* (Bruguière, 1789)
***Helix palustris* Gmelin-**
*Stagnicola palustris* (Müller, 1774)
**T*Helix petraea* Montagu-**
*Melarhaphe neritoides* (Linnaeus, 1758)
***Helix polita* Pulteney-**
*Melanella polita* (Linnaeus, 1758)
***Helix pomatia* Linnaeus-**
*Helix pomatia* Linnaeus, 1758
***Helix putris* Linnaeus-**
*Succinea putris* (Linnaeus, 1758)
***Helix radiata* da Costa-**
*Discus rotundatus* (Müller, 1774)
***Helix spinulosa* Lightfoot-**
*Acanthinula aculeata* (Müller, 1774)
***Helix spirorbis* Linnaeus-**
*Anisus spirorbis* (Linnaeus, 1758)
***Helix stagnalis* Linnaeus and *Helix fragilis* Linnaeus-**
*Lymnaea stagnalis* (Linnaeus, 1758)
**T*Helix subcarinata* Montagu-**
*Tornus subcarinatus* (Montagu, 1803)
**T*Helix trochiformis* Montagu-**
*Euconulus fulvus* (Müller, 1774)
**T*Helix umbilicata* Montagu-**
*Pyramidula umbilicata* (Montagu, 1803)
***Helix virgata* Pulteney-**
*Cernuella virgata* (da Costa, 1778)
***Helix viviparia* Linnaeus-**
*Viviparus viviparus* (Linnaeus, 1758)
***Helix vortex* Linnaeus-**
*Anisus vortex* (Linnaeus, 1758)
***Ligula distorta* Montagu-**
*Thracia convexa* (Wood, 1815)
**T*Ligula prismatica* Montagu-**
*Abra prismatica* (Montagu, 1808)
**T*Mactra boysii* Montagu-**
*Abra alba* (Wood, 1802)
**T*Mactra cinerea* Montagu-**
*Mactra stultorum* (Linnaeus, 1758)
***Mactra compressa* Pulteney-**
*Scrobicularia plana* (da Costa, 1778)
***Mactra hians* Pulteney-**
*Lutraria oblonga* (Gmelin, 1791)
***Mactra solida* Linnaeus-**
*Spisula solida* (Linnaeus, 1758)
***Mactra stultorum* Linnaeus-**
*Mactra stultorum* (Linnaeus, 1758)
***Mactra subtruncata* da Costa-**
*Spisula subtruncata* (da Costa, 1778)
**T*Mactra tenuis* Montagu-**
*Abra tenuis* (Montagu, 1803)
**T*Mactra triangularis* Montagu-**
*Goodallia triangularis* (Montagu, 1803)
**T*Mactra truncata* Montagu-**
*Spisula solida* (Linnaeus, 1758)
***Mactra truncata* Montagu-**
*Spisula subtruncata* (da Costa, 1778)
**T*Murex adversus* Montagu-**
*Marshallora adversa* (Montagu, 1803)
**T*Murex attenuatus* Montagu-**
*Mangelia attenuata* (Montagu, 1803)
***Murex corneus* Linnaeus-**
*Colus gracilis* (da Costa, 1778)
***Murex despectus* Linnaeus-**
*Neptunea antiqua* (Linnaeus, 1758)
***Murex erinaceus* Linnaeus-**
*Ocenebra erinaceus (Linnaeus, 1758)*
**TMurex gracilis Montagu-**
*Comarmondia gracilis* (Montagu, 1803)
**TMurex linearis Montagu-**
*Raphitoma linearis* (Montagu, 1803)
**TMurex muricatus Montagu-**
*Trophonopsis muricatus* (Montagu, 1803)
**TMurex nebula Montagu-**
*Bela nebula* (Montagu, 1803(
**TMurex purpureus Montagu-**
*Raphitoma purpurea* (Montagu, 1803)
***Murex reticulatus* da Costa-**
*Bittium reticulatum* (da Costa, 1778)
**T*Murex rufus* Montagu-**
*Propebela rufa* (Montagu, 1803)
**T*Murex septangularis* Montagu-**
*Haedropleura septangularis* (Montagu, 1903)
**T*Murex tubercularis* Montagu-**
*Cerithiopsis tuberularis* (Montagu, 1803)
**T*Murex turricula* Montagu-**
*Propebela turricula* (Montagu, 1803)
***Mya arenaria* Linnaeus-**
*Mya arenaria* (Linnaeus, 1758)
**T*Mya distorta* Montagu-**
*Thracia distorta* (Montagu, 1803)
**T*Mya inaequivalvis* Montagu-**
*Corbula gibba* (Olivi, 1792)
***Mya margaritifera* Linnaeus-**
*Margaritifera margaritifera* (Linnaeus, 1758)
**T*Mya ovalis* Montagu-**
*Unio tumidus* Philippson, 1788
**T*Mya pholadia* Montagu-**
*Rocellaria dubia* (Pennant, 1777)
***Mya praetenuis* Pult-**
*Cochlodesma pratenue* (Pulteney, 1799)
Mya pubescens Pulteney-
*Thracia phaseolina* (Lamarck, 1818)
***Mya pubescens* Pulteney-**
*Thracia pubescens* (Pulteney, 1799)
**T*Mya striata* Montagu-**
*Lyonsia norwegica* (Gmelin, 1791)
**T*Mya suborbicularis* Montagu-**
*Kellia suborbicularis* (Montagu, 1803)
***Mya truncata* Linnaeus-**
*Mya truncata* (Linnaeus, 1758)
***Mytilis discors* Linnaeus-**
*Musculus subpictus* (Cantraine, 1835)
***Mytilis incurvatus* Pennant-**
*Mytilus edulis* Linnaeus, 1758

***Mytilus anatinus* Linnaeus-**
*Anodonta anatina* (Linnaeus, 1758)
**[T]*Mytilus avonensis* Montagu-**
*Anodonta anatina* (Linnaeus, 1758)
***Mytilus barbatus* Linnaeus-**
*Modiolus barbatus* (Linnaeus, 1758)
**[T]*Mytilus discrepans* Montagu-**
*Musculus discors* (Linnaeus, 1767)
***Mytilus edulis* Linnaeus-**
*Mytilus edulis* Linnaeus, 1758
***Mytilus incurvatus* Pennant-**
*Mytilus edulis* Linnaeus, 1758
***Mytilus modiolus* Linnaeus-**
*Modiolus modiolus* (Linnaeus, 1758)
***Mytilus pellucidus* Pennant-**
*Mytilus edulis* Linnaeus, 1758
***Mytilus praecisus* Montagu-**
*Sphenia binghami* Turton, 1819
***Mytilus* rugosus Linnaeus-**
*Hiatella arctica* (Linnaeus, 1758)
**[T]*Nautilus lacustris* Montagu-**
*Segmentina nitida* (Müller, 1774)
***Nerita fluviatilis Linnaeus-***
*Theodoxus fluviatilis* (Linnaeus, 1758)
***Nerita glaucina* Linnaeus-**
*Euspira catena* (da Costa, 1778)
***Nerita pallidula* da Costa-**
*Lacuna pallidula* (da Costa, 1778)
**[T]*Patella apertura* Montagu-**
*Diodora graeca* (Linnaeus, 1758)
***Patella chinensis* Linnaeus-**
*Calyptraea chinensis* (Linnaeus, 1758)
***Patella fissura* Linnaeus-**
*Emarginula fissura* (Linnaeus, 1758)
***Patella fluviatilis* Gmelin-**
*Ancylus fluviatilis* Müller, 1774
***Patella lacustris* Linnaeus-**
*Acroloxus lacustris* (Linnaeus, 1758)
***Patella militaris* Linnaeus-**
*Capulus ungaricus* (Linnaeus, 1758)
***Patella pellucida* Linnaeus-**
*Patella pellucida* Linnaeus, 1758
***Pecten distortus* da Costa-**
*Talochlamys pusio (*Linnaeus, 1758)
***Pecten jacobaeus* Linnaeus-**
*Pecten jacobaeus* (Linnaeus 1758)
***Pecten maximus* Linnaeus-**
*Pecten maximus* (Linnaeus, 1758)
***Pecten obsoletus* Pennant-**
*Palliolum tigerinum* (Müller, 1776)
***Pecten opercularis* Linnaeus-**
*Aequipecten opercularis* (Linnaeus, 1758)
***Pecten varius* Linnaeus-**
*Mimachlamys varia* (Linnaeus, 1758)
***Pholas candidus* Linnaeus-**
*Barnea candida* (Linnaeus, 1758)
***Pholas crispata* Linnaeus-**
*Zirfaea crispata* (Linnaeus, 1758)
***Pholas dactylus* Linnaeus-**
*Pholas dactylus* Linnaeus, 1758
***Pholas parvus* Pennant-**
*Barnea parva* (Pennant, 1777)
***Pholas striatus* Linnaeus-**
*Martesia striata* (Linnaeus, 1758)
***Pinna pectinata* Linnaeus / *Pinna ingens* Pennant-**
*Atrina fragilis* (Pennant, 1777)
***Solen antiquatus* Pulteney-**
*Azorinus chamasolen* (da Costa, 1778)
***Solen fragilis* Pulteney-**
*Tagelus divisus* (Spengler, 1794)
***Solen legumen* Linnaeus-**
*Pharus legumen* (Linnaeus, 1758)
**[T]*Solen novacula* Montagu-**
*Ensis siliqua* (Linnaeus, 1758)
***Solen pellucidus* Pennant-**
*Phaxas pellucidus* (Pennant, 1777)
**[T]*Solen pinna* Montagu-**
*Pandora pinna* Montagu
***Solen vagina* Linnaeus-**
*Solen vagina* Linnaeus, 1758
***Solen vespertinus* Gmelin-**
*Gari depressa* (Pennant, 1777)
***Strombus costatus* da Costa-**
*Cerithideopsis costata* (da Costa, 1778)
***Strombus pes pelecani* Linnaeus-**
*Aporrhais pespelecani* (Linnaeus, 1758)
***Tellina bimaculata* Linnaeus-**
*Heterodonax bimaculatus* (Linnaeus, 1758)
***Tellina carnaria* Linnaeus-**
*Strigilla carnaria* (Linnaeus, 1758)
***Tellina crassa* Gmelin-**
*Arcopagia crassa* (Pennant, 1777)
***Tellina donacina* Linnaeus-**
*Moerella donacina* (Linnaeus, 1758)
***Tellina fabula* Gmelin-**
*Fabulina fabula* (Gmelin, 1791)
***Tellina fausta* Pulteney-**
*Arcopagia fausta* (Pulteney, 1799)
***Tellina fervensis* Gmelin-**
*Gari fervensis* (Gmelin, 1791)
**[T]*Tellina flexuosa* Montagu**
*Thyasira flexuosa* (Montagu, 1803)
***Tellina lactea* Linnaeus-**
*Loripes orbiculatus* (Poli, 1791)
***Tellina laeta* Pulteney-**
*Tellina angulosa* Gmelin, 1791 ?
**[T]*Tellina laskeyi* Montagu**
Uncertain
***Tellina proficua* Pulteney-**
*Semele proficua* (Pulteney, 1799)
**[T]*Tellina radula* Montagu**
*Lucinoma borealis* (Linnaeus, 1767)
**[T]*Tellina rotundata* Montagu-**
*Diplodonta rotundata* (Montagu, 1803)
***Tellina solidula* Pulteney-**
*Limecola balthica* (Linnaeus, 1758)

***Tellina squalida* Pulteney-**
*Bosemprella incarnata* (Linnaeus, 1758)
***Tellina striata* Gmelin-**
*Eurytellina lineata* (Turton, 1819)
***Tellina tenuis* da Costa-**
*Macomangulus tenuis* (da Costa, 1778)
***Teredo navalis* Linnaeus-**
*Nototeredo norvagica* (Spengler, 1792)
***Teredo navalis* Linnaeus-**
*Nototeredo norvagica* (Spengler, 1792)
***Trochus cinereus* da Costa-**
*Steromphala cineraria* (Linnaeus,1758)
***Trochus crassus* Pulteney-**
*Phorcus lineatus* (da Costa, 1778)
***Trochus exiguus* Pulteney-**
*Jujubinus exasperatus* (Pennant, 1777)
***Trochus magus* Linnaeus-**
*Gibbula magus* (Linnaeus, 1758)
***Trochus striatus* Linnaeus-**
*Jujubinus striatus* (Linnaeus, 1758)
T***Trochus tenuis* Montagu-**
*Calliostoma granulatum* (Born, 1778)
T***Trochus tumidus* Montagu-**
*Gibbula tumida* (Montagu, 1803)
T***Trochus umbilicatus* Montagu-**
*Steromphala umbilicalis* (da Costa, 1778)
***Trochus ziziphinus* Linnaeus-**
*Calliostoma zizyphinum* (Linnaeus, 1758)
T***Turbo biplicatus* Montagu-**
*Alinda biplicatus* (Montagu, 1803)
T***Turbo bryereus* Montagu-**
*Schwartziella bryerea* (Montagu, 1803)
T***Turbo canalis* Montagu-**
*Lacuna vincta* (Montagu, 1803)
***Turbo carychium* Müller–**
*Carychium minimum* (Müller, 1774)
T***Turbo cingillus* Montagu**
*Cingula trifasciata* (J. Adams, 1800)
***Turbo clathratulus* Turton-**
*Epitonium clathratulum* (Kanmacher, 1798)
***Turbo clathrus* Linnaeus-**
*Epitonium clathrus* (Linnaeus, 1758)
T***Turbo crassior* Montagu-**
*Lacuna crassior* (Montagu, 1803)
T**Turbo decussatus Montagu-**
*Parthenina decussata* (Montagu, 1803)
T***Turbo dispar* Montagu-**
*Littorina dispar* (Montagu, 1816)
***Turbo elegans* Gmelin-**
*Pomatius elegans* (Müller, 17774)
T***Turbo elegantissimus* Montagu-**
*Turbonilla lactea* (Linnaeus, 1758)
***Turbo fontinalis* Pulteney-**
*Valvata piscinalis* (Müller, 1774)
***Turbo interruptus* Adams-**
*Rissoa parva* (da Costa, 1778)
T***Turbo interstinctus* Adams in-Montagu-**
*Parthenina interstinctus* (Adams, 1797)
T***Turbo jugosus* Montagu-**
*Littorina saxatilis* (Olivi, 1792)
T***Turbo laminatus* Montagu-**
*Cochlodina laminata* (Montagu, 1803)
***Turbo littoreus* Linnaeus-**
*Littorina littorea* (Linnaeus, 1758)
***Turbo muscorum* Linnaeus-**
*Pupilla muscorum* (Linnaeus, 1758)
***Turbo obtusatus* Linnaeus-**
*Littorina littorea* (Linnaeus, 1758)
***Turbo parvus* da Costa-**
*Rissoa parva* (da Costa, 1778)
***Turbo pullus* Linnaeus-**
*Tricolia pullus* (Linnaeus, 1758)
T***Turbo punctura* Montagu**
*Alvania punctura* (Montagu, 1803)
T***Turbo quadrifasciatus* Montagu-**
*Lacuna vincta* (Montagu, 1803)
***Turbo ruber* Montagu not Adams-**
*Barleeia unifasciata* (Montagu, 1803)
***Turbo rudis* Donovan-**
*Littorina saxatilis* (Olivi, 1792)
***Turbo sexdentatus* Montagu-**
*Vertigo pygmaea* (Draparnaud, 1801)
T***Turbo spiralis* Montagu-**
*Spiralinella spiralis* (Montagu, 1803)
T***Turbo striatulus* Montagu-**
*Alvania carinata* (da Costa, 1778)
***Turbo striatus* Adams 1797 sensu Montagu-**
*Onoba semicostata* Montagu, 1803)
T***Turbo subtruncatus* Montagu-**
*Truncatella subcylindrica* (Linnaeus, 1767)
T***Turbo tenebrosus* Montagu-**
*Littorina saxatilis* (Olivi, 1792)
T***Turbo tenebrosus* Montagu-**
*Littorina saxatilis* (Olivi, 1792)
***Turbo terebra* Linnaeus-**
*Turritella communis* Risso, 1827
T***Turbo truncatus* Montagu-**
*Truncatella subcylindrica* (Linnaeus, 1767)
***Turbo ulvae* Pennant-**
*Peringia ulvae* (Pennant, 1777)
T***Turbo unicus* Montagu-**
*Graphis albida* (Kanmacher, 1798)
T***Turbo unidentatus* Montagu-**
*Odostomia unidentata* (Montagu, 1803)
T***Turbo unifasciatus* Montagu-**
*Barleeia unifasciata* (Montagu, 1803)
T***Turbo vinctus* Montagu-**
*Lacuna vincta* (Montagu, 1803)
T***Turbo vitreus* Montagu-**
*Hyala vitrea* (Montagu, 1803)
T***Turbo zetlandicus* Montagu-**
*Alvania zetlandica* (Montagu, 1816)
***Turbo ziczac* Gmelin-**
*Echinolittorina ziczac* (Gmelin, 1791)
***Venus aurea* Gmelin-**
*Politapes aureus* (Gmelin, 1791)

***Venus chione* Linnaeus-**
*Callista chione* (Linnaeus, 1758)
***Venus compressa* Montagu**
*Astarte sulcata* (da Costa, 1778)
***Venus decussata* Linnaeus-**
*Ruditaptus decussatus* (Linnaeus, 1758)
***Venus dysera* Gmelin-**
*Chione cf. elevata* Say, 1822
***Venus exoleta* Linnaeus-**
*Dosinia exoleta* (Linnaeus, 1758)
***Venus granulata* Gmelin-**
*Leukoma granulata* (Gmelin, 1791)
***Venus guineensis* Gmelin-**
*Lamelliconcha circinata* (Born, 1778)
***Venus islandica* Linnaeus-**
*Arctica islandica* (Linnaeus, 1767)
***Venus laminosa* Montagu-**
*Chamelea striatula* (da Costata, 1778)
***Venus laminosa* Montagu-**
*Chamelea striatula* (da Costata, 1778)
***Venus laminosa* Montagu-**
*Chamelea striatula* (da Costata, 1778)
[T]***Venus minima* Montagu-**
*Gouldia minima* (Montagu, 1803)
***Venus ovata* Pennant-**
*Timoclea ovata* (Pennant, 1777)
[T]***Venus paphia* Montagu-**
*Timoclea ovata* (Pennant, 1777)
[T]***Venus perforans* Montagu-**
*Venerupis corrugata* (Gmelin, 1791)
[T]***Venus pullastra* Montagu-**
*Venerupis corrugata* (Gmelin, 1791)
***Venus scotica* Maton & Rackett-**
*Astarte sulcata* (da Costa, 1778)
***Venus striatula* da Costa-**
*Chamelea striatula* (da Costa, 1778)
***Venus tigerina* Linnaeus-**
*Codakia orbicularis* (Linnaeus, 1785)
***Venus triangularis* Montagu**
*Gouldia minima* (Montagu, 1803)
***Venus undata* Pennant-**
*Mysia undata* (Pennant, 1777)
***Venus verrucosa* Linnaeus-**
*Venus verrucosa* Linnaeus, 1758
***Venus virginea* Linnaeus-**Montagu
confused *Politapes aurea* and *P. rhomboides*
[T]***Voluta bidentata* Montagu-**
*Auriculinella bidentata* (Montagu, 1808)
[T]***Voluta denticulata* Montagu-**
*Myosotella denticulata* (Montagu, 1803)
***Voluta pallida* Linnaeus-**
*Hyalina pallida* Linnaeus, 1758
***Voluta triplicata* Donovan-**
*Tralia ovula* (Bruguière, 1789)
**Unpublished-***Littorina* sp.
**Unpublished (new No 3)-***Otina ovata* (Brown, 1827)
**Unpublished-***Botula fusca* (Gmelin, 1791)
**Unpublished-***Epitonium turtonis* (Turton, 1819)
**Unpublished-***Eulima* sp.
**Unpublished-***Littoraria pintado* (Wood, 1828)
**Unpublished-***Polinices* sp.
**Unpublished-***Roxania utriculus* (Brocchi, 1814)
**Unpublished-***Turbonilla lactea* (Linnaeus, 1758)
**Unpublished-***Pholadidea loscombiana* Turton, 1819
**Unpublished-***Epitonium* principalis MS

## Discussion

The Montagu collection in RAMM was donated by Montagu's son Henry d'Orville in 1874 and at that time the register numbering sequence started at 3639 and ended at 4537. Rosemary Brind who compiled the first list of types (1979) noted that numbers 4135 to 4537 were missing. Oliver et al. (2017) surmised that these shells may have been retained by JG Jeffreys but could provide no evidence. Relevant to this Harriet Wood of the National Museum of Wales has uncovered a letter from JRle B Tomlin to JD Dean written in 1935. Both Tomlin and Dean were interested in historic collections and Tomlin was prompted to visit Exeter to examine their collection. At this time Tomlin also noted the missing material and in his letter clearly suggests that this was lent to JG Jeffreys but never returned. Consequently some Montagu material should be present in the Smithsonian within the Jeffreys collection. As noted in our earlier paper recognising the provenance of lots in the Jeffreys collection is difficult, as the original labels have been removed. The relevant portion of this letter is reproduced here with a transcript. It seems clear now that the missing lots will not be found in RAMM.

"Some years ago, when I was in Exeter, I took the trouble to go thoroughly into the matter of the Montagu spp..-on what Jeffreys reported in J. of Conch. II, 1–4. Unluckily the shells were all sent to J. with the help of the Curator. I ran down the entry in their records of the sending of the parcel to Jeffreys (I think it was in 1878). But there is no record of their return: the old scoundrel pinched them + no doubt they are now in Washington. I looked carefully through their collection and none of the spp mentioned in J. C. are forthcoming. I think the matter ought to be taken up and restitution demanded from the U. S. Nat. Mus. If properly and courteously done I think the things would be returned"

The remaining collection is contained in 316 lots covering register numbers 3639–4134 but no longer representing the number of shells noted in the register and it would appear that many, especially of the smaller shells, have becomed dislodged from the mounts and lost, some time prior to 1979. The majority of the shells verified by Brind in 1979 are present. The number of type bearing lots is now 92.

Although Montagu continued to publish up to his untimely death our review suggests that he was still studying shells and recognizing species not included in his earlier works, such as *Otina* and *Roxania*.

We have examined 23 non-native species included by Montagu (1803, 1808) but not described by him. Most

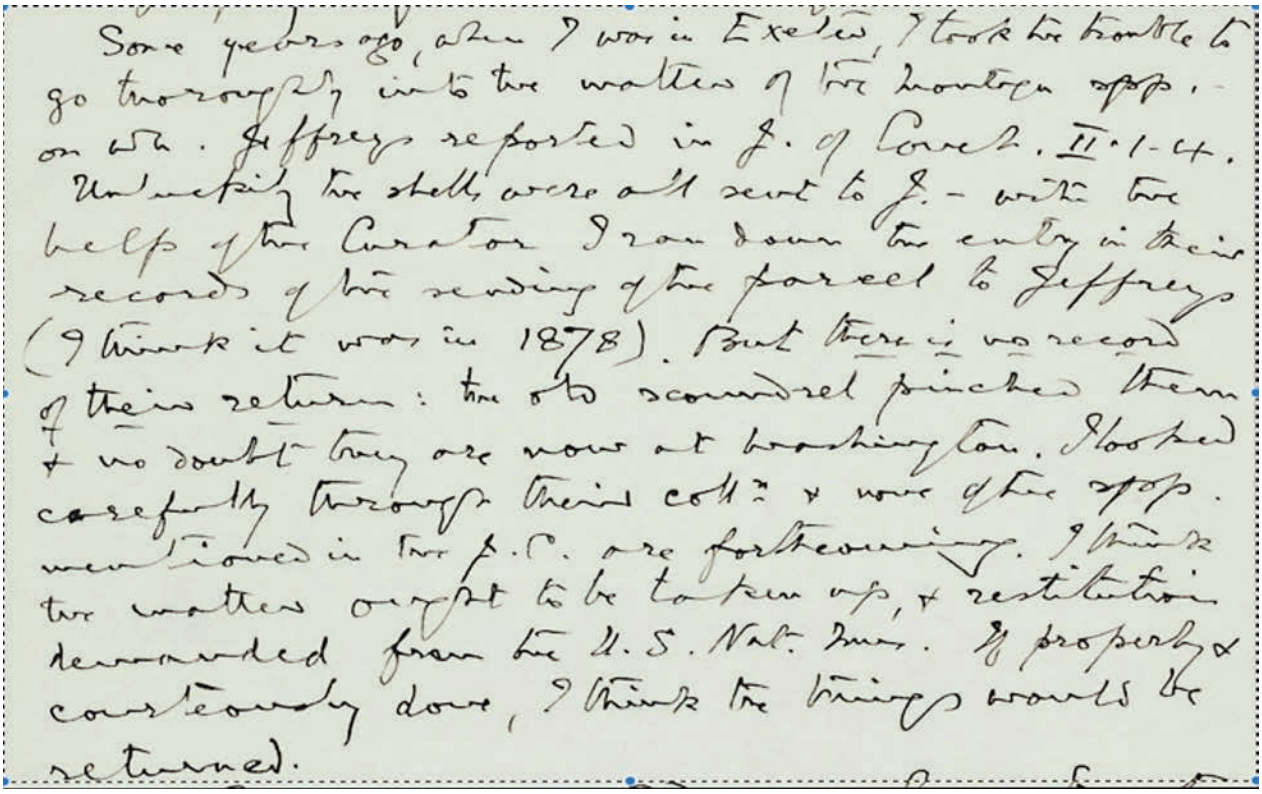

Some years ago, when I was in Exeter, I took the trouble to go thoroughly into the matter of the Montagu spp. on wh. Jeffreys reported in J. of Conch. II. 1-4. Unfortunately the shells were all sent to J. – with the help of the Curator I run down the entry in their records of the sending of the parcel to Jeffreys (I think it was in 1878). But there is no record of their return: the old scoundrel pinched them & no doubt they are now at Washington. I looked carefully through their coll.n & none of the spp. mentioned in the J.C. are forthcoming. I think the matter ought to be taken up, & restitution demanded from the U.S. Nat. Mus. If properly & courteously done, I think the things would be returned.

**Figure 54.** Part of a letter from J R Le B Tomlin to J D Dean concerning the fate of part of the Montagu collection in RAMM, Exeter; dated 25/May/1935.

were reputedly collected from the south coast of England, mainly Dorset, and many by Mr Bryer although some were given by Pulteney, Boys and Laskey.These shells for the major part have been identied correctly suggesting that there was consistency between these early collectors. The underlying issue with the non-British shells included by Montagu is not their identity their provenance as discussed by Oliver et al. (2017). Although the source as ballast was considered by Oliver et al. (2017) it was the co-occurrence of species found on the south coast of England and in Scotland that gave rise to the suspicion that the shells were not found naturally. The species are exclusively from the Caribbean region and all but two represented by empty shells showing signs of being long dead. If there had been an underhand attempt to introduce exotic species then one might expect such shells to be from numerous localities and to be in good condition. The Caribbean (West Indian) origin fits well with the bulk of the trade during the late 18th century and given the number of ships lost at this time (Boult 2003) it may not be unreasonable to assume that access to the ballast of a wrecked ship could produce this assemblage of shells. Where suspicion still arises is the co-occurrence of these species reported from Scotland by Laskey (1811) and in Montagu's (1808) list of North British Shells.

The lack of accuracy of records kept by collectors may also have added to our suspicions. For example Pulteney (1799) records *Subulina octona* from Weymouth but gives no details as to where. Weymouth is repeated by Miss Pocock for the shells sent to Lyons in Tenby and the shells collected by Dillwyn give only Bantry Bay as the locality. Nowhere is there any mention of this being a terrestrial species, typically found in hot houses. Once introduced into the British literature it would appear that such species were in demand by collectors and shells were then sourced but spuriously given the collection site as that of the original finding.

It must also be reminded that wooden shipping of the 18th century was a much different environment for possible translocation of organisms. The ships built up a much larger volume of fouling organisms enabling non-attached species such as snails to remain protected. This could explain how Montagu had two species of Caribbean littorinids with intact opercula in his collection.

The vast majority of Montagu's determinations are accurate according to the literature of the time. Montagu is honest in his admissions of being uncertain about certain taxa e.g. his confusion with species of *Thracia*. Although the nomenclature is often mistaken and Montagu was prone to introducing new names for known species he seldom splits species on minute differences. The RAMM collection is however far from complete and lacks may of the minute marine and terrestrial species such as those in the families Pyramidellidae and Vertiginidae. In some cases the extant material does not conform with the description given such as *Turbo sexdentatus* where none of the shells have six teeth and do not conform with the current concept of being a junior synonm of *Vertigo antivertigo*. This indicates that caution must be used when considering the type status of specimens and questions how consistent Montagu was in his identifications especially of these smaller species.

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## References

Adams J (1797a) The specific characters of some minute shells discovered on the coast of Pembrokeshire, with an account of a new marine animal. Transactions of the Linnean Society of London 3: 64–69. https://doi.org/10.1111/j.1096-3642.1797.tb00559.x

Adams J (1797b) Descriptions of *Actinia crassicornis* and some British shells. Transactions of the Linnean Society of London 3: 252–254. https://doi.org/10.1111/j.1096-3642.1797.tb00568.x

Adams J (1800a) Descriptions of some minute British shells. Transactions of the Linnean Society of London 5: 1–5. https://doi.org/10.1111/j.1096-3642.1800.tb00573.x

Adams J (1800b) Description of some marine animals found on the coast of Wales. Transactions of the Linnean Society of London 5: 7–13. https://doi.org/10.1111/j.1096-3642.1800.tb00574.x

Brind R (1979) The Montagu Collection of Mollusca at the Royal Albert Memorial Museum, Exeter. Biology Curators Group Newsletter 2(4): 135–137.

Boss KJ (1966) The subfamily Tellininae in the Western Atlantic. The genus *Tellina* (I). Johnsonia, 4(45): 217–272.

Boss KJ (1968) The subfamily Tellininae in the Western Atlantic. The genera *Tellina* (Part II) and *Tellidora*. Johnsonia, 4(46): 273–344.

Boult E (2003) Christian's Fleet A Dorset Shipping Tragedy.Tempus Publishing, Stroud, 160 pp.

da Costa EM (1778) Historia Naturalis Testaceorum Britanniae, or, The British Conchology. Millan, White, Elmsley & Robson, London 254 pp. [17 pls]

Dillwyn LW (1817) A descriptive catalogue of Recent shells, arranged according to the Linnean method; with particular attention to the synonymy. John and Arthur Arch., 1–580 [Vol. 1]; 581–1092 [Vol. 2], 29 pp [index].

Donovan E (1801–1804) The natural history of British shells, including figures and descriptions of all the species hitherto discovered in Great Britain, systematically arranged in the Linnean manner, with scientific and general observations on each. Printed for the author and F & C Rivington, London, vol. 1 [1800], pl. 1–36; vol. 2 [1801], pl. 37–72; vol. 3 [1801], pl. 73–108; vol. 4 [1803], pl. 109–144; vol. 5 [1804], pl. 145–180. [all with unpaginated text and indexes]

Forbes E, Hanley SC (1848–1853) A History of British Mollusca and their shells.van Voorst, London. Vol. 1: i–lxxx [1853], 1–486 [1848], pl. A-W, AA-ZZ, AAA-ZZZ [dates uncertain]; Vol. 2: 1–480 [1 dec. 1849], 481–557 [1850]; Vol. 3: 1–320 [1850], 321–616 [1851]; Vol. 4: 1–300 [1852], pl. 1–114F. [dates uncertain]

Frias Martins (AM 1996) Anatomy and systematics of the Western Atlantic Ellobiidae (Gastropoda: Pulmonata). Malacologia 37(2): 163–332.

Huber M (2010) Compendium of bivalves. A full-color guide to 3,300 of the world's marine bivalves. A status on Bivalvia after 250 years of research. Hackenheim: ConchBooks. 901 pp. [1 CD-ROM]

Jeffreys JG (1830) A synopsis on the testaceous pneumonobranchous Mollusca of Great Britain. Transactions of the Linnean Society of London 16: 323–392. https://doi.org/10.1111/j.1095-8339.1829.tb00139.x

Jeffreys JG (1833) A supplement to the 'Synopsis of testaceous pneumonobranchous Mollusca of Great Britain'. Transactions of the Linnean Society of London 16: 505–524. https://doi.org/10.1111/j.1095-8339.1829.tb00153.x

Jeffreys JG (1862–1869) British Conchology. van Voorst, London, Vol. 1: pp. CXIV + 341 [1862]. Vol. 2: pp. 479 [1864]. Vol. 3: pp. 394 [1865]. Vol. 4: pp. 487 [1867]. Vol. 5: pp. 259 [1869]

Jeffreys JG (1879) Notes on Colonel Montagu's collection of British shells. Journal of Conchology 2: 1–4

Laskey J (1811) Account of the North British Testacea. Memoirs of the Wernerian Natural History Society 1(18): 370–416.

Lucas M (1985) On the application of the law of priority to the name of a European species of *Lutraria*. La Conchiglia 17 (192/193), 6–8.

Maton WG, Rackett T (1807) A descriptive catalogue of the British Testacea. Transactions of the Linnean Society of London 8: 17–250. https://doi.org/10.1111/j.1096-3642.1807.tb00304.x

MolluscaBase (2018) Accessed at http://www.molluscabase.org [accessed on 2018–04–11]

Montagu G (1803) Testacea Britannica or Natural History of British Shells, Marine, Land, and Fresh-Water, Including the Most Minute: Systematically Arranged and Embellished with Figures. J White, London, Vol. 1: xxxvii, 1–291; Vol. 2: 293–606.

Montagu G (1804) Description of several marine animals found on the south coast of Devonshire. Transactions of the Linnean Society, London, 7: 61–85. https://doi.org/10.1111/j.1096-3642.1804.tb00282.x

Montagu G (1808) Description of several marine animals found on the south coast of Devonshire. Transactions of the Linnean Society of London 9: 81–114. https://doi.org/10.1111/j.1096-3642.1818.tb00327.x

Montagu G (1808) Supplement to Testacea Britannica with Additional Plates. Woolmer, Exeter, 183 pp. [pl. 17–30]

Montagu G (1813) Descriptions of several new or rare animals, principally marine, discovered on the South coast of Devonshire. Transactions of the Linnean Society of London 11(1): 1–26. https://doi.org/10.1111/j.1096-3642.1813.tb00035.x

Montagu G (1816) An account of some new and rare marine British shells and animals. Transactions of the Linnean Society of London 11(2): 179–204. https://doi.org/10.1111/j.1096-3642.1813.tb00047.x

Oliver PG, Morgenroth H, Salvador A (2017) Type specimens of Mollusca described by Col. George Montagu in the Royal Albert Memorial Museum & Art Gallery, Exeter and The Natural History Museum, London. Zoosystematics and Evolution 93(2): 363–412. https://doi.org/10.3897/zse.93.13073

Pulteney R. (1799) Catalogue of the birds, shells, and some of the more rare plants of Dorsetshire. J. Nichols, London, 92 pp.

Turton W (1819) A Conchological Dictionary of the British Islands. J. Booth, London, xxvii, 1–272 pp. [28 pls]

Wood W (1815) General conchology or a description of shells, arranged according to the Linnean system. John Booth, London, Vol. 1: lxi, 7, 1–246. [60 pl]

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# Taxonomic study of the leafmining genus *Liocrobyla* Meyrick, 1916 from China (Lepidoptera, Gracillariidae, Ornixolinae) with a description of one new species

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## Abstract

Three species of the leafmining genus *Liocrobyla* Meyrick, 1916 from China are treated. *Liocrobyla indigofera* **sp. n.** feeding on the plant genus *Indigofera* is described as new to science. *Liocrobyla lobata* Kuroko, 1960 feeding on *Pueraria montana* var. *lobata* is newly recorded in China. *Lespedeza bicolor* is documented as a new host plant for *L. desmodiella* Kuroko, 1982. Photographs of adult habitus, male and female genitalia, as well as host plants, leaf mines and biology are provided.

## Key Words

biology
distribution
host plant
new record
new taxon

## Introduction

The genus *Liocrobyla* Meyrick, 1916 belongs to the subfamily Ornixolinae Kuznetzov & Baryshnikova, 2001, which was confirmed by a recent phylogenetic study (Kawahara et al. 2017). *Liocrobyla* is endemic to the Old World and hitherto includes eight species (De Prins and De Prins 2018), with six species known from Asia: *L. paraschista* Meyrick, 1916, *L. brachybotrys* Kuroko, 1960, *L. lobata* Kuroko, 1960, *L. desmodiella* Kuroko, 1982, *L. kumatai* Kuroko, 1982 and *L. minima* (Noreika, 1992) (Meyrick 1916, Kuroko 1960, Kuroko 1982, Noreika and Puplesis 1992), one from Solomon Islands: *L. saturata* Bradley, 1961 (Bradley 1961) and one from South Africa: *L. tephrosiae* Vári, 1961 (Vári 1961). Larvae of all species with known host associations are blotch leaf miners on various genera of the plant family Fabaceae (De Prins and De Prins 2018). Only one species, *L. desmodiella* Kuroko, 1982, is currently known in China without host association (Bai and Li 2011).

The purpose of this paper is to report one new species and one species of *Liocrobyla* recorded for the first time from China and to document a new host for the known species. All the species are provided with information on their host plant associations, leaf mines, and morphology of adults and their genital diagnostic characters.

## Methods

Leaves containing mines with larvae were placed in sealed plastic bags or rearing containers with moist cotton as previously described (Liu and Yan 2017). Pupae in rearing containers were placed outdoors to

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overwinter and were transferred into the laboratory in early spring. All adults were collected by rearing from immature stages.

Adult photographs were taken with a Leica S6D stereo microscope. Genitalia were dissected and mounted according to the methods introduced by Li (2002), but stained with Eosin Y and/or Chlorazol Black. The illustrations were prepared by using a Leica DM1000 microscope and refined in PHOTOSHOP CS4 software. Photographs of host plant and mines were taken in the field using a Canon PowerShot G10 digital camera or a Canon EOS camera. Plant nomenclature follows APG (2016) and The Plant List (2013).

The types of the new species are deposited in the Zoological Collection, Shandong Normal University, Jinan, China (SDNU). Other specimens are deposited in the Insect Collection, Nankai University, Tianjin, China or SDNU as stated in the text.

### Abbreviations

| | |
|---|---|
| **ELKU** | Entomological Laboratory, Kyushu University, Fukuoka, Japan |
| **NKU** | Insect Collection, Nankai University, Tianjin, China |
| **SDNU** | Zoological Collection, Shandong Normal University, Jinan, China |
| **TD** | Type depository |
| **TL** | Type locality |

## Results

### *Liocrobyla desmodiella* Kuroko, 1982

Figures 1, 5, 8, 11 and 13–17

*Liocrobyla paraschista* Meyrick: Kuroko, 1960: 2. Misidentification.
*Liocrobyla desmodiella* Kuroko, 1982: 185; Ermolaev 1987: 370; Bai and Li 2011: 480. TL: Japan (Kyushu). TD: ELKU.

**Diagnosis.** This species resembles *Liocrobyla lobata* Kuroko, 1960 in the general appearance of the forewing patterns, but can be distinguished by the blackish-grey forewing ground colour, which is brownish-grey in *L. lobata*. In the male genitalia, this species can be separated by the valva having a remarkable concavity at distal 1/4 and bearing a small ventro-apical hook; in *L. lobata*, the valva is almost parallel-sided and bears straight spines. The ninth tergite of the male *L. desmodiella* bears a pair of sclerotized lines originated from the middle of the posterior margin, which is absent in *L. lobata*.

**Material examined. China**: Tianjin: 3♂, 1♀, Mt. Baxian, Ji County, 40.180°N, 117.550°E, 400 m, 2014.vi.24, leaf mines collected on *Lespedeza bicolor*, emerged 2014.vii.09, leg. Tengteng Liu, genitalia slide nos. LTT12611♂, LTT12612♀ (NKU).

**Adult** (Fig. 1). Forewing length 3.0–3.5 mm. Head white on frons and face, with a tuft of black scales at base of antenna, vertex white with a black median line. Maxillary palpus black, about 1/3 length of labial palpus. Labial palpus white, black on distal part of second segment, with a mid-ventral black spot on third segment. Antennae black on scape, yellowish-fuscous and with black rings on other segments. Thorax yellowish-fuscous, tegula blackish grey. Forewing ground colour blackish grey, a greyish-fuscous stripe from costal 1/3 to 1/2, then curved downwards by white colour to near distal end of cell; two white stripes on distal 1/3 and 2/5 on costa obliquely to middle of wing; two longitudinal striae near apex, two longer striae near lower angle of cell; an yellowish-fuscous stripe along dorsum from base to tornus, with three to four black spots above; cilia white with two black lines around apex, dark grey on dorsum. Abdomen: blackish grey dorsally, white ventrally.

The forewing pattern of the specimens reared from *Lespedeza bicolor* is congruent to the Japanese specimens reared from *L. cyrtobotrya* (Kuroko 1960).

**Male genitalia** (Figs 5 and 8). Tegumen narrowed towards apex, with distal 1/6 triangular. Vinculum with a small round protrusion anteriorly. Valva concave at distal 1/4 and bearing a small ventro-apical hook and some 12 smaller teeth below. Phallus shorter than valva. The ninth tergite more or less semicircular, having paired sclerotized lines originated from the middle of the posterior margin.

**Female genitalia** (Fig. 11). Seventh sternite sharply projected postero-laterally, thus U-shaped on posterior margin. Antrum sclerotized short tube. Ductus bursae membranous, densely covered with granules on inner wall, about twice the length of antrum; ductus seminalis originated from ductus bursae near antrum, covered with dense teeth on opening, a sclerotized line extending from ductus bursae to ductus seminalis then curved back to near its beginning. Corpus bursae a membranous bag, about twice the length of ductus bursae.

**Biology** (Figs 13–17). The larval behaviour in the mine on *L. bicolor* is similar to that on *L. cyrtobotrya* as described by Kuroko (1960). Leaf mines placed on upper side of leaflet and stretched across midrib; a black tunnel made of silk and frass aside midrib from upper to lower side of leaflet, covered by dry leaflet epidermis on lower side opening. One mine per leaflet.

**Host plants.** Fabaceae: *Lespedeza bicolor* Turcz. in China, **new record**; *L. cyrtobotrya* Miq., *Desmodium oldhamii* Oliv., *Ohwia caudata* (Thunb.) H. Ohashi and *Hylodesmum podocarpum* subsp. *oxyphyllum* (DC.) H. Ohashi & R.R. Mill in Japan (Kuroko 1960, The Plant List 2013; see remarks for the nomenclature changes of the host plants).

**Figures 1–4.** Adult of *Liocrobyla* spp. **1.** *L. desmodiella*; **2.** *L. lobata*; **3**) *L. indigofera* sp. n., male, holotype, before dissection; **4**. *L. indigofera* sp. n., female, paratype, before dissection. Scale bars: 2.0 mm.

**Distribution.** China: Tianjin (**new record)**; Sichuan and Zhejiang (Bai and Li 2011), Japan (Kuroko 1982), Russia Far East (Ermolaev 1987).

**Remarks.** The host plant *Lespedeza bicolor* was newly recorded for this species. Kuroko (1982) stated in Japanese that the species identified as *Liocrobyla paraschista* Meyrick, 1916 in Kuroko (1960) was a misidentification and he named it as a new species, *L. desmodiella* Kuroko, 1982. Because of the misidentification of the moth, the host plants recorded in Japan, *L. cyrtobotrya*, *D. oldhamii*, *Desmodium racemosum* (Thunb.) DC. and *D. caudatum* (Thunb.) DC., originally associated with *Liocrobyla paraschista* by Kuroko (1960), are actually belonging to *L. desmodiella*. Ermolaev (1987) recorded *L. desmodiella* in the Russian Far East but just followed Kuroko (1960) by listing its host plants, thus the host plants of this species in Russia are still of uncertainty. Moreover, *D. racemosum* and *D. caudatum* have been treated as synonyms of *Hylodesmum podocarpum* subsp. *oxyphyllum* and *Ohwia caudata* in The Plant List (2013), respectively.

### *Liocrobyla lobata* Kuroko, 1960

Figures 2, 6, 9 and 18–22

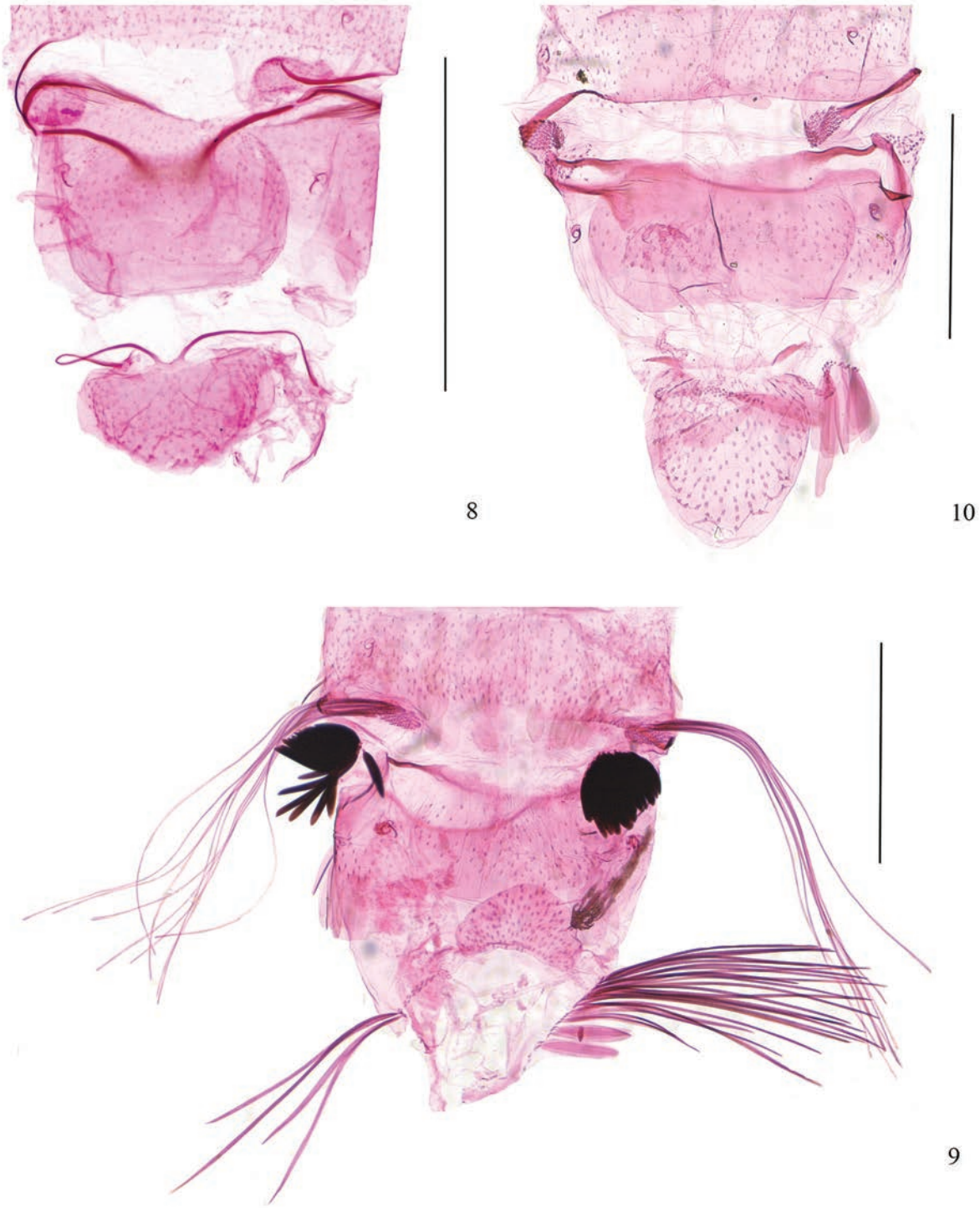
*Liocrobyla lobata* Kuroko, 1960: 5; Kuroko 1982: 184; Park 1983: 62. TL: Japan (Kyushu). TD: ELKU.

**Diagnosis.** See *Liocrobyla desmodiella* for detail.

**Material examined.** China: Shandong Province: 4♂, Mt. Kunyu National Nature Reserve, 37.292°N, 121.740°E, 400 m, Yantai City, 2017.vii.18, leaf mines collected on *Pueraria montana* var. *lobata*, emerged vii.29, leg. Tengteng Liu & Zhenquan Gao, genitalia slide nos. LIU0028, registered nos. SDNU.YT17170702.3–6 (SDNU).

**Adult** (Fig. 2). Head white on frons and face, tinged blackish fuscous on base of antennae, vertex white with a brownish-grey median line. Maxillary palpus black mixed with white, about 1/4 length of labial palpus. Labial palpus white, black on distal part of second segment, with a mid-lateral black spot on third segment. Antennae

**Figures 5–7.** Male genitalia of *Liocrobyla* spp. **5.** *L. desmodiella*, slide no. LTT12611 (NKU); **6.** *L. lobata*, slide no. LIU0028; **7.** *L. indigofera* sp. n., slide no. LIU0030, holotype. Scale bars: 0.2 mm.




**Figures 8–10.** Male pregenital structures of *Liocrobyla* spp. **8.** *L. desmodiella*, slide no. LTT12611 (NKU); **9.** *L. lobata*, slide no. LIU0028; **10.** *L. indigofera* sp. n., holotype, slide no. LIU0030. Scale bars: 0.5 mm.

with scape white on front and black dorsally, other segments brownish grey with darker rings. Thorax yellowish fuscous, tegula brownish grey. Forewing ground colour brownish grey, darker towards costa, a white stripe from costal 1/3 to before 1/2, then curved downwards to near tornus; two white stripes beyond distal 1/3 and 2/5 on costa obliquely to middle of wing; two longitudinal striae near apex, with the costal one indistinct, one white spot on tornus; a white stripe along dorsum from base to tornus, partially edged with black scales and largely covered by brownish-fuscous scales on dorsum; cilia white with two black lines around apex, grey on dorsum. Abdomen: light fuscous dorsally, white ventrally.

**Male genitalia** (Figs 6 and 9). Tegumen slender, almost same in width. Vinculum narrowly triangular. Valva almost

**Figures 11–12.** Female genitalia of *Liocrobyla* spp. **11.** *L. desmodiella*, slide no. LTT12612 (NKU); **12.** *L. indigofera* sp. n., slide no. LIU0029, paratype. Scale bars: 0.5 mm.

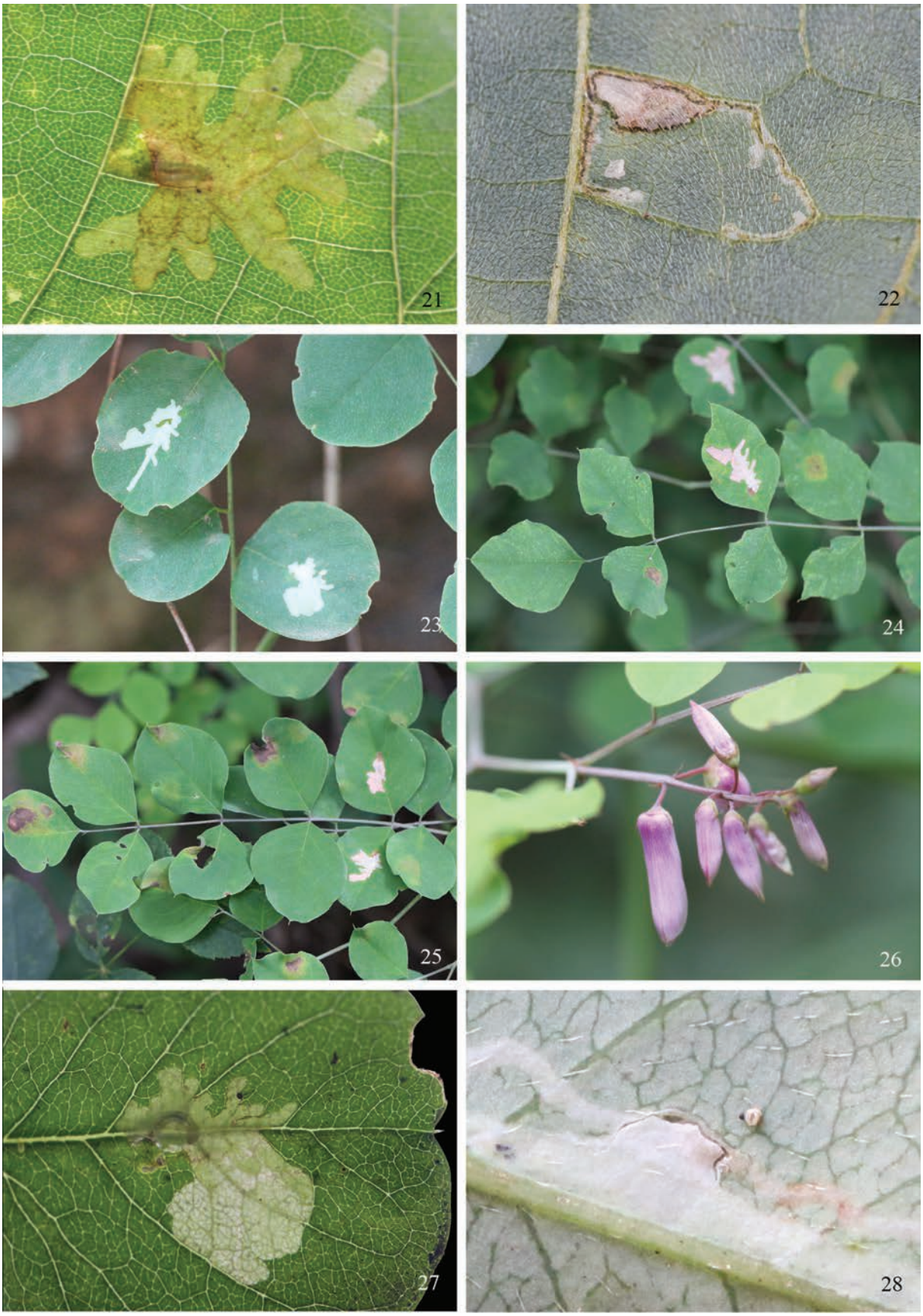
identical in width, bears some 15 minute teeth along dorsal margin and two longer ventro-apical spines. Phallus shorter than valva. Paired clusters of slender scales and black short scales on membrane between seventh and eighth terga. Ninth segment with tergite heart-shaped, a line of slender scales along lateral side (Fig. 9).

**Biology** (Figs 18–22). Mines not restricted to a fixed placement, with apparent digital galleries. A white tunnel made of silk only from upper to lower side of leaflet. Often more than one mine per leaf (Fig. 19).

**Host plants.** Fabaceae: *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Pradeep.

**Distribution.** China: Shandong (**new record)**, Japan (Kuroko 1960), Korea (Park 1983).

**Figures 13–20.** Biology of *Liocrobyla* spp. **13–17.** *L. desmodiella*, **13.** host plant, *Lespedeza bicolor*; **14.** leafmine; **15.** frass opening on upper side of leaf; **16.** frass opening on lower side of leaf; **17.** cocoon and pupal exuviae; **18–20**. *L. lobata*, **18.** host plant, *Pueraria montana* var. *lobata*; **19.** leafmines; **20.** an opened mine with a larva exposed.




**Figures 21–28.** Host plants, leafmines and biology of *Liocrobyla* spp. **21–22**, *L. lobata*, **21.** leafmine with an alive larva; **22.** frass opening on lower side of leaf; **23–28.** *L. indigofera* sp. n., **23.** leafmines each on a leaf of *Indigofera kirilowii*; **24–25.** leafmines on *I. tinctoria*; **26.** raceme of *I. tinctoria*; **27.** leafmine with a living larva, identical mine to the lower-right mine in Fig. 23; **28.** frass opening on lower side of leaf, identical mine to Fig. 27.

**Remarks.** *Pueraria lobata* has been treated as a synonym of *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Pradeep in The Plant List (2013).

### *Liocrobyla indigofera* sp. n.

http://zoobank.org/2D79B9E2-C197-4377-B9F0-43F507168183
Figures 3, 4, 7, 10, 12 and 23–28

**Diagnosis.** The new species resembles *L. lobata* in the male genitalia, but can be separated by the phallus longer than the valva and the minute cornutus; in *L. lobata*, the phallus is shorter than valva and the cornutus is more than 1/12 length of the phallus. In *L. indigofera*, the head is dark grey and the forewing ground colour is blackish fuscous, while in *L. lobata*, the head is white and the forewing ground colour is brownish grey.

**Type material. Holotype**, ♂, China: Shandong Province: Mt. Laoshan, 36.204°N, 120.609°E, 400 m, Qingdao City, 2017.vii.01, leafmine collected on *Indigofera kirilowii*, pupated vii.05, emerged vii.18, leg. Tengteng Liu & Zhenquan Gao, genitalia slide no. LIU0030♂, registered no. SDNU.QD170710.2 (SDNU); **Paratype**: 1♀, genitalia slide no. LIU0029♀, registered no. SDNU. QD170710.1, other data same as holotype (SDNU).

**Other material.** Leaf mines collected on *Indigofera tinctoria* Linn., Mt. Laoshan, 120.609°E, 36.204°N, 400 m, Qingdao City, 2017.vii.01, leg. Tengteng Liu.

**Adult** (Figs 3 and 4). Forewing length 3.0 mm. Head with frons white, vertex dark grey with a black median line. Maxillary palpus black, pointed apically, about 1/4 length of labial palpus. Labial palpus white, with black rings at middle and before apex. Antennae with scape dark dorsally, white ventrally, other segments dark with black rings. Thorax yellowish gray, with a dark central line, tegula blackish-fuscous; legs white with blackish-fuscous rings. Forewing blackish-fuscous, fuscous along dorsum, stripes and spots white; a sinuous stripe from costal 1/3 to apex of disc divided into three or occasionally two parts (which is continuous in *L. lobata*); one stria at costal 2/3; a transverse outwards arched stripe at 4/5, separated at middle, with violet reflection; two spots above the fuscous stripe on dorsum, with outer one larger; two minute spots near apex; cilia white, with three black lines. Hind wing blackish-grey. Abdomen: blackish-grey dorsally, silvery white ventrally.

**Male genitalia** (Figs 7 and 10). Tegumen weakly sclerotized. Valva more or less rectangular, divided by a sclerotized ridge, dorsal part more sclerotized than costal one; costal part densely covered with setae apically, dorsal part with two long digital processes apically and one or two minute processes below apex. Vinculum narrowly triangular. Phallus longer than valva, curved beyond middle, pointed apically, vesica roughened, with a minute inversed cornutus. Ninth tergite more or less oval (Fig. 10).

**Female genitalia** (Fig. 12). Posterior and anterior apophyses triangular, about as long as eighth tergite. Antrum sclerotized. Ductus bursae about twice the length of seventh segment, sinuous at middle, strongly sclerotized ventrally, membranous dorsally; ductus seminalis originated from ductus bursae near antrum, sclerotized and sinuous basally. Corpus bursae oval, membranous.

**Biology** (Figs 23–28). Larval mine is a white blotch, with several digital galleries, always located in the middle of the upper side of a leaflet (Figs 23 and 27). A frass opening on the lower side of the leaflet always locates close to the midrib, covered by a piece of white dry leaf tissue (Fig. 28). A single mine per leaflet (Figs 23–25).

**Host plants.** Fabaceae: *Indigofera kirilowii* Palib., *I. tinctoria* L.

**Distribution.** China (Shandong).

**Etymology.** The specific name is derived from the genus name of the host plants.

## Discussion

Individual species of leaf miners, at least in many groups of Lepidoptera, are typically host-specific at a plant genus or family level (Regier et al. 2015), a fact which greatly facilitates the process of identification of a moth specimen with host association. The process involves two main parts: recognizing the family or genus of a moth specimen and identifying the host plant. The first part is generally easier for a lepidopterist than identifying a plant species. Besides the great differences between disciplines that results in this difficulty, botanists are undertaking revisions for the nomenclature of plants (The Plant List 2013), thus making the difficulty even greater.

Host plant specimens are recommended to be identified by a botany taxonomist and the nomenclature of the host plant is recommended to be checked in The Plant List which may be the most comprehensive single information resource covering all plants (The Plant List 2013). The nomenclature of host plant names derived from the old literature have possibly changed, such as the host plant names of *Liocrobyla desmodiella* and *L. lobata* in the present study. The Plant List could thus be treated as advisory and a lepidopterist should check the nomenclature of these plant names derived from old literature.

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## References

APG (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society 181(1): 1–20. http://dx.doi.org/10.1111/boj.12385

Bai HY, Li HH (2011) Three newly recorded genera and four newly recorded species of Gracillariidae (Lepidoptera) in China. Acta Zootaxonomica Sinica 36(2): 477–481. [In Chinese]

Bradley JD (1961) Microlepidoptera from the Solomon Islands. Additional records and descriptions of Microlepidoptera collected in the Solomon Islands by the Rennell Island Expedition 1953–54. Bulletin of the British Museum (Natural History), Entomology Series 10(4): 113–168.

De Prins J, De Prins W (2018) Global taxonomic database of Gracillariidae (Lepidoptera). http://www.gracillariidae.net/ [accessed: 20 April 2018]

Ermolaev VP (1987) *Liocrobyla* Meyrick a new to the fauna of the USSR genus of the gracillariid moths (Lepidoptera, Gracillariidae) from Southern Primorye Territory. Entomologicheskoe Obozrenie 66(2): 369–372.

Kawahara AY, Plotkin D, Ohshima I, Lopez-Vaamonde C, Houlihan P, Breinholt JW, Kawakita A, Xiao L, Regier JC, Davis DR, Kumata T, Sohn J-C, De Prins J, Mitter C (2017) A molecular phylogeny and revised higher-level classification for the leaf-mining moth family Gracillariidae and its implications for larval host use evolution. Systematic Entomology 42: 60–81. http://dx.doi.org/10.1111/syen.12210

Kuroko H (1960) Notes on the *Liocrobyla* of Japan, with descriptions of two new species (Lepidoptera, Gracilariidae). Esakia 1: 1–6. http://hdl.handle.net/2324/2337

Kuroko H (1982) Gracillariidae. In: Inoue H, Sugi S, Kuroko H, Moriuti S, Kawabe A (Eds) Moths of Japan. Kodansha Co. Ltd., Tokyo, 176–202.

Kuznetzov VI, Baryshnikova SV (2001) Review of the Palaearctic genera of the family Gracillariidae (Lepidoptera) with description of a new subfamily Ornixolinae Kuznetzov et Baryshnikova, subfam. n. Entomological Review 81: 26–46.

Li HH (2002) The Gelechiidae of China (I) (Lepidoptera: Gelechioidea). Nankai University Press, Tianjin, China.

Liu T, Yan J (2017) Review of the Palearctic *Atemelia* Herrich-Schäffer (Lepidoptera, Yponomeutoidea, Praydidae), with description of a new leafmining species. Zootaxa 4250(4): 327–336. http://dx.doi.org/10.11646/zootaxa.4250.4.3

Meyrick E (1916–1923) Exotic Microlepidoptera. Vol. 1. Thornhanger, Marlborough, Wilts, 640 pp. https://biodiversitylibrary.org/page/9808404

Noreika R, Puplesis R (1992) Description of new species of moths of the family Gracillariidae (Lepidoptera) from Azerbaijan and Middle Asia and synonymy of *Gracillaria impictipennella* Grsm. Entomologicheskoe Obozrenie 71(2): 414–421.

Park KT (1983) Microlepidoptera of Korea. Insecta Koreana 3: 1–189.

Regier JC, Mitter C, Kristensen NP, Davis DR, Van Nieukerken EJ, Rota J, Simonsen TJ, Mitter KT, Kawahara AY, Yen SH, Cummings MP, Zwick A (2015) A molecular phylogeny for the oldest (nonditrysian) lineages of extant Lepidoptera, with implications for classification, comparative morphology and life-history evolution. Systematic Entomology 40(4): 671–704. http://dx.doi.org/10.1111/syen.12129

The Plant List (2013) Version 1.1. Published on the Internet. http://www.theplantlist.org/ [accessed: 21 January 2018]

Vári L (1961) South African Lepidoptera. Vol. I. Lithocolletidae. Transvaal Museum Memoir 12: 1–238.

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# Discovery of a rare hybrid specimen known as Maria's bird of paradise at the Staatliches Naturhistorisches Museum in Braunschweig

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Private collector
Walter Behrens
Haus der Natur

## Abstract

The discovery of a rare hybrid specimen of Maria's bird of paradise (*Paradisaea maria*, i.e., *P. guilielmi* × *P. raggiana augustaevictoriae*) in the ornithological collection of the Staatliches Naturhistorisches Museum in Braunschweig (SNMB) is reported. Until today only six male specimens (deposited in the natural history museums in Berlin and New York) and presumably one female have been identified in collections world-wide. The male specimen in Braunschweig corresponds well in its plumage colouration with an historical illustration and photographs of the original type specimen from the 19th century housed at the Berlin collection. It shows intermediate characteristics between both parental species, viz. the Emperor bird of paradise (*P. guilielmi*) and the Raggiana bird of paradise (*P. raggiana augustaevictoriae*). In addition, we try to elucidate the circumstances how this rare specimen of hybrid origin, which formerly belonged to the natural history collection of the factory owner Walter Behrens from Bad Harzburg, came to the SNMB. Our unexpected discovery highlights the importance to maintain, support and study also smaller private natural history collections, since they may house historical voucher specimens of high scientific value.

## Zusammenfassung

Es wird über die Entdeckung eines seltenen Exemplars des Hybrid-Paradiesvogels *Paradisaea maria* (d.h. *Paradisaea guilielmi* × *P. raggiana augustaevictoriae*), in der ornithologischen Sammlung des Staatlichen Naturhistorischen Museums in Braunschweig (SNMB) berichtet. Bis heute sind lediglich sechs männliche (aus den Museen in Berlin und New York) und vermutlich ein weibliches Exemplar in internationalen Naturkundesammlungen bekannt geworden. Das männliche Exemplar aus Braunschweig entspricht in seiner Gefiederfärbung einer historischen Abbildung und Fotos des ursprünglichen Typusexemplars aus dem 19. Jahrhundert, das sich im Berliner Museum befindet. Es zeigt deutlich intermediäre Merkmalsausprägungen zwischen den beiden Elternarten, dem Kaiserparadiesvogel (*P. guilielmi*) und dem Raggi-Paradiesvogel (*P. raggiana augustaevictoriae*). Die Umstände, wie dieser seltene Hybrid-Paradiesvogel, der ehemals Teil der Sammlung des Fabrikanten Walter Behrens aus Bad Harzburg war, in die SNMB-Sammlung gelangte, werden erläutert. Unsere unerwartete Entdeckung unterstreicht die Bedeutung, auch kleinere private naturkundliche Sammlungen zu bewahren, zu erhalten und zu erforschen, da sie historische Belegexemplare von hoher wissenschaftlicher Bedeutung enthalten können.

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# Introduction

## Maria's bird of paradise

Hardly any other group of birds has ever since their first discovery exalted the imagination of people as the birds of paradise (family Paradisaeidae). Due to their mainly colourful and conspicuous plumage, birds of paradise have been most admired commodities since the early 16th century. They were discovered and specifically collected for the domestic demand in curiosities of natural history and exotics by European circumnavigators during their adventurous journeys to the Indo–Australian Archipelago (Stresemann 1954, Swadling 1996). Although known long before binary nomenclature was consistently used since the mid-18th century (Mužinić et al. 2009), the first bird of paradise species, which was formally described by Linnaeus (1758), was the Greater bird of paradise (*Paradisaea apoda*). In the following decades, more and more members of this fascinating bird family were discovered and described.

In 1894, Anton Reichenow (1847–1941), then ornithologist and deputy director of the Museum für Naturkunde (= Museum of Natural History) in Berlin, described another new species, *P. maria*, based on a single male specimen from the Finisterre Mountain Range of the Huon Peninsula in north–eastern New Guinea, dedicating the species epithet to his wife Maria (the daughter of J. L. Cabanis, see below). Three years later, Reichenow (1897, pl. V) published an artistic plate of Maria's bird of paradise (Fig. 1). Some years earlier, Jean Louis Cabanis (1816–1906), then director of the Museum für Naturkunde in Berlin, had described two new species of birds of paradise (Cabanis 1888), viz. *P. guilielmi* and *P. augustaevictoriae* (Fig. 2a, b), in honour of the last German Emperor and King of Prussia, Wilhelm II (1859–1941), and his wife Augusta Victoria of Schleswig-Holstein (1858–1921). Illustrations of both majestic birds of paradise were published the following year in volume 37, plates 1 and 2, of the Journal für Ornithologie (nowadays the Journal of Ornithology). While the Raggiana bird of paradise (of which the taxon *augustaevictoriae* is considered a subspecies today) is widespread in the South and Northeast of Papua New Guinea, the Emperor bird of paradise is restricted to the Huon Peninsula, where it inhabits lower mountains and hills from ca. 450 m to 1,500 m. Below this altitude, *P. guilielmi* is replaced by the Raggiana bird of paradise in the East of its range, where both species occur sympatrically (Frith and Beehler 1998).

## Hybridisation in birds of paradise

Hybridisation between different bird species is a relatively common phenomenon. So far about 4,000 combinations of hybrid origin have been identified, about half of which are crossbreedings in captivity (Mc Charty 2006). According to this author, however, the total number of hybridisations in birds is estimated to be much higher, since hybrid specimens are usually difficult to identify.

**Figure 1.** Plate of the male hybrid bird of paradise *Paradisaea maria* from Reichenow (1897). Reproduced from the Biodiversity heritage library (http://biodiversitylibrary.com/).

For the family Paradisaeidae, with currently 42 recognized members (the last was described in 1992), so far 25 hybrid combinations have been identified (Table 1), most of which were described as separate species in the past (Fuller 1995, Frith and Beehler 1998, Lepage 2015). Based on the phylogenetic species concept even approximately 90 different species of paradisaeids were postulated by Cracraft (1992). This conceptual approach, however, is currently not accepted (Lepage 2015). Notably, Mayr (1945, 1963) assumed that one in every 20,000 wild specimens of birds of paradise is a hybrid (i.e. 0.005%), while for birds in general he considered only one in every 60,000 specimens to be of hybrid origin (i.e. <0.002%). Frith and Beehler (1998), however, judged Mayr's assumption "as no more than an informed guess". Among almost 5,000 voucher specimens of birds of paradise these authors examined, more than 88 male and merely three female hybrid specimens (i.e. about 2%) were identified (Table 1).

Reichenow (1901) himself was among the first authors to speculate about the putative hybrid origin of one of

**Figure 2.** Plates of *Paradisaea guilielmi* (**A**) and *P. raggiana augustaevictoriae* (**B**), the presumed parental species of *P. maria*, related to the original descriptions by Cabanis (1888). Reproduced from the Biodiversity heritage library (http://biodiversitylibrary.com/).

the species he himself had previously described (see also Rothschild 1898). Subsequently, Rothschild (1910) and Stresemann (1923: 40) suggested, that also *P. maria* is a hybrid form between *P. guilielmi* und *P.* (*raggiana*) *augustaevictoriae*. The latter discussed a second specimen from the Berlin museum (ZMB 951) that was allegedly collected by Dr Bürgers during the German Empress Augusta (now Sepik) River Expedition in 1912–1913. Subsequently, however, Stresemann (1925) corrected that the original label of this voucher specimen had been mixed up and that it was actually collected by H. Andechser, a planter from Singana, at the southern slopes of the Herzoggebirge (the Herzog mountains) south of Lae at the base of the Huon Peninsula. Later, Stresemann (1930a) even mentioned five specimens of Maria's bird of paradise, which he had examined in the ornithological collections in Berlin (the holotype ZMB 31049, Fig. 3) and Tring (UK), respectively. In the early 1930s, the four specimens from Walter Rothschild's (1868–1937) famous bird collection at Tring were sold to the American Museum of Natural History in New York (LeCroy 2014).

A dubious taxon phenotypically very similar to *P. maria* is *P. duivenbodei* described by Ménégaux (1913a, b). Until today, it is known only from a single male specimen, the holotype MNHN 863, in the Muséum National d'Histoire Naturelle in Paris. It has "(…) brown uppertail coverts not marked with straw yellow streaking and its flank plumes are more yellow than red (…)" (Frith and Beehler 1998). Walter Rothschild had examined the type specimen in Paris and expressed his doubt about the taxonomic validity of *P. duivenbodei* to his French colleague. In turn, Ménégaux (1913c) again discussed in detail the minor morphological differences of his new species as compared with obviously closely-related birds of paradise such as *P. maria*. Finally, he concluded that *P. duivenbodei* should be retained until further evidence would prove the opposite since only three specimens of *P. maria* were known at that time. Also Stresemann (1923) considered the taxon *duivenbodei* a synonym of *P. maria*. Today, however, it is considered a hybrid between *P. guilielmi* and *P. minor finschi* (Frith and Beehler 1998, see Table 1). The origin of the holotype is un-

**Table 1.** Summary of known hybrid specimens of birds of paradise in international museum collections. In few cases, different hybrid forms have been identified (and described) twice based on the same parental species combination, but apparently with varying influence of each species involved. *A third specimen, the holotype, was destroyed during WWII; [#] The hybrid status is uncertain. > Minimum number of specimens known. Data derived from Frith and Beehler (1998).

| Parental species combination | Hybrid species name | No. of known specimens | |
|---|---|---|---|
| | | males | females |
| **Intrageneric hybrids** | | | |
| *Astrapia mayeri* x *A. stephaniae* | '*Astrachia barnesi*' | > 12 | |
| *Paradisaea guilielmi* x *P. raggiana augustaevictoriae* | '*Paradisea maria*' | > 6 | (1[#]) |
| *Paradisaea guilielmi* x *P. minor* | '*Paradisea duivenbodei*' | 1 | |
| *Paradisaea raggiana augustaevictoriae* x *P. raggiana intermedia* | '*Paradisaea granti*' and '*P. apoda subintermedia*' | 1 | |
| *Paradisaea raggiana salvadorii* x *P. rudolphi margaritae* | '*Paradisea bloodi*' | 1 | |
| *Paradisaea raggiana augustaevictoriae* x *P. minor finschi* | '*Paradisea mixta*' | > 4 | |
| *Paradisaea raggiana salvadorii* x *P. minor finschi* | unnamed | only through observations | |
| *Paradisaea apoda novaeguineae* x *P. raggiana salvadorii* | '*Paradisea apoda luptoni*' | numerous | |
| *Cicinnurus magnificus* x *C. regius* | '*Diphyllodes gulielmi III*' | > 25 | |
| *Cicinnurus regius* x *C. magnificus* | '*Cicinnurus lyogyrus*' and '*C. goodfellowi*' | > 3 | |
| **Intergeneric hybrids** | | | |
| *Astrapia nigra* x *Epimachus f. fastuosus* | '*Epimachus astrapioides*' and '*Astrapimachus ellioti*' | 1 | |
| *Epimachus f. fastuosus* x *Astrapia nigra* | '*Epimachus ellioti*' | 2 | |
| *Paradigalla carunculata* x *Epimachus f. fastuosus* | '*Pseudastrapia lobata*' | 1 | |
| *Paradigalla carunculata* x *Lophorina s. superba* | '*Loborhamphus nobilis*' | 3 | |
| *Epimachus fastuosus* x *Lophorina superba feminina* | *Epimachus fastuosus atratus* x *Lophorina superba feminina* | 1 | |
| *Parotia sefilata* x *Lophorina superba* | '*Parotia duivenbodei*' | 2 | |
| *Paradigalla carunculata* x *Parotia sefilata* | '*Loborhamphus ptilorhis*' | 1 | |
| *Lophorina superba* x *Cicinnurus magnificus* | '*Lamprothorax wilhelminae*' | 3 | |
| *Parotia carolae* x *Lophorina superba* | '*Lophorina superba pseudoparotia*' | | 1 |
| *Parotia l. lawesii* x *Paradisaea rudolphi margaritae* | unnamed | | 1 |
| *Ptiloris intercedens* x *Lophorina superba minor* | '*Paryphephorus* (*Craspedophora*) *duivenbodei*' | 2* | |
| *Seleucidis melanoleuca* x *Paradisaea minor* | '*Paradisea mirabilis*' | 5 | |
| *Ptiloris m. magnificus* x *Paradisaea m. minor* | '*Janthothorax bensbachi*' | 1 | |
| *Seleucidis melanoleuca* x *Ptiloris magnificus* | '*Craspedophora mantoui*' and '*C. bruyni*' | > 12 | |
| *Cicinnurus m. magnificus* x *Paradisaea m. minor* | '*Neoparadisea ruysi*' | 1 | |
| **Number of known hybrid specimens in total** | | **> 88** | **3** |

certain, since it was received from the well-established Dutch merchant Maarten Dirk van Renesse van Duivenbode (1804–1878), a trader of birds and other naturalia on Ternate, Moluccas, in the Dutch East Indies (Frodin 2007). Notably, three hybrid forms of birds of paradise have been named after him (Table 1). Allegedly, the sole specimen of *P. duivenbodei* was collected by a person called M. Seng near Yaour (= Yaur; Frith and Beehler 1998 assumed that it originated from [Mois or Meos] Waar Island) in Geelvink (todays Cenderawasih, which means bird of paradise in a local language) Bay in north–western New Guinea (Ménégaux 1913a, c). Stresemann (1930a, b) had already speculated that it actually originated from the Huon Peninsula of north–eastern Papua New Guinea, the distribution range of *P. guilielmi*.

In the course of the reorganisation and re-conception of parts of the permanent exhibitions at the Staatliches Naturhistorisches Museum in Braunschweig (= State Natural History Museum in Brunswick), a random bird of paradise specimen (which received the new collection number SNMB N49068), labelled as *Paradisaea maria*, was selected from the ornithological collections in order to enrich the new show depot which shall demonstrate the representation of a traditional natural history museum of the 19th century. During the literature search to gather specific information about this bird of paradise, it turned out that *P. maria* is a rather rare hybrid taxon with only few known specimens world-wide. Therefore, the intention of this contribution is to provide information about the existence of further specimens of this hybrid bird of paradise in German natural history collections and to trace back the way how one of them finally arrived at the natural history museum in Brunswick.

**Figure 3.** The holotype (ZMB 31049) of *Paradisaea maria* Reichenow, 1894, in dorsal, lateral, and ventral view. Photos by H. J. Goetz (Museum für Naturkunde, Berlin).

## Material and methods

In order to confirm the identification attached to voucher specimen SNMB N49068 it was compared with photographs of the ZMB holotype of *P. maria* (ZMB 31049) and naturalistic illustrations from Reichenow (1897) and those of similar birds of paradise (see Figs. 1–2 and those in Frith and Beehler 1998).

Collection acronyms are as follows:

AMNH: American Museum of Natural History, New York, USA; MNHN: Muséum National d'Histoire Naturelle, Paris, France; SNMB: Staatliches Naturhistorisches Museum (State Natural History Museum), Brunswick, Germany; ÜMB: Überseemuseum (Overseas Museum) Bremen, Germany: ZMB: Museum für Naturkunde (Museum of Natural History, formerly Zoologisches Museum, i.e. Zoological Museum), Berlin, Germany.

## Results

### The newly discovered specimen(s) of Maria's bird of paradise

The original scientific determination attached by the specimen's label (Fig. 4) as *Paradisaea maria* could be confirmed during our investigations. The assigned German vernacular name (translated as carmine red bird of paradise), however, does not exist for any *Paradisaea* species (e.g. see appendix in Apel et al. 2011). Only *P. rubra* is commonly known as the Red bird of paradise but it is morphologically distinct from the remaining species of the genus (Frith and Beehler 1998). Until today, only six males and presumably one female (AMNH 679107) of *P. maria* have been identified in natural history museums world-wide (Frith and Frith 2010 mention eight specimens): Two specimens are deposited in the ZMB collection in Berlin and four in the AMNH collection in New York (Frith and Beehler 1998, see details above). They originate from Sattelberg (the Saddle Mountain near Finschhafen) and the Finisterre Mountains on the Huon Peninsula of Papua New Guinea, respectively.

The stuffed specimen is mounted on a branch with a wooden socket and labelled Karminroter Paradiesvogel, Männchen, *Paradisaea maria*, Neuguinea (= carmine red bird of paradise, male, *Paradisaea maria*, New Guinea). The information written with black ink on the underside of the pedestal is as follows: "*Paradisea maria* Rchw. Neu-Guinea Köper Docke u. Co. Bremen C. N. 3423". With a pencil "Weber det[ermined]." is added vertically at the side with an arrow pointing from the last line of words, which is covered by a white label, to the first

**Figure 4.** The male specimen SNMB N49068 of *P. maria* in the Staatliches Naturhistorisches Museum in Braunschweig. The information written on the underside of the pedestal in black ink is as follows: "*Paradisea maria* R[ei]ch[eno]w. Neu-Guinea [= New Guinea] Köper Docke u. Co. Bremen C. N. 3423. Weber [?] det. [?]". The white label written with pencil, which partly sticks above the former ink writing, reads as follows: "Real. Kat. 13143 *Paradisea maria* Rchw. ♂ Deutsch-N.-G. Eing. N° 3423" (= Realia Catalogue No. 13143, *Paradisea maria* Reichenow, ♂, German New Guinea, Entrance No. 3423). Photos by M. Forthuber.

line with the species name. This additional label reads as follows: "Real. Kat. 13143 *Paradisea maria* Rchw. ♂ Deutsch–N.–G. Eing. No 3423" (= Realia Catalogue No. 13143, *Paradisaea maria* Reichenow, ♂, German New Guinea, Entrance No. 3423) (Fig. 4).

Since the specimen lacked an N-number as is typical for the Braunschweig natural history collection since 1871, when chosen for the new exhibition, a search in the SNMB inventory catalogues was not successful. Due to the reference to the city of Bremen on the underside of the socket, we thus contacted the Überseemuseum, to inquire whether the specimen formerly belonged to their ornithological collection and this time our request received a positive response (M. Stiller, pers. comm.). From the information available the following circumstances can be reconstructed: Obviously, specimen SNMB N49068 formerly belonged to the collection of the Überseemuseum in Bremen, where it was registered in 1917 under the entrance number 3423 together with a second specimen of the same taxon and the same attached information (Fig. 5). Both specimens had the catalogue numbers [ÜMB] 13143 and [ÜMB] 13144 (not examined). The latter specimen, a bird skin indicated by a "b" in the entrance catalogue for "Balg", which means skin (in contrast to the letter a meaning "aufgestellt" = mounted for the other specimen), is still present in the ÜMB collection (M. Stiller, pers. comm.).

Specimen SNMB N49068 (Fig. 4), a male with unknown origin and collector (but see discussion below), shows intermediate character traits in plumage colouration between both parental species. Thus, large parts of head and throat are highly-iridescent dark green (vs. green colouration extending almost on entire head and breast in *P. guilielmi* and being limited to the throat in *P. raggiana*), with the eyes being largely bordered by the green colouration (vs. being entirely encompassed in *P.*

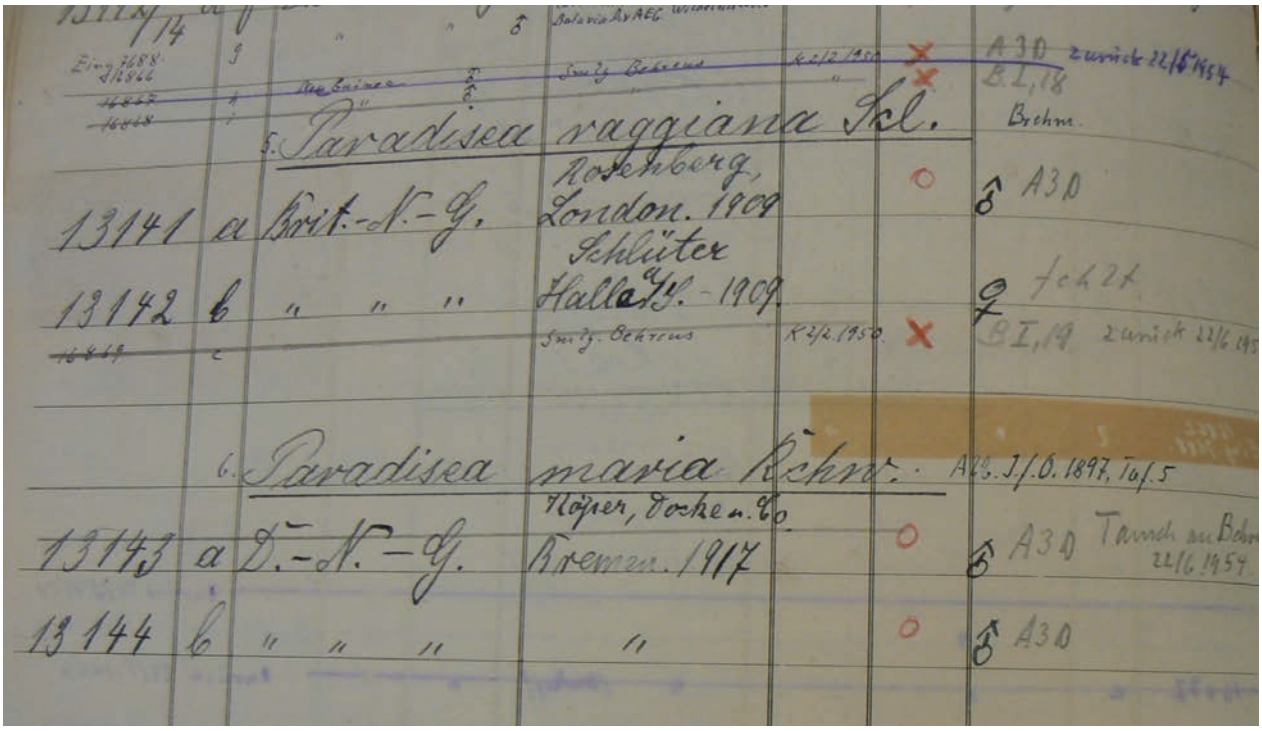


**Figure 5.** Catalogue page from the Überseemuseum in Bremen with the entries of two specimens of *P. maria* (catalogue numbers 13143 and 13144), the former of which was, probably among others, exchanged with Walter Behrens on 22 June 1954 and which is now SNMB N49068 at the Braunschweig collection. Both male specimens were transported to Bremen by the trading company Köper, Docke u. Co. in 1917. Photo by M. Stiller.

*guilielmi* and merely touched anteriorly in *P. raggiana*). The narrow yellow collar separating the green throat from the brown upper breast as seen in *P. raggiana* is missing in *P. guilielmi* and only faintly visible laterally in *P. maria*. Bright yellow colouration of rear part of head and nape extends onto mantle and upper part of wings. Remaining upper wing plumes are medium brown as are the lower breast and belly, while the upper wings and belly are dark brown, the latter merging white, in *P. guilielmi*. In turn, *P. raggiana* has medium brown upper wing plumes, while the belly is light brown coloured. Elongated, filamental flank plumes are crème-coloured washed light brown in *P. maria* vs. mostly white with little yellow ventrally and light brown with apricot orange ventrally in *P. guilielmi* and *P. raggiana*, respectively.

## Discussion

### Possible origin and collector of the specimens

The person called Weber, who, according to the writing under the pedestal, probably determined the specimen as *P. maria*, was Adolf Weber (according to Roselaar 2003, his initials were G. A. Weber), a former technical assistant and later inspector of the ornithological collection of the Überseemuseum (then called the Municipal Museum for Natural History, Ethnology, and Trade) in Bremen (Duncker 1953). After the First World War he reorganized the entire bird skin collection (Abel 1970). The name Köper, Docke & Co. refers to a former export-import company based in Bremen and which obviously transported the specimens from the former German colony in Northeast New Guinea (the so-called Kaiser–Wilhelmsland which existed until the First World War) to the harbour of Bremen. Although the collector's name is unknown, it seems likely that both birds of paradise were hunted or acquired by the former director of the museum, Hugo Schauinsland (1857–1937), who visited among others also New Guinea during his journey to Asia and the Pacific in 1913 to 1914 (Backmeister–Collacott et al. 2007). Due to the difficulties in global trade activities accompanying the First World War, it is known that some overseas collections amassed by Schauinsland arrived at the museum with a delay of several years. Although his material was usually transported back home free of charge by the Bremen-based North German Lloyd shipping company (Backmeister–Collacott et al. 2007), it seems reasonable that Köper, Docke & Co. managed at least this transport during the difficult years of war.

Noteworthy, in the ornithological collection catalogue another potential hybrid bird of paradise specimen is mentioned. It is listed as "presumably a hybrid between *Paradisea maria* and *P. guilielmi*" and belongs to the zoological material collected at Friedrich–Wilhelmshafen (nowadays Madang, the capital of the Papua New Guinean province of the same name) during the above mentioned expedition of Hugo Schauinsland (M. Stiller, pers. comm.). Another collector of zoological specimens

for the museum was Ludwig Cohn (1873–1935). He also travelled around New Guinea before the outbreak of the First World War and could likewise have been the collector of the two birds of paradise (Abel 1970). But apart from the collector's identity and exact origin of the specimens, the question remains how and when one of these extraordinary hybrid birds of paradise became part of the natural history museum in Brunswick.

## How the hybrid bird of paradise came into the SNMB collection

According to an entry in the catalogue of the Überseemuseum in Bremen (Fig. 5), the specimen was exchanged with a person named Behrens on 22 June 1954. The same person had donated a collection of birds of paradise to the Bremen museum in 1950, but four years later all specimens plus the voucher specimen in question together with additional specimens were returned to Behrens (M. Stiller, pers. comm.). Duncker (1953) specified this by mentioning that Behrens' bird collection was on loan at the Überseemuseum in the early 1950s. Some of these specimens, particularly birds of paradise and species lacking in the museum collection, were purchased from the owner.

So who was this person called Behrens with an interest in birds of paradise? Based on our investigations it is most likely that Walter Behrens (1892–1964) from Harlingerode at the northern base of the Harz Mountains is meant. Behrens, who was the owner of a factory for wooden boxes, became a member of the German Ornithological Society in 1940 (DOG 1940), and also possessed a private natural history collection (Knolle 2015, Knolle and Peinemann 2015). Since the late 1950s, parts of his collection were exhibited at the newly established local natural history museum Haus der Natur (= house of nature) in Bad Harzburg (Knolle 2015), to which Harlingerode administratively belongs since 1972. The remaining specimens of the collection were stored in the museum's attic and in Behrens' private house (Anonymous 1961). In 1961, these 1,300 (or according to Knolle 1,800) specimens were transferred into the possession of the city of Goslar, the neighbouring municipality of Bad Harzburg. There, three years later and only a few weeks before Walter Behrens' death on 26 November 1964, about half of the specimens were exhibited in the second newly founded local natural history museum called Haus der Tiere – Exoten (= house of animals – exotics) (Giesecke 2013). The other half were stored behind the scenes and were meant to be exchanged with the specimens on display from time to time. Most probably, in one of these two museums the mounted hybrid belonged to the specimens on display, as birds of paradise, among many other animals, were mentioned as highlights of the museum exhibitions in several newspaper articles (e.g., Anonymous 1964).

The museum in Bad Harzburg was temporarily closed in 1975 (Hevers 2008). It re-opened with a changed concept and reorganized exhibitions. Further on, only native species from northern Germany were displayed under the title Wald und Wild im Naturpark Harz (= forest and game in the Harz Mountains nature park) (Knolle 2015). The fate of the remaining and exotic specimens of the Behrens collection is unknown, but in this regard Hevers (2008) mentioned that the bird collection of Karl Fuest (1857–1938), originally exhibited in Wolfenbüttel castle since 1929 and transferred to the Haus der Natur in Bad Harzburg in 1956, was donated to the State Natural History Museum in Braunschweig in 1975. Perhaps also various of Walter Behrens' specimens were included in this transfer but this is not recorded. Alternatively, they were translocated to the Haus der Tiere in Goslar or deposited in the museum's attic. A few years later, however, by the end of December 1982, also the museum in Goslar was closed due to financial reasons and low visitor numbers (Anonymous 1982). In another newspaper article, dated 11 October 1983, the fate of the natural history collection is discussed (Anonymous 1983). According to this report, 2,000 specimens (700 molluscs, 150 mammals and 1,150 birds) should have been transferred to the natural history museum in Braunschweig as a permanent loan. It is likewise mentioned that besides the Braunschweig museum also the Landesmuseum (= State Museum) in Hannover was interested to buy the collection, but this plan failed due to the financial situation of the state of Lower Saxony (Anonymous 1983). Already in 1961, Professor Steininger, the former head of the natural history section at the State Museum in Hannover, had emphasized the high value of the Behrens collection (Anonymous 1961). Obviously, the transfer to Braunschweig was not, or only partly, realized, since no records thereof exist in the museum's catalogues and archives (Ahrens 2004) and we were unable to locate any further specimens from Walter Behrens' former private collection. The reason for this might be the fact that (some of) his specimens lacked personal collection numbers and labels as is the case in the hybrid bird of paradise which only shows the original information from the Bremen Überseemuseum.

There are, however, several specimen labels with reference to Walter Behrens and the Haus der Natur in the collection and archives of the State Museum in Hannover. Additionally, there exists a correspondence concerning a loan request between Behrens and Ernst Schäfer, the former curator at the museum (C. Schilling and A. Böhme, pers. comm.). Therefore, it is also reasonable that Maria's bird of paradise may have been exchanged with or donated to the Braunschweig collection after it had been transferred from Goslar or Bad Harzburg to Hannover. Since the specimen was not assigned a SNMB collection number when it came to Braunschweig, it was first inventoried on 13 October 2014, when it was chosen for the new permanent exhibition. Although some speculations remain how the hybrid bird of paradise finally entered the SNMB collection, the whereabouts of Walter Behrens' famous natural history collection could (at least partly) be answered by our investigations and we hope to stimulate further research into this forgotten private collection. The following details which we were able to gather while writing up this article, demonstrate that the private collection of Walter Behrens indeed contained valuable and rare specimens.

**The importance of small and private natural history collections**

Walter Behrens was a dedicated collector of natural history specimens with good connections to various museums, such as Bremen (see above) and Moscow (Anonymous 1964). But unfortunately, we were unable to find any specific publications by himself or another author about his private collection. Merely several articles in local newspapers report about their extent and mention some details. Thus, it is reported that the collection contained among others one specimen each of the extinct Tasmanian Tiger (*Thylacinus cynocephalus*) and the Passenger Pigeon (*Ectopistes migratorius*) (Anonymous 1961, 1964). While it is recorded that the latter specimen, which Walter Behrens had once purchased from an unaware Czech for merely 10 Deutschmarks, was eventually auctioned in 1957 for 22,000 Deutschmarks (Anonymous 1964), the thylacine is neither in the collection in Braunschweig nor in Hannover. Soon, however, it turned out that this male taxidermied specimen was sold to the natural history museum in Münster in 1962, where it still exists next to a second specimen from the University of Münster (S. Sleightholme, pers. comm.). In addition, Duncker (1953) mentioned several specimens of rare or extinct bird species from the Behrens' collection such as the Huia (*Heteralocha acutirostris*) and the Ivory–billed Woodpecker (*Campephilus principalis*).

These prominent specimens together with our unexpected find of a rare hybrid bird of paradise in the Staatliches Naturhistorisches Museum in Braunschweig highlight the importance of Walter Behrens' collection. It represents a nearly forgotten example of a small natural history collection and demonstrates the need to support and maintain also such private (museum) collections since they often house specimens of high scientific value for various research topics (e.g., Steinheimer 2003, Casas-Marce et al. 2012, Winker and Withrow 2013, Koch et al. 2017). Often, however, taxonomically well-trained staff and financial resources to appropriately maintain and study these minor and mainly local, but nevertheless essential collections are limited, putting the long-term existence of rare and valuable voucher specimens at risk. This is particularly true in developing countries (Cracraft 2000, Paknia et al. 2015). But also, for instance, in Europe, which has a long and rich tradition of natural history collections (Greuter et al. 2005, Romano et al. 2015, Beck 2018), this huge cultural world heritage is sometimes threatened due to neglect and needs more attention (Kovar–Eder and Niedernostheide 2014, Andreone 2015).

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## References

Abel H (1970) Vom Raritätenkabinett zum Bremer Überseemuseum. Die Geschichte einer hanseatischen Sammlung aus Übersee anläßlich ihres 75jährigen Bestehens. Verlag Friedrich Röver, Bremen.

Ahrens S (2004) 250 Jahre Naturhistorisches Museum in Braunschweig: Eine „Pflanzstätte für die naturgeschichtliche Bildung". Staatliches Naturhistorisches Museum, Braunschweig.

Andreone F (2015) Natural history: save Italy's museums. Nature 517: 271. https://doi.org/10.1038/517271b

Anonymous (1961) Invasion der Exoten im „Schwan" – Stadt Goslar übernimmt die Sammlung Behrens/Seltene Kostbarkeiten. Goslarsche Zeitung, 29. November 1961.

Anonymous (1964) Ratsprominenz bei den Exoten zu Gast. „Haus der Tiere" im Weißen Schwan wurde gestern eröffnet – „Ausgestopfte" aus aller Herren Länder. Goslarsche Zeitung, 04. November 1964.

Anonymous (1982) Das „Haus der Tiere" soll ab 1. Januar geschlossen werden. Goslarsche Zeitung, 27.September 1982.

Anonymous (1983) „Goslarer" vor der Auswanderung: Exoten ins Braunschweiger Museum. Goslarsche Zeitung, 11 October 1983.

Apel M, Glaw K, Simon G (2011) Natur- und Kulturgeschichte der Paradiesvögel. Museum Mensch und Natur, Schloss Nymphenburg, München.

Backmeister-Collacott I, Burkhardt U, Determann E (2007) Schauinsland! Ansichten aus Übersee, Überseemuseum Bremen.

Beck LA (2018) Zoological Collections of Germany – The Animal Kingdom in its Amazing Plenty at Museums and Universities. Springer, Heidelberg. https://doi.org/10.1007/978-3-319-44321-8

Cabanis J (1888) Vorläufige Notiz über 2 neue Paradies-Vögel. Journal für Ornithologie 36: 119. https://doi.org/10.1007/BF02002966

Casas-Marce M, Revilla E, Fernandes M, Rodríguez A, Delibes M, Godoy JA (2012) The Value of Hidden Scientific Resources: Preserved Animal Specimens from Private Collections and Small Museums. BioScience 62(12): 1077–1082. https://doi.org/10.1525/bio.2012.62.12.9

Cracraft J (1992) The species of the Birds-of-Paradise (Paradisaeidae): applying the phylogenetic species concept to a complex pattern of diversification. Cladistics 8: 1–43. https://doi.org/10.1111/j.1096-0031.1992.tb00049.x

Cracraft JL (2000) Charting the biosphere: Building global capacity for systematics science. In: Raven PH, Williams T (Eds) Nature and Human Society: the Quest for a sustainable World. National Academy Press, Washington, D.C., 374–386.

DOG (1940) Mitgliederverzeichnis der Deutschen Ornithologischen Gesellschaft 1940.

Duncker H (1953) Mitteilungen aus der Bremer Vogelsammlung. Abhandlungen des Naturwissenschaftlichen Vereins zu Bremen 33: 211–246.

Frith CB, Beehler BM (1998) The Birds of Paradise, Paradisaeidae. Bird families of the world. Oxford University Press, Oxford, New York, Tokyo.

Frith CB, Frith DW (2010) Birds of paradise: Nature, Art and History. Frith & Frith publications, Malanda.

Frodin DG (2007) Biological exploration of New Guinea. In: Marshall AJ, Beehler BM (Eds) The Ecology of Papua, Part I. Periplus Editions, Singapore, 14–107.

Fuller E (1995) The lost birds of paradise, Swan Hill Press, Shewsbury.

Giesecke H (2013) Nun muß sich alles wenden. Goslarer Allerlei 1948–1970 aus gesammelten Zeitungsberichten. Books on demand.

Greuter W, Naumann CM, Steininger FF, Breyer R, Häuser CL, Haas F (2005) Schatzkammern des Lebens und der Erde - Naturwissenschaftliche Forschungssammlungen in Deutschland. E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller), Stuttgart.

Hevers J (2008) Historische Vogelschau – Vogelkästen und Federbilder. Staatliches Naturhistorisches Museum, Braunschweig.

Knolle F (2015) Zur Geschichte des Hauses der Natur in Bad Harzburg. Braunlager Zeitung 10(100): 8.

Knolle F, Peinemann H (2015) Nachtrag zur Geschichte des Hauses der Natur. Braunlager Zeitung 10(101): 14.

Koch A, Bayer S, Joger U (2017) Die Kobra aus Querum: ein kurioses Zeugnis historischer Völkerschauen im Naturhistorischen Museum Braunschweig? Natur im Museum 7: 30–32.

Kovar-Eder J, Niedernostheide N (2014) Deutschlands naturkundliche Sammlungen - Erhaltung der Vielfalt als gesamtgesellschaftliche Aufgabe. Mitteilungen und Berichte aus dem Institut für Museumsforschung 52: 1–72.

LeCroy M (2014) Type specimens of birds in the American Museum of Natural History. Part 12. Passeriformes: Ploceidae, Sturnidae, Buphagidae, Oriolidae, Dicruridae, Callaeidae, Grallinidae, Corcoracidae, Artamidae, Cracticidae, Ptilonorhynchidae, Cnemophilidae, Paradisaeidae, and Corvidae. Bulletin of the American Museum of Natural History 393: 1–165. https://doi.org/10.1206/885.1

Lepage D (2015) Avibase – the world bird database. www.avibase.bsc-eoc.org [Last accessed 08.12.2015]

Linnaeus C (1758) Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Laurentius Salvius, Holmiæ (= Stockholm).

Mayr E (1945) Birds of paradise. Natural History 54: 264–276.

Mayr E (1963) Animal species and evolution. Harvard University Press, Cambridge. https://doi.org/10.4159/harvard.9780674865327

Mc Charty EM (2006) Handbook of Avian Hybrids of the World. Oxford University Press, Oxford.

Ménégaux HA (1913a) Description de deux nouveaux paradisers (*Paradisea Duivenbodei* et *P. raggiana sororia*) des collections du Muséum. Bulletin du Muséum national d'histoire naturelle 19: 145–147.

Ménégaux HA (1913b) Description de deux nouveaux paradisiers (*Paradisea duivenbodei* et *P. ragg. sodoria*). Revue française d'ornithologie 3(48): 49–51.

Ménégaux HA (1913c) A propos de deux nouveaux paradisiers (*P. duivenbodei et P. raggiana sororia*). Revue française d'ornithologie 3(55): 172–176.

Mužinić J, Bogdan JF, Beehler B (2009) Julije Klović: the first colour drawing of Greater Bird of Paradise *Paradisaea apoda* in Europe and its model. Journal of Ornithology 150: 645–649. https://doi.org/10.1007/s10336-009-0388-0

Paknia O, Rajaei ShH, Koch A (2015) Lack of well-maintained natural history collections and taxonomists in megadiverse developing countries hampers global biodiversity exploration. Organisms Diversity and Evolution 15: 619–629. https://doi.org/10.1007/s13127-015-0202-1

Reichenow A (1894) *Paradisea maria* n. sp. Ornithologische Monatsberichte 2: 22–23.

Reichenow A (1897) Zur Vogelfauna von Kaiser Wilhelms Land. Journal für Ornithologie 45(3): 201–224. https://doi.org/10.1007/BF02206692

Reichenow A (1901) Ein merkwürdiger Paradiesvogel. Ornithologische Monatsberichte 9: 185–186.

Romano M, Cifelli RL, Vai GB (2015) Natural history: first museologist's legacy. Nature 517: 271. https://doi.org/10.1038/517271c

Roselaar CS (2003) An inventory of major European bird collections. Bulletin of the British Ornithologists' Club 123A: 253–337.

Rothschild W (1898) Paradiseidae. In: Schulze FE (Ed.) Das Tierreich. R. Friedländer und Sohn, Berlin.

Rothschild W (1910) Mr Rothschild also exhibited a male of the Bird of Paradise known as *Paradisea maria* (…). Bulletin of the British Ornithologists' Club 27: 36.

Steinheimer FD (2003) Bamberg's Natural History Museum - The scientific Significance of small Collections. Bonner Zoologische Beiträge 51: 141–146.

Stresemann E (1923) Dr. Bürger's ornithologische Ausbeute im Stromgebiet des Sepik. Ein Beitrag zur Kenntnis der Vogelwelt Neuguineas. Archiv für Naturgeschichte, Abteilung A 89(7): 1–96.

Stresemann E (1925) Die Heimat der *Paradisaea maria* Reichenow. Ornithologische Monatsberichte 33: 128.

Stresemann E (1930a) Welche Paradiesvogelarten der Literatur sind hybriden Ursprungs? Novitates Zoologicae 36: 6–15.

Stresemann E (1930b) Welche Paradiesvogelarten der Literatur sind hybriden Ursprungs? Proceedings of the seventh international Ornithological Congress, Amsterdam: 284.

Stresemann E (1954) Die Entdeckungsgeschichte der Paradiesvögel. Journal für Ornithologie 3–4: 263–291. https://doi.org/10.1007/BF01961423

Swadling P (1996) Plumes from paradise: trade cycles in outer Southeast Asia and their impact on New Guinea and nearby islands until 1920. Papua New Guinea National Museum, Boroko.

Winker K, Withrow JJ (2013) Natural history: Small collections make a big impact. Nature 493: 480. https://doi.org/10.1038/493480b

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# Three new species of the '*Geophagus*' *brasiliensis* species group from the northeast Brazil (Cichlidae, Geophagini)

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## Abstract

Morphological characters and phylogenetic trees generated by analyses of segments of two mitochondrial genes cytochrome b and cytochrome c oxidase I support recognition of three new species of the '*Geophagus*' *brasiliensis* species group from coastal basins of northeast Brazil. All new species were diagnosed by exclusive morphological characters and exclusive nucleotide transformations. *Geophagus rufomarginatus* sp. n., from the Rio Buranhém Basin, is distinguished from all other species of the group by dorsal-fin lappets with red edges, the presence of longitudinal series of small light blue spots between the anal-fin spines and rays, and non-denticulated gill-rakers; it is closely related to *G. brasiliensis* and *G. iporangensis*. *Geophagus multiocellus* sp. n., from the Rio de Contas Basin, is distinguished from all other species of the group by having small pale blue spots with minute bright blue dots at their centres, that are often vertically coalesced to form short bars on the caudal fin. *Geophagus santosi* sp. n., from the Rio Mariana Basin, is distinguished from all other species of the group by having blue stripes parallel to the dorsal and anal fin rays on their longest portions. *Geophagus multiocellus* and *G. santosi* belong to the same clade of *G. itapicuruensis*. The clade composed by the Rio Paraguaçu Basin species was recovered as the sister group of the other species of the '*G.*' *brasiliensis* species group.

## Introduction

The Atlantic Forest is a biodiversity hotspot biome which has suffered degradation and drastic reduction throughout the history of human occupation and development of economic activities (Myers et al. 2000). Consequently, many components of its endemic biodiversity are currently under threat of extinction. This South American natural province (Morrone 2006) shows a high rate of endemism in its remaining fragments (Myers et al. 2000), harboring some endemic species of freshwater fishes, including cichlids (Kullander 2003, Lucena and Kullander 2006, Ottoni 2013, Ottoni and Costa 2008).

The cichlid tribe Geophagini is broadly distributed in South America and presents the greatest diversity among tribes of Neotropical cichlids, comprising 15 genera (López-Fernández et al. 2010). Included species occupy a wide range of ecological niches and exhibit remarkable morphological and behavioural adaptations (López-Fernández et al. 2013, Arbour and López-Fernández 2014). The type genus *Geophagus* Haeckel, 1940 has been diagnosed by the morphology of vertebrae, comprising the presence of epipleural ribs on caudal vertebrae, which are associated with expansions of the swim bladder, and caudal vertebrae more numerous than abdominal (Kullander 1986). However, these morphological features are not shared by all species of the genus. Presently, the genus has been divided into three species groups (Kullander 1998; López-Fernández and Taphorn 2004). Species that fit Kullander's (1986) generic diagnosis were assembled into the *Geophagus sensu stricto* species group, which includes the type species of the genus *Geophagus altifrons* Haeckel, 1840 and other species distributed in northern South America, including the Amazonas, Orino-

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co and Parnaíba river basins. Nevertheless, two species groups do not have those morphological characteristics: the '*Geophagus*' *steindachneri* species group, with a trans-Andean distribution between southern Panama and Maracaibo lake region in Venezuela, and the '*Geophagus*' *brasiliensis* species group, geographically widespread in eastern South America, mostly in the Atlantic Forest (Kullander 2003, Mattos et al. 2015). Recent phylogenetic studies (López-Fernández et al. 2010, Ilves et al. 2017) indicate that these three species groups together do not form a monophyletic lineage, and consequently, authors when describing new species of the last two groups have tentatively assigned them to '*Geophagus*', thus using the genus name between apostrophes to designate their uncertain position (Kullander 1998, López-Fernández et al. 2010, Ilves et al. 2017).

Currently, the '*G.*' *brasiliensis* species group comprises five valid species (Kullander 2003; Mattos et al. 2015): *G. iporangensis* Haseman, 1911, from the Rio Ribeira do Iguape Basin; *G. itapicuruensis* Haseman, 1911, from the Rio Itapicuru Basin; *G. obscurus* (Castelnau, 1855), from the coastal section of the Rio Paraguaçu Basin (Lucena and Kullander 2006); *G. diamantinensis* Mattos, Costa & Santos, 2015 from the upper section of the Rio Paraguaçu Basin; and '*G.*' *brasiliensis*, occurring in a broad area along the coastal basins between Bahia state, northeast Brazil, and the La Plata province, northeast Argentina (Kullander 2003, Mattos et al. 2015). The distribution of this species group covers a broad area of the Atlantic Forest and a small area of the Caatinga, a semiarid northeast Brazilian biome (Mattos et al. 2015). This study is the first analysis in which all valid species of the '*G.*' *brasiliensis* species group were sampled and analysed in a molecular phylogenetic framework, besides including populations of three unidentified species from the Atlantic Forest of northeast Brazil exhibiting unique morphological features, which are herein recognised and described as new.

# Material and methods

## Material

Measurements and counts follow Kullander (1986, 1990) and Kullander & Nijssen (1989). Measurements are presented as percentages of standard length (SL), except subunits of head, which are presented as percentages of head length (HL). Osteological preparations (C&S) were made according to Taylor and Van Dyke (1985). Osteological nomenclature follows Costa (2006). Material examined is deposited in the following ichthyological collections: Ichthyology collection of the Center for Agrarian and Environmental Sciences, Chapadinha (CICCAA); Museu Nacional, Rio de Janeiro (MNRJ); Institute of Biology, Federal University of Rio do Janeiro, Rio de Janeiro (UFRJ); Museum of Zoology of the State University of Feira de Santana, Feira de Santana (UEFS). Comparative material is listed in Mattos et al. (2015). The distribution map was generated using QGIS Geographic Information System, Open Source Geospatial Foundation Project, and the information of this map was based on our examined material and data provided by Mattos et al. 2015. Specimens were euthanized by immersion in a buffered solution of tricaine methane sulphonate (MS-222) at a concentration of 250 mg/L, for a period of 10 minutes, following the guidelines of the Journal of the American Veterinary Medical Association (AVMA Guidelines) (Leary et al. 2013) and European Commission DGXI consensus for fish euthanasia (Close et al. 1996, 1997). Tissue specimens for molecular analysis (DNA) were fixed and preserved in absolute ethanol just after collection.

## Species delimitation

The species delimitation methodology followed in this study aims to fulfil goals of integrative taxonomy. The character-based methodology for species delimitation was the Population Aggregation Analysis. It employs a unique combination of morphological character states to diagnose species. This method of species delimitation was formally described by Davis and Nixon (1992).

The PAA applied for molecular data in this study aimed the unique substitution nucleotide for each gene analysed (Costa and Amorim 2014, Costa et. al. 2014, Costa et. al. 2017). The character-state optimization among the '*G.*' *brasiliensis* species group and another included genus were performed using PAUP4 by most parsimonious reconstruction method (Swofford 1993). The relative numeric position was determined for each transformation through sequence alignment with the complete mitochondrial genome of *Astronotus ocellatus* (Agassiz, 1831) (Mabuchi et al. 2007). Plesiomorphic state for each species was presented before arrow and apomorphic state after the arrow.

The tree-based approach used for molecular data was proposed by Wiens and Penkrot (2002), in which species are delimited through well supported clades of haplotypes with concordant geographic distribution. The significance of the branches for species delimitation was evaluated by the support values, bootstrap values equal or higher than 70% as significant (Hillis and Bull 1993) and posterior probability of the branches values equal or higher than 0.95 as significant (Alfaro and Holder 2006).

## DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscular tissue of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen). Sequence fragments of cytochrome b (CYTB) with 1,100 bp and cytochrome c oxidase I (COI) with 680 bp were obtained. To amplify these DNA fragments, we used primers available in the literature (Farias et al. 2001). Polymerase chain reaction (PCR) was performed in 50 µl reaction mixtures containing 5× Green GoTaq Reaction Buffer (Promega), 3.2 mM MgCl2, 1 µM of each primer, 75 ng of total genomic DNA, 0.2 mM of each dNTP and 1U of Taq polymerase. The thermocycling profile was: (1) 1 cycle of 4 minutes

at 95 °C; (2) 35 cycles of 1 minute at 92 °C, 1 minute at 48–50 °C and 1 minute at 72 °C; and (3) 1 cycle of 4 minutes at 72 °C. Negative controls were used to check DNA contamination in all PCR reactions. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 10 µl reaction volumes containing 1 µl BigDye 2.5, 1.55 µl 5× sequencing buffer (Applied Biosystems), 2 µl of the amplified products (10–40 ng), and 2 µM of primer. The thermocycling profile was 30 cycles of 10 seconds at 96 °C, 5 seconds at 54 °C and 4 minutes at 60 °C. The sequencing reactions were purified, and the samples were run on an ABI 3130 Genetic Analyzer.

### Phylogenetics analysis

Sequences were edited using MEGA 6.0 (Tamura et al. 2013) and aligned using ClustalW (Chenna et al. 2003); subsequently sequences were translated into amino acids residues to verify the absence of premature stop codons or indels. The best-fit model of sequence evolution was calculated by JModelTest 2.1.7 (Darriba et al. 2012), which provided the same evolutive model for both genes fragments, General Time Reversible (Nei and Kumar 2000), with 5 rate categories and by assuming that a certain fraction of sites is evolutionarily invariable.

Phylogenetic analyses were performed using PAUP4 for maximum parsimony (MP), MrBayes v.3.2.1 (Ronquist et al. 2012) for Bayesian inference (BI) and GARLI v.2.0 (Zwickl 2006) for maximum likelihood (ML) methods for the mitochondrial concatenated dataset. MP was performed with branch-and-bound search algorithm; and tree branch support was given by bootstrap analysis, using a heuristic search with 1000 replicates and the same settings used in the MP search. ML searches for the best tree were performed in five independent replications with at least 10,000 generations, since no topology improvement was observed by adding more generations. ML tree branch support was calculated with 1000 non-parametric bootstrap replicates (Felsenstein 1985). BI was performed with the following settings: two Markov chain Monte Carlo (MCMC) runs of four chains each for 30 million generations, a sampling frequency of 1000. All parameters between partitions except topology and branch lengths were unlinked. The convergence of the MCMC chains were graphically assessed by evaluating the stationary phase of the chains using Tracer v. 1.5 (Rambaut et al. 2014). Consensus topology and posterior probabilities were obtained after applying a burn-in of the first 25% of the generated trees.

The molecular data matrix includes 27 terminal taxa of in-group terminals representing twelve populations scattered throughout the eastern range of the '*G.*' *brasiliensis* specie group distribution, including topotypes of all species. List of ingroup specimens and respective Gen-Bank accession numbers are shown in Table 1. Outgroups comprise seven species of five Geophagini genera closely related to the '*Geophagus*' *brasiliensis* species group (Smith et al. 2008, López-Fernández et al. 2010). Additional sequences of CYTB and COI for the following out-group taxa were obtained from GenBank (accession number): *Biotodoma wavrini* (Gosse, 1963) (GU736928/ EU888075); *Geophagus steindachneri* Eigenmann & Hildebrand, 1922 (AF370660/ DQ119217); *Geophagus surinamensis* (Bloch, 1791) (GU736944/ JN026709); *Gymnogeophagus gymnogenys* (Hensel, 1870) (GU736950/ EU888086); *Mikrogeophagus altispinosus* (Haseman, 1911) (GU736953/ EU888090); and *Mikrogeophagus ramirezi* (Myers & Harry, 1948) (AF370664/ KU568932.1).

**Table 1.** Vouchers and GenBank accession numbers for new sequenced material of *Geophagus*.

| Species | DNA sample voucher | GeneBank accesion number | | GenSeq Nomenclature |
|---|---|---|---|---|
| | | COI | CYTB | |
| *G. brasiliensis* | UFRJ 8365.1 | MH538060 | KT373984 | genseq-3 |
| | UFRJ 8365.2 | MH538061 | KT373985 | genseq-3 |
| | UFRJ 7925.1 | MH538062 | KT373988 | genseq-4 |
| | UFRJ 8251.1 | MH538063 | KT373987 | genseq-4 |
| | UFRJ 7738.2 | MH538064 | KT373986 | genseq-4 |
| *G. iporangensis* | UFRJ 8628.1 | MH538065 | MH538045 | genseq-4 |
| | UFRJ 8628.2 | MH538066 | MH538046 | genseq-4 |
| | UFRJ 8617.1 | MH538067 | MH538047 | genseq-3 |
| | UFRJ 8617.2 | MH538068 | MH538048 | genseq-3 |
| *G. rufomarginatus* | URFJ 9518.1 | MH538069 | MH538049 | genseq-2 |
| | URFJ 9519.1 | MH538070 | MH538050 | genseq-3 |
| | URFJ 9519.2 | MH538071 | MH538051 | genseq-3 |
| | URFJ 1103.1 | MH538072 | MH538052 | genseq-2 |
| | URFJ 1103.2 | MH538073 | MH538053 | genseq-2 |
| *G. itapicuruensis* | UFRJ 9442.1 | MH538074 | KT374000 | genseq-3 |
| | UFRJ 9442.2 | MH538075 | KT374001 | genseq-3 |
| | UFRJ 9442.3 | MH538076 | KT374002 | genseq-3 |
| | UFRJ 9442.4 | MH538077 | KT374003 | genseq-3 |
| *G. multiocellus* | UFRJ 8254.1 | MH538078 | MH538057 | genseq-2 |
| | UFRJ 8254.2 | MH538079 | MH538058 | genseq-2 |
| | UFRJ 8254.3 | MH538080 | MH538059 | genseq-2 |
| *G. santosi* | UFRJ 9998.1 | MH538081 | MH538054 | genseq-2 |
| | UFRJ 9998.2 | MH538082 | MH538055 | genseq-2 |
| | UFRJ 9998.3 | MH538083 | MH538056 | genseq-2 |
| *G. diamantinensis* | UFRJ 8245.1 | MH538084 | KT373992 | genseq-2 |
| | UFRJ 8245.2 | MH538085 | KT373993 | genseq-2 |
| | UFRJ 8245.3 | MH538086 | KT373994 | genseq-2 |
| | UFRJ 8245.4 | MH538087 | KT373995 | genseq-2 |
| *G. obscurus* | UFRJ 9440.1 | MH538088 | KT373998 | genseq-3 |
| | UFRJ 9440.2 | MH538089 | KT373999 | genseq-3 |
| | UFRJ 10026.1 | MH538090 | KT373996 | genseq-3 |
| | UFRJ 10026.1 | MH538091 | KT373997 | genseq-3 |

## Results

The MP, ML and IB analyses generated trees with the same topology (Fig. 1). All species of the '*G.*' *brasiliensis* species group were recovered as exclusive lineages:

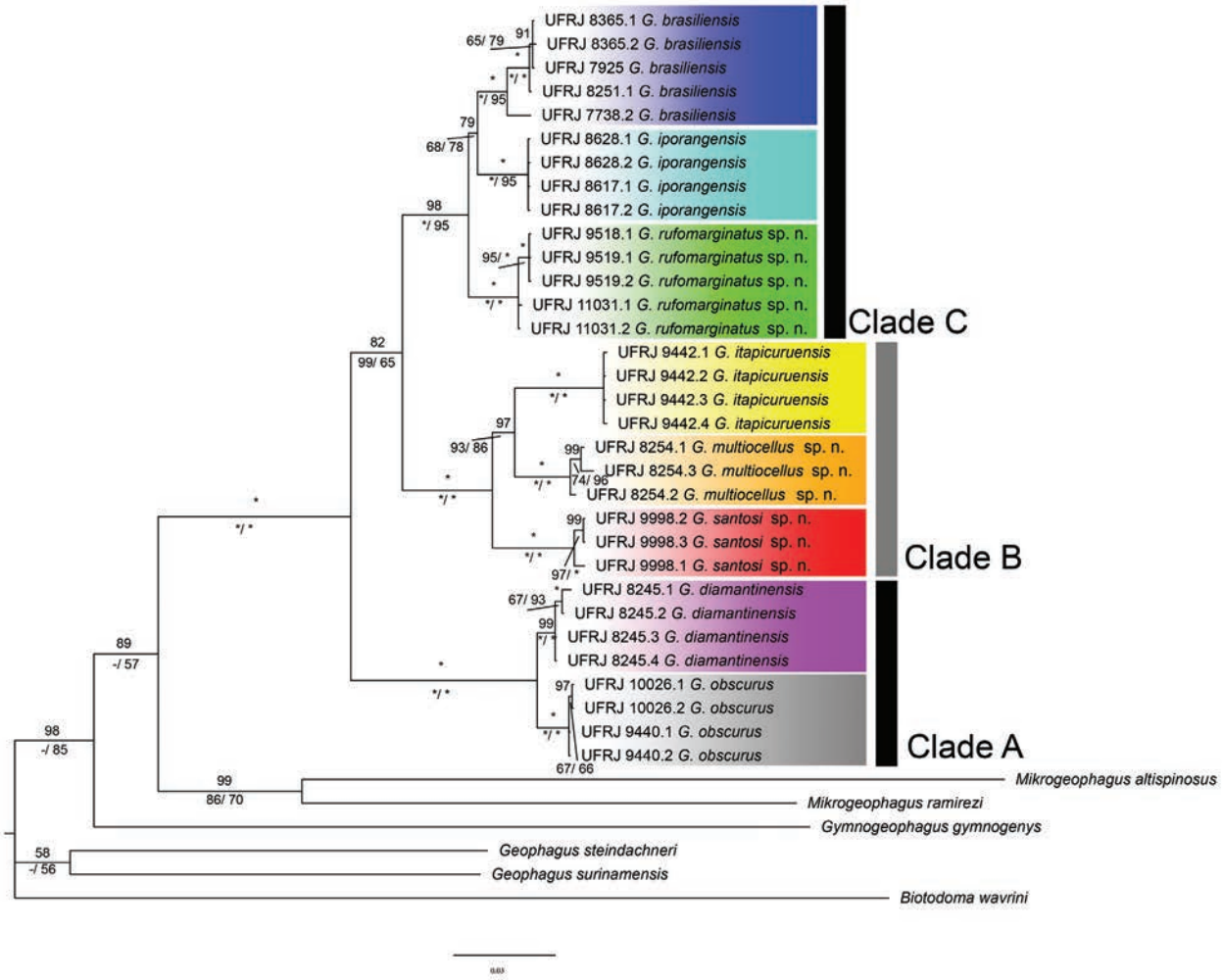


**Figure 1.** Tree topology estimated by Bayesian inference analysis for the '*Geophagus*' *brasiliensis* species group. Numbers before terminal species names are voucher numbers. Numbers above branches indicates Bayesian posterior probabilities and below bootstrap values of the Maximum Parsimony and Maximum Likelihood analyses, respectively, separated by bar. Dashes indicate values below 50 and asterisks indicate maximum support values.

*G. brasiliensis*, *G. iporangensis*, *G. itapicuruensis*, *G. obscurus* and *G. diamantinensis*. Additionally, three new species were congruently supported as exclusive lineages for the Rio de Contas, Rio Buranhém and Rio Mariana basins. The PAA analyses also supported the recognition of all the species mentioned above. The three new species were also supported by unique colour patterns and unique nucleotide substitutions.

The '*G.*' *brasiliensis* species group was recovered as a monophyletic group. Three main strongly supported clades were recovered within the '*G.*' *brasiliensis* species group: the clade A endemic to the Rio Paraguaçu Basin, comprising *G. obscurus* and *G. diamantinensis*; the clade B endemic to an area encompassing the Rio de Contas, Rio Itapicuru, and Rio Mariana basins and comprising *G. itapicuruensis* and two new species; and The clade C geographically widespread clade comprising *G. brasiliensis*, *G. iporangensis* and the new species from the Rio Buranhém Basin.

### *Geophagus rufomarginatus* sp. n.

http://zoobank.org/E402678B-DABD-4DF3-B013-84D00A71F5C6
Fig. 2, Table 2

**Material.** *Holotype*. UFRJ 9994, 97.8 mm SL; Brazil, Bahia state: Porto Seguro municipality: small stream crossing the road BA-001, Rio Buranhém Basin, 16°26'17"S, 39°10'47"W, altitude about 10 m asl; A. M. Katz, F. R. Pereira and J. L. O. Mattos, 20 July 2016.

Paratypes. UFRJ 11198, 6, 89.5–104.1 mm SL, 1, 94.3 mm SL (d&c); UFRJ 11031, 2, 15.9–41.6 mm SL (DNA); CICCAA 01378, 2, 94.3–97,6 mm SL; collected with holotype. UFRJ 9741, 1, 103.5 mm SL; UFRJ 9518, 7, 20.4–40.9 mm SL; Brazil, Bahia state: Eunápolis municipality: Rio Buranhém crossing the road BR-101, Rio Buranhém Basin, 16°24'47"S, 39°35'14"W, altitude about 65 m asl; F. R. Pereira and F. P. Ottoni, 23 June 2013. UFRJ 9519, 6, 17.3–40.7 mm SL (DNA); Brazil, Bahia state: Rio Buranhém under BA-001 road bridge, between

**Figure 2.** *Geophagus rufomarginatus*, UFRJ 9994, holotype, 96.8 mm SL; Brazil: Bahia: Rio Buranhém Basin. Scale bar 10 mm. Photograph by J.L.O. Mattos.

the towns of Porto Seguro and Trancoso, Rio Buranhém Basin, 16°23'32"S, 39°17'08"W, altitude about 20 m asl; F. R. Pereira and F. P. Ottoni, 24 June 2013.

**Diagnosis.** *eophagus rufomarginatus* is distinguished from all other species of '*G.*' *brasiliensis* group by having: dorsal-fin lappets with red edges (vs. grey or dark brown), presence of longitudinal series of small light blue spots between anal-fin spines and rays (vs. never this pattern), and non-denticulated gill-rakers (vs. denticulated). In addition, it is distinguished from all other species of the '*G.*' *brasiliensis* species group, except *G. obscurus* and *G. santosi*, by having an oblique iridescent blue zone between the humeral region and the anterior portion of the dorsal-fin base (vs. iridescent blue zone absent). It is also distinguished from *G. diamantinensis* by the absence of a dark brown mark on the humeral region (vs. presence); presence of iridescent blue to green spots on the opercular region (vs. whole opercular region golden); and absence of a horizontal dark brown band on the snout (vs. presence).

In addition, *G. rufomarginatus* is also distinguished from all other species of '*G.*' *brasiliensis* group by 13 unique nucleotide substitutions: COI 285 (T > C), COI 330 (T > C), COI 333 (T > C), COI 591 (A > C), COI 642 (C > T), CYTB 60 (C > T), CYTB 129 (C > T), CYTB 186 (C > T), CYTB 309 (C > T), CYTB 324 (A > G), CYTB 886 (T > C), CYTB 906 (A > G), CYTB 958 (C > T); it is similar to *G. iporangensis* and *G. brasiliensis* and distinguished from all other species of the '*G.*' *brasiliensis* group by four unique nucleotide substitutions: COI 700 (T > C), CYTB 165 (C > T), CYTB 582 (A > G), CYTB 1078 (A > C).

**Description.** Morphometric data appear in Table 2. Medium sized species, largest specimen examined 104.2 mm SL. Body relatively slender and compressed. Dorsal profile slightly convex on head, convex from nape to end of dorsal-fin base, approximately straight on caudal peduncle; no adipose nuchal protuberance. Ventral profile straight to slightly convex from lower jaw to pelvic-fin insertion, slightly convex between belly and end of anal-fin base, nearly straight on caudal peduncle. Caudal peduncle approximately as deeper as long. Greatest body depth at level of first dorsal-fin spine. Snout moderately pointed; nostrils located between tip of snout and anterior margin of orbit. Mouth subterminal, distal tip of maxilla not reaching vertical through anterior margin of orbit. Lower lip fold moderately deep. Lower jaw slightly shorter than upper one. Eye near dorsal profile of head. Opercle not serrated.

Insertion of first dorsal-fin spine slightly anterior to vertical line through posterior-most margin of opercle. Tip of dorsal-fin pointed, reaching 30–90% of caudal-fin length, shorter and rounded in specimens 40.0 mm SL or smaller. Tip of anal fin pointed, reaching 30–50% of caudal-fin length, shorter and rounded in specimens 43.0 mm SL or smaller. Caudal fin subtruncate. Pectoral fin trapezoidal with rounded extremity, posterior margin posteriorly surpassing flank blotch. Tip of pelvic-fin pointed, short, reaching insertion of 1st anal-fin spine in larger specimens, shorter and rounded in specimens 50.0 mm SL or smaller, reaching between urogenital papilla and insertion of first anal-fin spine. Pelvic-fin filaments absent. Anal-fin origin at vertical between insertion of 13th dorsal-fin spine and 1st dorsal- fin ray. Dorsal fin XIV + 12–13 (23); anal fin III + 9–10 (23); pectoral-fin rays 15 (23); pelvic fin I + 5 (26). Caudal-fin rays iv + 16 + iv (5).

**Table 2.** Morphometric data of *G. rufomarginatus*. H, holotype; SD, standard deviation. Values of holotype included in range.

| | H | range (n=8) | mean | SD |
|---|---|---|---|---|
| Standard length (mm) | 96.83 | 89.5–104.1 | – | – |
| **Percentage of standard length** | | | | |
| Body depth | 43.7 | 42.7–44.6 | 43.4 | 0.7 |
| Predorsal length | 47.3 | 43.7–47.3 | 45.8 | 1.3 |
| Dorsal-fin base length | 55.9 | 55.4–57.7 | 56.2 | 0.7 |
| Last dorsal-fin spine length | 15.3 | 15.1–16.7 | 15.8 | 0.6 |
| Prepelvic length | 43.4 | 41.6–44.5 | 43.1 | 1.0 |
| Pelvic-fin length | 34.9 | 31.7–34.9 | 32.9 | 1.2 |
| Pelvic-fin spine length | 16.2 | 15.0–16.8 | 15.9 | 0.7 |
| Pectoral-fin length | 31.5 | 31.4–32.8 | 32.1 | 0.5 |
| Anal-fin base length | 17.9 | 14.5–20.1 | 18.2 | 1.8 |
| Last anal-fin spine length | 15.3 | 14.5–16.0 | 15.5 | 0.5 |
| Caudal peduncle length | 13.5 | 13.5–17.3 | 14.8 | 1.4 |
| Caudal peduncle depth | 14.1 | 12.9–14.8 | 14.0 | 0.6 |
| Head length | 40.2 | 35.2–41.6 | 39.4 | 2.1 |
| **Percentage of head length** | | | | |
| Snout length | 50 | 46–52 | 49.4 | 2.3 |
| Preorbital depth | 69 | 64–69 | 67.6 | 1.6 |
| Head width | 46 | 46–50 | 48.1 | 1.5 |
| Head depth | 89 | 87–93 | 90.5 | 2.3 |
| Orbital diameter | 23 | 23–27 | 24.4 | 1.3 |
| Interorbital width | 26 | 26–31 | 28.5 | 1.8 |
| Upper jaw length | 35 | 31–35 | 33.5 | 1.4 |
| Lower jaw length | 30 | 28–30 | 29.2 | 0.9 |

Side of head covered with cycloid scales, ventral surface of head and snout without scales. Chest, trunk and caudal peduncle covered with ctenoid scales. Scales on head smaller than scales on chest and flank. Dorsal and anal fins without scales. About one fourth of caudal fin covered with small, delicate scales. Two scale rows between lateral lines. Scales of dorsal-fin origin row 5; scales of anal-fin origin row 6; longitudinal series of scales 26; cheek scale row 5; upper lateral line scales 18, lower lateral line scales 11 + 2; circum-peduncular scale rows 16.

Premaxillary teeth conical, hyaline with red tip, slightly curved posteriorly; one regular outer row of teeth, increasing in size on symphysis; proximal teeth smaller and irregularly arranged. Dentary teeth with similar arrangement, but slightly smaller. Five branchiostegal rays. Urohyal with strong anterior constriction. Gill-rakers on first branchial arch: first ceratobranchial 12, articulation 1, first epibranchial 9. Ceratobranchial rakers short, blunt and denticulated, except on fourth ceratobranchial proximal margin and fifth ceratobranchial distal margin, conical and non-denticulated. Anterior teeth of third pharyngobranchial and fifth ceratobranchial small, thin and slightly curved anteriorly, proximal posterior teeth large, robust and circular in cross section. Distal posterior teeth of the fifth ceratobranchial laterally compressed and with one or two cuspids. Five dentigerous plate on fourth pharyngobranchial. Fifth ceratobranchial subtriangular, with concave posterior margin. One supraneural. Proximal radial of dorsal fin 25 + 1; proximal radial of anal fin 10 + 1; pleural ribs 13, epipleural ribs 12; vertebrae 14 + 14.

**Colouration in life.** Flank yellowish brown with seven broad dark brown bars and one dark brown longitudinal stripe; dark brown bars and stripe often overlapped and without visible limits in live specimens, conspicuously delimited in preserved specimens. Pale blue iridescence on anteroventral portion of flank and small metallic blue dots on centre of scales of middle portion of flank and caudal peduncle. Rounded dark brown spot on fifth trunk bar, sometimes inconspicuous in live specimens; similar and smaller spot on middle of posterior portion of caudal peduncle. Oblique iridescent blue zone between humeral region and anterior portion of dorsal-fin base. Dorsum yellowish brown, chest and belly pinkish white.

Head greyish brown with ventral region lighter, branchiostegal region light red. Infra-orbital area with small metallic blue dots, most of them coalesced. Opercular region background colour yellowish brown. Opercular and temporal regions with few elliptical, small and large metallic blue spots spread through opercle. Iris golden brown, with dark brown bar through orbit not aligned to any portion of supra-orbital and infra-orbital stripes. Dark brown supra-orbital stripe extending from nape to posterodorsal margin of orbit, and dark brown infra-orbital stripe, approximately vertical, running between ventral margin of orbit and preopercle angle. Dorsal fin brownish yellow on basal portion, becoming reddish orange on distal and posterior portions, with metallic blue dots aligned between rays; marginal lappets with red edges; dark brown pigmentation concentrated at first two dorsal-fin spines. Anal fin reddish orange with small metallic blue spots, to brownish yellow with metallic blue lines parallel to rays and spine on anteriormost portion of fin; intense blue iridescence on distal portion of anal fin. Caudal fin reddish orange, to brownish yellow on posterodorsal corner, with small metallic blue rounded dots, vertically coalesced to form metallic blue bars on anterior portion; posterior margin dark bluish grey. Pectoral fin yellowish hyaline. Pelvic-fin spine light yellowish brown, anterior pelvic-fin rays light yellowish brown with metallic bluish stripes parallel to rays, region around last rays hyaline.

**Colouration in alcohol.** Similar to colouration in life, except for metallic marks becoming dark brown on flank and light grey on fins; red and dark brown pigmentation fainted.

**Distribution.** Known only from the middle and lower sections of the Rio Buranhém Basin, at altitudes of about 65 m above sea level or less, Bahia state, northeast Brazil (Fig. 3).

**Etymology.** From the Latin *marginatus* (edge, border, margin) and *rufo* (red), an allusion to the colour pattern in life of the dorsal-fin lappets.

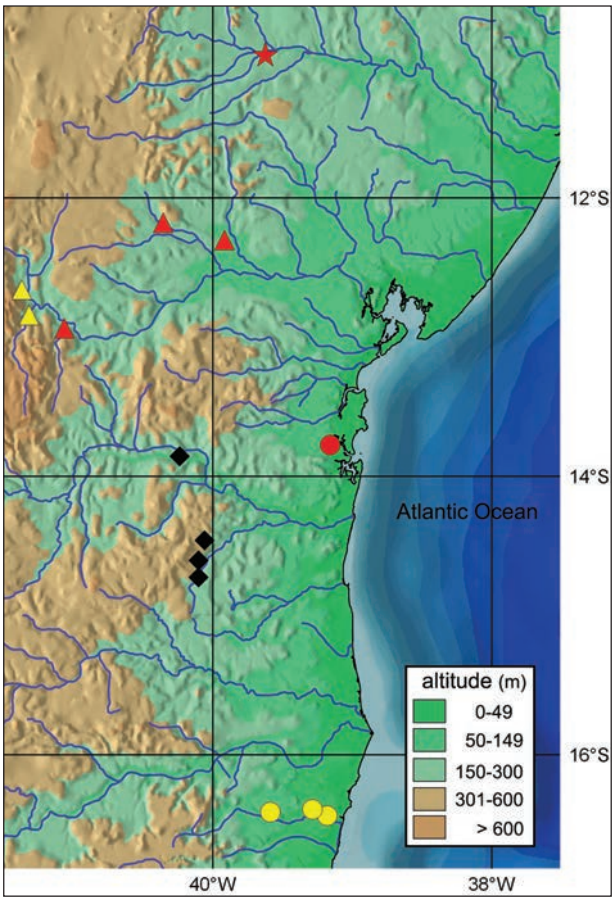


**Figure 3.** Geographical distribution of the '*Geophagus*' *brasiliensis* species group in northeast Brazil: red star, *G. itapicuruensis*; red triangle, *G. obscurus*; yellow triangle, *G. diamantinensis*; red circle, *G. santosi*; black diamond, *G. multiocellus*; yellow circle, *G. rufomarginatus*. Raw data set source was obtained from Natural Earth public domain (http://www.naturalearthdata.com).

### *Geophagus multiocellus* sp. n.

http://zoobank.org/873C4147-778E-4798-A8C8-FD89EE459969
Figs 4, 5, Table 3

**Material.** *Holotype*. UFRJ 11764, 101.4 mm SL; Brazil: Bahia state: Iguaí municipality: Guaíra balneary, Rio Cambiriba, Rio Gongogi drainage, Rio de Contas Basin, 14º36′17″ S 40º06′09″ W, altitude about 345 m asl; W. J. E. M. Costa et. al., 18 June 2011.

Paratypes. UFRJ 8217, 6, 57.4–102.9 mm SL; UFRJ 8254, 5, 26.5–41.9 mm SL (DNA); CICCAA 01379, 2, 78.9–82.7 mm SL; collected with holotype. UFRJ 8222, 5, 63.3–68.4 mm SL; UFRJ 8246, 2, 35.1–35.7 mm SL (DNA); Brazil: Bahia state: Nova Canaã municipality: small stream crossing the road BA-262, between the villages of Nova Canãa and Poções, Rio de Contas Basin, 14º43′33″ S 40º14′17″ W, altitude about 545 m asl; W. J. E. M. Costa et. al., 18 June 2011. MNRJ32263, 5, 7.8–9.6 mm SL, 1, 8.2 mm SL (C&S); Brazil: Bahia state: Poções municipality, stream of Rio Valentim drainage, Rio de Contas River Basin, 14°27'38"S, 40°03'34"W (approx.), altitude about 365 m asl; M. Cetra and M. Trindade. 02 February 2007. MNRJ 22302, 47, 6.2–101.1 mm SL, 2, 7.2–7.9 mm SL (C&S); Brazil; Bahia state: Jequié municipality: Rio de Contas Basin, 13°51'22"S, 40°4'58"W (approx.), altitude about 270 m asl; P. A. Buckup, A. T. Aranda and F. A. G. Melo. 12 August 2001.

**Diagnosis.** *Geophagus multiocellus* is distinguished from all other species of the '*G.*' *brasiliensis* group by having small pale blue spots with minute bright blue dots on its centre, often vertically coalesced to form short bars on the caudal fin (vs. never a similar pattern). In addition, it is distinguished from *G. rufomarginatus*, *G. obscurus* and *G. santosi* by the absence of an oblique iridescent blue zone between humeral region and anterior portion of dorsal-fin base (vs. iridescent blue zone present); from *G. rufomarginatus* by having dorsal-fin lappets with grey or dark brown edges (vs. red); presence of denticles on gill-rakers of the first branchial arch (vs. absence); absence of longitudinal series of small light blue spots between anal-fin spines and rays (vs. presence); from *G. santosi* by having blue bands crossing anal-fin rays (vs. blue bands parallel to fin rays); from *G. itapicuruensis* by having XIV dorsal-fin spines (vs. XIII); lateral spot rounded (vs. elliptical); absence of a horizontal dark brown band on snout (vs. presence); and from *G. brasiliensis* by having longitudinal blue bands crossing the anal-fin rays (vs. transversal blue bands crossing the anal-fin rays); mouth subterminal (vs. subdorsal).

*Geophagus multiocellus* is also distinguished from all other species of the '*G.*' *brasiliensis* group by ten unique nucleotide substitutions: COI 279 (C > T), COI 363 (G > A), CYTB 30 (C > T), CYTB 147 (A > G), CYTB 195 (C > T), CYTB 841 (C > T), CYTB 873 (C > T), CYTB 945 (A > G), CYTB 1014 (T > C) CYTB 1023 (A > G); it is similar to *G. itapicuruensis* and *G. santosi* and distinguished from all other species of the '*G.*' *brasiliensis* group by three unique nucleotide substitutions: COI 678 (A > G), CYTB 114 (A > G), CYTB 927 (A > G).

**Description.** Morphometric data appear in Table 3. Medium sized species, largest specimen examined 102.9 mm SL. Body relatively slender and compressed. Dorsal profile slightly convex on head, convex from nape to end of dorsal-fin base, approximately straight on caudal peduncle; no adipose nuchal protuberance. Ventral profile convex from lower jaw to pelvic-fin insertion, nearly straight between belly and insertion of first anal-fin spine, about straight on anal-fin base, gently concave on caudal peduncle. Caudal peduncle approximately as deeper as long. Greatest body depth slightly anterior to first dorsal-fin spine insertion. Snout moderately pointed; nostrils located between tip of snout and anterior margin of orbit. Mouth subterminal, distal tip of maxilla not reaching vertical through anterior margin of orbit. Lower lip fold

**Figure 4.** *Geophagus multiocellus*, UFRJ 11764, holotype, 101.4 mm SL; Brazil: Bahia: Rio de Contas Basin. Scale bar 10 mm. Photograph by J.L.O. Mattos.

moderately deep. Lower jaw slightly shorter than upper one. Eye near dorsal profile of head. Opercle not serrated.

Insertion of first dorsal-fin spine slightly anterior to vertical line through posterior-most margin of opercular series. Tip of dorsal fin pointed, reaching 35–50% of caudal-fin length, shorter and rounded in specimens 41.0 mm SL or smaller. Tip of anal fin pointed, reaching 20–50% of caudal-fin length, shorter and rounded in specimens 41.0 mm SL or smaller. Caudal fin subtruncate. Pectoral fin trapezoidal with rounded extremity, posterior margin posteriorly reaching vertical through posterior margin of flank blotch.

Tip of pelvic fin pointed, short, reaching insertion of 3rd anal-fin spine in larger specimens; shorter and rounded in specimens 50.0 mm SL or smaller, reaching between urogenital papilla and insertion of first anal-fin spine. Pelvic-fin filaments absent. Anal-fin origin at vertical between insertion of 13th dorsal-fin spine and 1st dorsal-fin ray. Dorsal fin XIV–XV + 11–12 (26); anal fin III + 8–9 (26); pectoral-fin rays 14–15 (26); pelvic fin I + 5 (26). Caudal-fin rays vi + 16 + iii (4).

Side of head covered with cycloid scales, ventral surface of head and snout without scales. Chest, trunk and caudal peduncle covered with ctenoid scales. Scales on head smaller than scales on chest and flank. Dorsal and anal fins without scales. About one fourth of caudal fin covered with small delicate scales. Two scale rows between lateral lines. Scales of dorsal-fin origin row 5; scales of anal-fin origin row 6; longitudinal series of scales 26; cheek scale row 5; upper lateral line scales 18, lower lateral line scales 11 + 2; circum-peduncular scale rows 16.

Premaxillary teeth conical, hyaline with red tip, slightly curved posteriorly; one regular, outer row of teeth, increasing in size on symphysis; proximal teeth smaller and irregularly arranged. Dentary teeth with similar arrangement, but slightly smaller. Five branchiostegal rays. Urohyal with strong anterior constriction. Gill-rakers on first branchial arch: first ceratobranchial 11, articulation 1, first epibranchial 8. Ceratobranchial rakers short, blunt and denticulated, except on fourth ceratobranchial proximal margin and fifth ceratobranchial distal margin, conical and non-denticulated. Anterior teeth of third pharyngobranchial and fifth ceratobranchial small, thin and slightly curved anteriorly, posterior teeth larger, robust and circular in cross section. Distal posterior teeth of the fifth ceratobranchial laterally compressed and with one or two cuspids. Five or six dentigerous plate on fourth pharyngobranchial, with three or four fused. Fifth ceratobranchial subtriangular, with concave posterior margin. One supraneural. Proximal radial of dorsal fin 25 + 1; proximal radial of anal fin 8 + 1; pleural ribs 12; epipleural ribs 12; vertebrae 14 + 14.

**Colouration in life.** Flank greyish brown with seven broad dark brown bars and one dark brown longitudinal stripe; dark brown bars and stripe often overlapped and without visible limits in live specimens, conspicuously delimited in preserved specimens. Longitudinal rows of golden spots on ventral part of flank, between pectoral-fin insertion and caudal-fin base; spots approximately occupying ventral half-length of scales. Rounded dark brown spot on fifth trunk bar, similar and smaller spot on middle of caudal peduncle. Humeral region with three metallic blue spots arranged in oblique row. Dorsum greyish brown, chest and belly greyish white.

Head greyish brown, ventral region lighter, branchiostegal region greyish white. Infra-orbital area with oblique row of small metallic greenish blue spots. Opercular region background colour greyish brown. Absence or up to five small elliptical metallic greenish blue spots spread through opercle. Iris golden brown, with greenish blue iridescence on anterior and posterior portions, and dark brown bar through orbit aligned with sub-orbit-

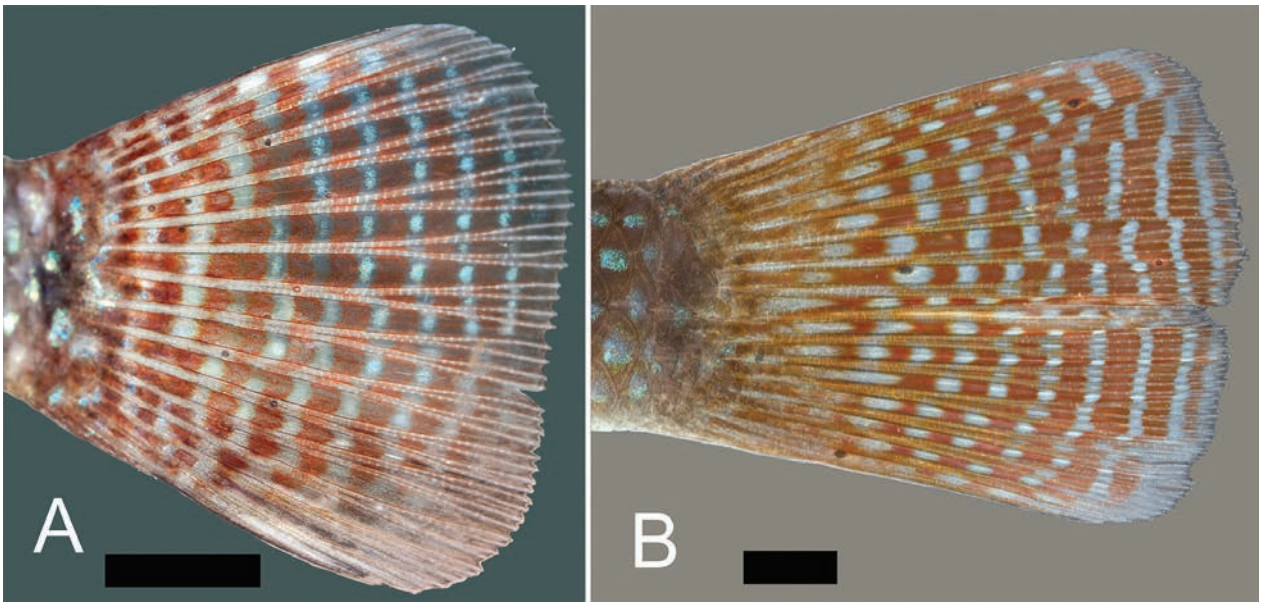


**Figure 5.** Caudal fin colour pattern. **A** *Geophagus multiocellus*, UFRJ 8217, topotype. **B** *Geophagus santosi*, UFRJ 9998, topotype. Scale bar 10 mm. Photographs by J.L.O. Mattos.

**Table 3.** Morphometric data of *G. multiocellus*. H, holotype; SD, standard deviation. Values of holotype included in range.

| | H | range (n=10) | mean | SD |
|---|---|---|---|---|
| Standard length (mm) | 101.0 | 57.4-102.9 | – | – |
| **Percentage of standard length** | | | | |
| Body depth | 40.8 | 39.6-41.2 | 40.3 | 0.5 |
| Predorsal length | 45.5 | 42.4-47.4 | 45.0 | 1.9 |
| Dorsal-fin base length | 52.8 | 52.5-56.7 | 53.9 | 1.6 |
| Last dorsal-fin spine length | 14.1 | 12.5-14.4 | 13.6 | 0.6 |
| Prepelvic length | 43.4 | 42.1-44.6 | 43.4 | 1.0 |
| Pelvic-fin length | 29.3 | 26.2-44.3 | 30.2 | 5.4 |
| Pelvic-fin spine length | 12.5 | 12.1-15.2 | 13.4 | 1.0 |
| Pectoral-fin length | 31.1 | 29.8-32.5 | 31.3 | 0.7 |
| Anal-fin base length | 17.5 | 16.6-18.4 | 17.4 | 0.6 |
| Last anal-fin spine length | 14.0 | 11.8-14.4 | 13.5 | 0.8 |
| Caudal peduncle length | 12.4 | 10.7-14.2 | 12.8 | 1.0 |
| Caudal peduncle depth | 13.5 | 13.2-14.6 | 13.9 | 0.5 |
| Head length | 38.0 | 35.1-38.4 | 37.1 | 1.1 |
| **Percentage of head length** | | | | |
| Snout length | 56 | 48-56 | 51.4 | 3.1 |
| Preorbital depth | 70 | 63-72 | 67.6 | 3.3 |
| Head width | 55 | 52-58 | 55.2 | 1.8 |
| Head depth | 91 | 87-98 | 90.6 | 2.9 |
| Orbital diameter | 24 | 24-31 | 27.1 | 1.9 |
| Interorbital width | 31 | 28-32 | 29.9 | 1.3 |
| Upper jaw length | 35 | 30-37 | 33.6 | 1.6 |
| Lower jaw length | 27 | 25-31 | 27.5 | 2.6 |

al stripe. Pale brown supra-orbital stripe extending from dorsal profile to postero-dorsal margin of orbit, and dark brown infra-orbital stripe, approximately vertical, running from ventral margin of orbit to sub-opercle. Dorsal fin brownish red; anterior portion with short, oblique metallic blue stripes, posterior region with transverse rows of small pale blue spots; dark brown pigmentation concentrated at first two dorsal-fin spines and distal half of third spine. Anal fin brownish red, with oblique metallic blue stripes, posterior-most region with longitudinal rows of small, elongated pale blue spots. Caudal fin brownish red with small pale blue spots with minute bright blue dots on its centre, often vertically coalesced to form short bars. Pectoral fin pale yellowish hyaline. Pelvic-fin spine light yellowish brown, anterior pelvic-fin rays light yellowish brown with metallic bluish stripes parallel to rays, region around last rays hyaline.

**Colouration in alcohol.** Similar to colouration in life, except for metallic blue marks becoming dark brown on flank and light grey on fins; red and dark brown pigmentation faded.

**Distribution.** Known only from the middle section of the Rio de Contas Basin, in altitudes between about 270 and 545 m above sea level, Bahia state, northeast Brazil (Fig. 3).

**Etymology.** From the Latin *multum* (several) and *ocellus* (little eyes, jewels), an allusion to the presence of small pale blue spots with minute bright blue dots on its centre on the caudal fin.

### *Geophagus santosi* sp. n.

http://zoobank.org/AEAE1FF0-0A2C-4F98-B9C3-8F5EBAC8AA6D
Figs 5, 6, Table 4

**Material.** *Holotype.* UFRJ 11765, 99.7 mm SL; Brazil: Bahia state: Ituberá municipality: Rio Mariana upstream of Cachoeira da Pancada, Área de Proteção Ambiental Michelin, 13°46’32"S, 39°09’29"W, altitude about 15 m asl; W. J. E. M. Costa et. al., 21 February 2014.

Paratypes. UFRJ 9998, 3, 92.0-113.4 mm SL (DNA); CICCAA 01380, 1, 99.5 mm SL; collected with holotype. UEFS 10336, 2, 78.1–94.4 mm SL, 1, 78.06 mm SL (C&S); UEFS 10519, 1, 115.0 mm SL, 1, 58.2 mm SL (C&S); UEFS 11585, 8, 69.5–148.4 mm SL; UEFS 10098, 7, 114.3–164.3 mm SL; Brazil: Bahia state: Ituberá municipality: Rio Mariana, Michelin APA, approximately 13°46'42"S, 39°09'32"W (approx.), altitude about 15 m asl; A. C. A. Santos et al., October 2007.

**Diagnosis.** *Geophagus santosi* is distinguished from all other species of the '*G.*' *brasiliensis* group by having dorsal and anal fins with blue stripes parallel to fin rays on their longest portion (vs. transverse blue bands crossing rays or fins with dots), and basal portion of caudal-fin with short, longitudinal bluish-white lines (vs. dots or bars). *Geophagus santosi* is similar to *G. rufomarginatus* and *G. obscurus*, and distinguished from all other species of the '*G.*' *brasiliensis* group, by the presence of an oblique iridescent blue zone between the humeral region and the anterior portion of the dorsal-fin base (vs. absence of an iridescent blue zone). Furthermore, it is also distinguished from *G. obscurus* by the presence of an oblique suborbital row of aligned, small iridescent blue marks, not extending to cheek (vs. suborbital iridescent blue marks irregularly arranged extending to the cheek) and chest profile straight in lateral view (vs. convex); from *G. rufomarginatus* by possessing dorsal-fin lappets with grey or dark brown edge (vs. red) and presence of denticles on the first branchial arch gill-rakers (vs. absence); from *G. itapicuruensis* by having XIV spines on dorsal fin (vs. XIII) and lateral spot rounded (vs. elliptical); from *G. diamantinensis* by the absence of a dark brown mark on the humeral region (vs. presence), absence of a horizontal dark brown band on the snout (vs. presence), and urohyal bone with strong constriction (vs. with gentle anterior constriction); and from *G. brasiliensis* by having a terminal mouth (vs. sub-dorsal).

*Geophagus santosi* is also distinguished from all species of '*G.*' *brasiliensis* group by 20 unique nucleotide substitutions: COI 143 (T > C), COI 291 (A > G), COI 523 (G > A) COI 564 (T > A) COI 589 (C >T), CYTB 69 (A >G), CYTB 78 (C > T), CYTB 231 (A >G), CYTB 279 (C > T), CYTB 297 (A > C), CYTB 327 (C > T), CYTB 447 (C >A), CYTB 606 (A > G), CYTB 609 (C > T), CYTB 687 (A > G), 735 (C > T), CYTB 801 (T > C), CYTB 852 (T > C), CYTB 915 (A > T), CYTB 1090 (A > G). It is similar to *G. itapicuruensis* and *G. multiocellus* and distinguished from all other species of '*G.*' *brasiliensis* group by three unique nucleotide substitutions: COI 678 (A > G), CYTB 114 (A > G), CYTB 927 (A > G).

**Description.** Morphometric data appear in Table 4. Medium sized species, largest specimen examined 164.3 mm SL. Body relatively slender and compressed. Dorsal profile slightly convex on head, convex from nape to end of dorsal-fin base, approximately straight on caudal peduncle; no adipose nuchal protuberance. Ventral profile convex from lower jaw to pelvic-fin insertion, gently straight between belly and insertion of first anal-fin spine, nearly straight on anal-fin base, nearly concave on caudal peduncle. Caudal peduncle slightly longer than deep. Greatest body depth at level of first dorsal-fin spine insertion. Snout moderately pointed; nostrils located between tip of snout and anterior margin of orbit. Mouth subterminal, distal tip of maxilla not reaching vertical through anterior margin of orbit. Lower lip fold moderately deep. Lower jaw slightly shorter than upper one. Eye near dorsal profile of head. Opercle not serrated.

Insertion of first dorsal-fin spine slightly anterior or aligned in a vertical line through posterior-most margin of opercular series. Tip of dorsal fin pointed, short, reaching 20–40% of caudal-fin length, even in larger specimens. Tip of anal fin pointed, reaching 20–40% of caudal-fin length. Caudal fin subtruncate. Pectoral fin trapezoidal with rounded extremity, posterior margin posteriorly surpassing flank blotch. Tip of pelvic-fin rounded or pointed, relatively short and reaching between urogenital papilla and insertion of 3rd anal-fin spine. Pelvic-fin filaments absent. Anal-fin origin at vertical between insertion of 13th and 14th dorsal-fin spine. Dorsal fin XIV + 13 (25); anal fin III + 9–10 (25); pectoral-fin rays 15–16 (25); pelvic fin I + 5 (25). Caudal-fin rays vi + 16 + vi (3).

Side of head covered with cycloid scales, ventral surface of head and snout without scales. Chest, trunk and caudal peduncle covered with ctenoid scales. Scales on head smaller than scales on chest and flank. Dorsal and anal fins without scales. About one fifth of caudal fin covered with small delicate scales. Two scale rows between lateral lines. Scales of dorsal-fin origin row 4; scales of anal-fin origin row 5; longitudinal series of scales 26–27; cheek scale row 5; upper lateral line scales 18, lower lateral line scales 9–11 + 2; circum-peduncular scale rows 16.

Premaxillary teeth conical, hyaline with red tip, slightly curved posteriorly; one regular, outer row of teeth, increasing in size on symphysis; proximal teeth smaller and irregularly arranged. Dentary teeth with similar arrangement, but slightly smaller. Five branchiostegal rays. Urohyal with strong anterior constriction. Gill-rakers on first branchial arch: first ceratobranchial 10, articulation 1, first epibranchial 8. Ceratobranchial rakers short, blunt and denticulated, except on fourth ceratobranchial proximal margin and fifth ceratobranchial distal margin, conical and non-denticulated. Anterior teeth of third pharyngobranchial and fifth ceratobranchial small, thin and slightly curved anteriorly, posterior teeth large, robust and circular in cross section. Distal posterior teeth of the fifth ceratobranchial laterally compressed and with one or two cuspids. Five or six dentigerous plate on fourth pharyngobranchial, two of them could merge. Fifth ceratobranchial subtriangular, with concave posterior margin and robust. One supraneural. Proximal radial of dorsal fin 24 + 1; proximal radial of anal fin 10 + 1; pleural ribs 12, epipleural ribs 11; vertebrae 14 + 14.

**Colouration in life.** Flank orangish brown with seven broad dark brown bars and one dark brown longitudinal

**Figure 6.** *Geophagus santosi*, UFRJ 11765, holotype, 110.6 mm SL; Brazil: Bahia: Mariana River. Scale bar 10 mm. Photograph by J.L.O. Mattos.

stripe; dark brown bars and stripe often overlapped and without visible limits in live specimens, conspicuously delimited in preserved specimens. Longitudinal rows of metallic light green spots on ventral part of flank, between pectoral-fin insertion and caudal-fin base; spots approximately occupying most scale area. Rounded dark brown spot on fifth trunk bar, similar and smaller spot on middle of caudal peduncle. Oblique iridescent blue zone between humeral region and anterior portion of dorsal-fin base. Dorsum dark orangish brown, chest and belly light pinkish white.

Head side dark orange, ventral surface white; branchiostegal region dark orangish grey. Infra-orbital area with row of four to six small metallic greenish blue dots, sometimes two or three dots coalesced. Opercular region background orangish brown; opercular and temporal regions with scattered metallic greenish blue spots. Iris yellowish brown, with greenish blue iridescence on anterior and posterior portions, and dark brown bar through orbit not aligned to supra-orbital and infra-orbital stripes. Dark brown supra-orbital stripe extending from nape to postero-dorsal margin of orbit, and dark brown infra-orbital stripe, approximately vertical, running between ventral margin of orbit and pre-opercle angle. Dorsal fin pale brown on anterior portion, pale yellow on middle, pale orange on posterior region; oblique series of elongate drop-shaped metallic green spots on anterior two thirds of fin, light blue stripes parallel to fin rays on longest region of fin, and longitudinal rows of rounded light blue spots on posterior portion of fin; dark brown pigmentation most concentrated at first two dorsal-fin spines and distal half of third spine. Anal fin reddish orange, to yellowish orange on basal portion, with longitudinal metallic blue stripes between rays, and metallic blue spots on posterior region. Caudal fin reddish orange with transverse rows of small bluish white spots often coalesced to form narrow bars; basal portion of fin light yellowish orange with short, longitudinal bluish white lines. Pectoral fin pale

**Table 4.** Morphometric data of *G. santosi*. H, holotype; SD, standard deviation. Values of Holotype included in range.

| | H | range (n=15) | mean | SD |
|---|---|---|---|---|
| Standard length (mm) | 110.6 | 78.1–153.4 | – | – |
| **Percentage of standard length** | | | | |
| Body depth | 41.1 | 37.3–43.9 | 41.2 | 1.9 |
| Predorsal length | 43.7 | 42.6–47.8 | 45.1 | 1.6 |
| Dorsal-fin base length | 54.3 | 51.9–58.2 | 54.5 | 1.5 |
| Last dorsal-fin spine length | 15.2 | 12.9–16.0 | 14.4 | 0.9 |
| Prepelvic length | 45.6 | 41.8–47.0 | 45.0 | 1.5 |
| Pelvic-fin length | 30.9 | 25.6–34.1 | 29.1 | 2.4 |
| Pelvic-fin spine length | 13.4 | 11.7–14.4 | 13.0 | 0.8 |
| Pectoral-fin length | 31.4 | 28.0–33.6 | 29.2 | 8.5 |
| Anal-fin base length | 18.7 | 16.8–19.1 | 17.8 | 0.8 |
| Last anal-fin spine length | 13.3 | 12.5–15.1 | 13.1 | 0.7 |
| Caudal peduncle length | 17.3 | 12.3–17.5 | 15.4 | 2.0 |
| Caudal peduncle depth | 14.4 | 13.4–15.3 | 14.3 | 0.5 |
| Head length | 38.9 | 36.8–41.4 | 39.0 | 1.5 |
| **Percentage of head length** | | | | |
| Snout length | 54 | 48–59 | 54.0 | 3.2 |
| Preorbital depth | 71 | 65–78 | 69.8 | 3.9 |
| Head width | 56 | 41–56 | 50.0 | 5.6 |
| Head depth | 89 | 84–95 | 88.5 | 3.6 |
| Orbital diameter | 23 | 18–27 | 23.7 | 2.8 |
| Interorbital width | 28 | 28–35 | 30.4 | 1.8 |
| Upper jaw length | 33 | 30–36 | 32.8 | 1.4 |
| Lower jaw length | 29 | 27–32 | 28.8 | 1.2 |

orangish hyaline. Pelvic-fin spine light orangish brown, anterior pelvic-fin rays light orangish brown with metallic greenish blue stripes parallel to rays, region around last rays hyaline.

**Colouration in alcohol.** Similar to colouration in life, except for metallic marks becoming dark brown on flank and light grey on fins; red and dark brown pigmentation fainted.

**Distribution.** Known only from the Rio Mariana, an isolated small coastal river of Bahia state, northeast Brazil (Fig. 3).

**Etymology.** The name *santosi* is in honour of Alexandre Clistenes Alcântara Santos, ichthyologist and friend, who is dedicated to the study of aquatic ecosystems of northeast Brazil.

## Discussion

This study demonstrated that short fragments of the mitochondrial genome, with a total of 1780 bp, were enough to produce phylogenetic trees strongly supporting mutually exclusive lineages designated as species, as well as recognizing species clades with high support values (Fig. 1). However, presently no morphological character is known to unambiguously diagnose those clades.

Among the three main clades of the '*G.' brasiliensis* species group, the two species endemic to the Rio Paraguaçu Basin, *G. diamantinensis* and *G. obscurus*, form a well-supported basal clade (clade A), restricted to semi-arid areas of northeastern Brazil (Figs. 1 and 3). Interestingly, the analyses support another clade endemic to northeastern Brazil, between about 11° and 15° S, comprising *G. itapicuruensis* + *G. santosi* + *G. multiocellus* (clade B) that is sister to a geographically disjunct clade comprising *G. brasiliensis* + *G. rufomarginatus* + *G. iporangensis* (clade C), occurring in a vast area between about 16° and 35° S. Although a biogeographic analysis is beyond the scope of this study, the occurrence of two distinct basal lineages in northeastern Brazil, highly suggests that the most recent common ancestor of the '*G.' brasiliensis* species group was geographically restricted to northeastern Brazil.

The analyses also indicated that the main clades of the '*G.' brasiliensis* species group cannot be associated with specific biomes or phytogeographical provinces, in contrast to that recently reported for fish groups inhabiting temporary pools (Costa et al. 2017). Although the two species of the clade A being endemic to a semi-arid Caatinga area, only *G. itapicuruensis* inhabits a typical Caatinga area among species of the clade B. The other species of the clade C, *G. santosi* and *G. multiocellus*, are found in a transitional area of the Atlantic Forest known as Agreste. On the other hand, species of the clade C are found in different biomes such as Atlantic Forest, Cerrado and Pampas. Palynological studies have demonstrated a succession of different vegetation formations along the Pleistocene/Holocene of northeastern Brazil (Oliveira et al. 1999). Since members of different lineages of the '*G.' brasiliensis* species group are presently found in habitats such as rain forests and semi-arid regions, we conclude that vegetation changes following different climatic periods may have not affected fishes inhabiting rivers.

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## References

Alfaro ME, Holder MT (2006) The posterior and the prior in Bayesian phylogenetics. Annual Review of Ecology, Evolution, and Systematics 37: 19–42. https://doi.org/10.1146/annurev.ecolsys.37.091305.110021

Arbour JH, López-Fernández H (2014) Ecological variation in South American geophagine cichlids arose during an early burst of adaptive morphological and functional evolution. Proceedings of the Royal Society B 280: 20130849. http://dx.doi.org/10.1098/rspb.2013.0849

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31: 3497–3500. https://doi.org/10.1093/nar/gkg500

Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D, Warwick C (1996) Recommendations for euthanasia of experimental animals: Part 1. Laboratory Animals 30: 293–316. https://doi.org/10.1258/002367796780739871

Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D, Warwick C (1997) Recommendations for euthanasia of experimental animals: Part 2. Laboratory Animals 3: 1–32. https://doi.org/10.1258/002367797780600297

Costa WJEM (2006) Descriptive morphology and phylogenetic relationships among species of the Neotropical annual killifish genera *Nematolebias* and *Simpsonichthys* (Cyprinodontiformes: Aplocheiloidei: Rivulidae). Neotropical Ichthyology 4: 1–26. http://dx.doi.org/10.1590/S1679-62252006000100001

Costa WJEM, Amorim PF (2014) Integrative taxonomy and conservation of seasonal killifishes, *Xenurolebias* (Teleostei: Rivulidae), and the Brazilian Atlantic Forest. Systematics and Biodiversity 12: 350–365. https://doi.org/10.1080/14772000.2014.918062

Costa WJEM, Amorim PF, Aranha GN (2014) Species limits and DNA barcodes in *Nematolebias*, a genus of seasonal killifishes threatened with extinction from the Atlantic Forest of south-eastern Brazil, with description of a new species (Teleostei: Rivulidae). Ichthyological Exploration of Freshwaters 24: 225–236.

Costa WJEM, Cheffe MM, Amorim PF (2017) Two new seasonal killifishes of the *Austrolebias adloffi* group from the Lagoa dos Patos Basin, southern Brazil (Cyprinodontiformes: Aplocheilidae). Vertebrate Zoology 67: 139–149.

Costa WJEM, Amorim PF, Mattos JLO (2017) Molecular phylogeny and timing of diversification in South American Cynolebiini seasonal killifishes. Molecular Phylogenetics and Evolution 116: 61–68. https://doi.org/10.1016/j.ympev.2017.07.020

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest2: More models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Davis JI, Nixon KC (1992) Populations, genetic variation, and the delimitation of phylogenetic species. Systematic Biology 41: 421–435. https://doi.org/10.1093/sysbio/41.4.421

Farias IP, Ortí G, Sampaio L, Schneider H, Meyer A (2001) The cytochrome b gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. Journal of Molecular Evolution 53: 89–103. https://doi.org/10.1007/s002390010197

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192. https://doi.org/10.1093/sysbio/42.2.182

Ilves KL, Torti D, López-Fernández H (2017) Exon-based phylogenomics strengthens the phylogeny of Neotropical cichlids and identifies remaining conflicting clades (Cichliformes: Cichlidae: Cichlinae). Molecular Phylogenetics and Evolution 118: 232–243. https://doi.org/10.1016/j.ympev.2017.10.008

Mabuchi K, Miya M, Azuma Y, Nishida M (2007) Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. BMC Evolutionary 7: 10. https://doi.org/10.1186/1471-2148-7-10

Kullander SO (1986) Cichlid fishes of the Amazon river drainage of Peru. Swedish Museum of Natural History, Stockholm, 431 pp.

Kullander SO (1998) A phylogeny and classification of the south american Cichlidae (Teleostei: Perciformes). In: Malabarba LR, Reis RE, Vari RP, Lucena ZM, Lucena CAS (Eds) Phylogeny and classification of Neotropical fishes, EDIPUCRS, Porto Alegre, 461–498.

Kullander SO (2003) Family Cichlidae (Cichlids). In: Reis RE, et al. (Eds) Check list of the freshwater fishes of South and Central America. EDIPUCRS, Porto Alegre, 605–654.

Lucena CAS, Kullander SO (2006) A review of the species of *Crenicichla* (Teleostei: Cichlidae) from the Atlantic coastal rivers of southeastern Brazil from Bahia to Rio Grande do Sul States, with descriptions of three new species. Neotropical Ichthyology 4: 127–146. https://doi.org/10.1590/S1679-62252006000200001

Kullander SO, Nijssen H (1989) The cichlids of Surinam (Teleostei: Labroidei). Brill, Leiden, 256 pp.

Leary S, Underwood W, Anthony R, Cartner S, Corey D, Grandin T (2013) AVMA Guidelines for the Euthanasia of Animals (2013 edn). http://works.bepress.com/cheryl_greenacre/14.

López-Fernández H, Arbour JH, Winemiller KO, Honeycutt RL (2013) Testing for ancient adaptive radiation in Neotropical cichlid fishes. Evolution 67: 1321–1337. https://doi.org/10.1111/evo.12038

López-Fernández H, Taphorn DC (2004) *Geophagus abalios*, *G. dicrozoster* and *G. winemilleri* (Perciformes: Cichlidae), three new species from Venezuela. Zootaxa 439: 1–27. https://doi.org/10.11646/zootaxa.439.1.1

López-Fernández HK, Winemiller O, Honeycut RL (2010) Multilocus phylogeny and rapid radiations in neotropical cichlid fishes (Perciformes: Cichlidae: Cichlinae). Molecular Phylogenetics and Evolution 55: 1070–1086. https://doi.org/10.1016/j.ympev.2010.02.020

Mattos JLO, Costa WJEM, Santos ACA (2015) *Geophagus diamantinensis*, a new species of the *G. brasiliensis* species group from Chapada Diamantina, north-eastern Brazil (Cichlid: Geophagini). Ichthyological Exploration of Freshwaters 26: 209–220.

Morrone JJ (2006) Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. Annual Review of Entomology 51: 467–494. https://doi.org/10.1146/annurev.ento.50.071803.130447

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York, 333 pp.

Oliveira PE, Barreto AMF, Suguio K (1999) Late Pleistocene/Holocene climatic and vegetational history of the Brazilian caatinga: the fossil dunes of the middle São Francisco River. Palaeogeography, Palaeoclimatology, Palaeoecology 152: 319–337. https://doi.org/10.1016/S0031-0182(99)00061-9

Ottoni FP (2013) *Australoheros sanguineus* sp. n. – a new cichlid species from the rio Cubatão basin, southern Brazil (Cichlidae: Heroini). Vertebrate Zoology 63: 161–169.

Ottoni FP, Costa WJEM (2008) Taxonomic revision of the genus *Australoheros* Rican & Kullander, 2006 (Teleostei: Cichlidae) with descriptions of nine new species from southeastern Brazil. Vertebrate Zoology 58: 207–232.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. http://tree.bio.ed.ac.uk/software/tracer/.

Smith WL, Chakrabarty P, Sparks JS (2008) Phylogeny, taxonomy, and evolution of Neotropical cichlids (Teleostei: Cichlidae: Cichlinae). Cladistics 24: 625–641. https://doi.org/10.1111/j.1096-0031.2008.00210.x

Swofford DL (2003) PAUP* Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–109. http://sfi.mnhn.fr/cybium/numeros/1985/92/01-Taylor%5b92%5d107-119.pdf

Wiens JJ, Penkrot TA (2002) Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Systematic Biolology 51: 69–91. https://doi.org/10.1080/106351502753475880

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Unpublished thesis, The University of Texas at Austin, 115 pp.

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# Two new skink-endoparasitic species of *Meteterakis* (Nematoda, Heterakidae, Meteterakinae) from East Asian islands

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*Plestiodon japonicus*
Japan
Taiwan

## Abstract

Here, two new nematodes of *Meteterakis* Karve, 1930 from Taiwan and the western Japanese Archipelago that are endoparasitic to scincid lizards are described. The Taiwanese *Meteterakis formosensis* **sp. n.** and the Japanese *Meteterakis occidentalis* **sp. n.** can be distinguished from other congeners by the following characteristics: spicules 437–537 µm in length in *M. formosensis* **sp. n.** and 359–538 µm in *M. occidentalis* **sp. n.**; spicules with narrow alae, funnel-shaped, proximal ends ventrally bent; prevulval flap well-developed; gubernaculum mass absent; preclocal sucker with diameter of 35–47 µm in *M. formosensis* **sp. n.** and of 32–36 µm in *M. occidentalis* **sp. n.**; 9–15 caudal papillae on both lateral sides in *M. formosensis* **sp. n.** and 10–14 in *M. occidentalis* **sp. n.**; and relatively narrow lateral alae, ending at region near proximal end of spicule in male or at region anterior to anus in female. *Meteterakis formosensis* **sp. n.** is distinguished from *M. occidentalis* **sp. n.** by possessing spicules with hyaline pointed distal ends and well-developed cuticular backing structures. The present study suggests that lateral alae can be used as diagnostic character among the *Meteterakis* species, and it revealed that meteterakine nematodes mature in the host's small intestine and then migrate to the rectum to oviposit.

## Introduction

*Meteterakis* Karve, 1930 is a parasitic nematode genus, which is specific to amphibians (frogs and caecilians) and reptiles (lizards and land turtles) (Baker 1984, 1987, Hasegawa and Asakawa 2004, Zhang and Zhang 2011, Junker et al. 2015), and currently consists of 27 species. They are distributed in South, Southeast and East Asian regions, as well as in Oceania and Sao Tome Island, which is in the Gulf of Guinea (Baker 1984, 1987, Junker et al. 2015). Four species of *Meteterakis* have been recorded from the East Asian islands, which consist of the islands from the Japanese Archipelago to Taiwan: *M. japonica* (Wilkie, 1930), inhabiting the eastern Japanese Archipelago and Shimokoshikijima Island, an islet west off Kyushu, western Japan; *M. amamiensis* Hasegawa, 1990, indigenous to the western Japanese Archipelago and northern the Ryukyu Archipelago (Kodakarajima and Amamioshima Islands); *M. ishikawanae* Hasegawa, 1987, described from the Okinawan Islands; and the Burmese *M. govindi* Karve, 1930, which is the type species of the genus, from southern Taiwan (Karve 1930, Wilkie 1930, Yamaguti 1935, 1941, Hasegawa 1987, 1990, 1992, Telford Jr 1997, Goldberg and Bursey 2002, Bursey et al. 2005, Norval et al. 2014, Sata 2015, 2018). Although *M. japonica* was once reported from Miyakojima Island in the southern Ryukyu Archipelago (Hasegawa 1984), the individuals from the islet can be distinguished from the "true" *M. japonica* by the absence of a gubernaculum (*M. japonica* possesses a gubernaculum; Wilkie 1930, Inglis 1958).

A molecular phylogenetic study revealed that the East-Asian insular *Meteterakis* nematodes are divided into two major clades (Sata 2018). One phylogroup contains *M. japonica* and *M. ishikawanae*, while the other major clade contains *M. amamiensis* and three distinct unidentified nematodes from Ishigakijima and Iriomotejima islands in

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the southern Ryukyu Archipelago and Taiwan. The study also highlights the deep-genetic divergence between the *M. amamiensis* populations inhabiting Kodakarajima and Amamioshima (type locality) Islands and those distributed in the western Japanese Archipelago (Sata 2018).

Clarifying the systematic accounts of the aforementioned unidentified species will lead to a better understanding of the species diversity and evolutionary history of *Meteterakis* parasites inhabiting the East Asian islands. In the present study, therefore, the taxonomic states of the unidentified Taiwanese species and the *M. amamiensis* populations in the western Japanese Archipelago are investigated, and each is described as a new species.

## Methods

The *Meteterakis* specimens examined in this study were obtained from the scincid lizard hosts, *Plestiodon chinensis* (Gray, 1838) and *Plestiodon japonicus* (Peters, 1864), which were collected from Taiwan and the western Japanese Archipelago (Fig. 1), respectively. The host lizard specimens were identified based on Okamoto and Hikida (2012) and Kurita et al. (2017). Hosts were collected and handled in accordance with the Regulations of Animal Experimentations at Kyoto University (approval numbers: H24014 and H2711). All captured lizards were euthanized by an injection of sodium pentobarbital. The body cavity of each specimen was dissected by a longitudinal incision, and then the digestive tract was removed. The excised organs were dissected longitudinally, and the lumens were investigated. The obtained nematode individuals were fixed with a hot 5% solution of glycerin in 70% ethyl alcohol. To clear the nematode specimens, they were placed in 50% solution of glycerin in 70% ethyl alcohol, then incubated 2–3 days at 60 °C to gradually evaporate the ethyl alcohol. The cleared specimens were observed with a light microscope (OLYMPUS BX53). The measurements in males were given for a holotype, followed by the range of paratypes in parentheses; for females, averages were provided, followed by the range of the paratypes in parentheses. All measurements were described in micrometers (μm) unless otherwise stated. Both the nematode and reptile specimens examined in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

For comparison, the following *Meteterakis* specimens deposited in the KUZ collection were examined: *M. amamiensis* (sensu Sata 2015): KUZ Z2015 from Yakushima Town, Kagoshima Prefecture, Japan (Yakushima Island), Japan (site 5); KUZ Z1769 from Kodakarajima, Toshima Village, Kagoshima Prefecture (Kodakarajima Island), Japan (site 6); KUZ Z673, Z674 from Amami City, Kagoshima Prefecture, Japan (Amamioshima Island) (site 7); KUZ Z1770 from Yamato Village, Kagoshima Prefecture, Japan (Amamioshima Island) (site 8); *M. japonica*: KUZ Z1762 from Odawara City, Kanagawa Prefecture, Japan; KUZ Z1763 from Izunokuni City, Shizuoka Prefecture, Japan; KUZ Z630–Z632 from Fukuroi City, Shizuoka Prefecture, Japan; KUZ Z636 from Kofu City, Yamanashi Prefecture, Japan; KUZ Z637, Z638 from Takasaki City, Gunma Prefecture, Japan; KUZ Z639 from Hachioji City, Tokyo, Japan; KUZ Z641, Z642, Z1765 from Tokushima City, Tokushima Prefecture, Japan; and KUZ Z645, Z1767 from Nagahama, Satsumasendai City, Kagoshima Prefecture, Japan (Shimokoshikijima Island) (Sata 2018).

## Systematics

### *Meteterakis formosensis* sp. n.

http://zoobank.org/51F7462B-95D2-43AD-A4DA-0ECBD7E019FA
Fig. 2

*Meteterakis* sp. 3; Sata 2018: figs 2, 3 (in part), table 1 (in part).

**Type materials.** Holotype: KUZ Z1779, whole specimen, adult male, obtained from the rectum of a *Plestiodon chinensis* specimen (KUZ R69425), collected from Mt. Guanyinshan, Bali District, New Taipei City, Taiwan (25°08'53.1"N, 121°25'47.3"E; elevation 199 m) (site 10 in Fig. 1) on 20 March 2013. Paratypes: KUZ Z1777, Z1778, Z1783, Z1992 and Z1993, whole specimens, five adult males; KUZ Z1780–Z1782, three adult females, obtained from the same host specimen of the holotype; KUZ Z1994, one prepared slide of male spicules; and KUZ Z1995, a section of the anterior end and a remaining body (KUZ Z1994 is also derived from this individual), obtained from the same host specimen of the holotype.

**Additional material.** The following scincid lizard specimens, which were collected from Taiwan, were also dissected to reveal the geographic range of the new Taiwanese taxon: *P. chinensis* from Taipei City (KUZ R51443, R51444 and R51449–R51453), from New Taipei City (KUZ R46132, R46134 and R46136–R46139), and from Miaoli County (KUZ R70946, R70948–R70951, R70953 and R70963); *Plestiodon leucostictus* (Hikida, 1988) from Hualien City, Hualien County (KUZ R69421 and R69424); *Plestiodon elegans* (Thompson, 1912) from Taipei City (KUZ R66354), from the Xindian District, New Taipei City (KUZ R30191, R36205), from Miaoli County (KUZ R50394, R70957 and R70964), from Yilan County, (KUZ R36552), and from Tainan City (KUZ R70090 and R70091); and *Eutropis longicaudata* (Hallowell, 1857) from Tainan City, (KUZ R70089). One *Meteterakis*-like specimen (KUZ Z2021) was obtained from a *P. chinensis* specimen (KUZ R70948).

**Type locality.** Taiwan, New Taipei City: Bali District, Mt. Guanyinshan.

**Type host.** *Plestiodon chinensis* (Gray, 1838) (Reptilia, Scincidae); site of infection: rectum and small intestine.

**Diagnosis.** Relatively stout body, with narrow lateral and caudal alae; lateral alae commencing from region anteri-

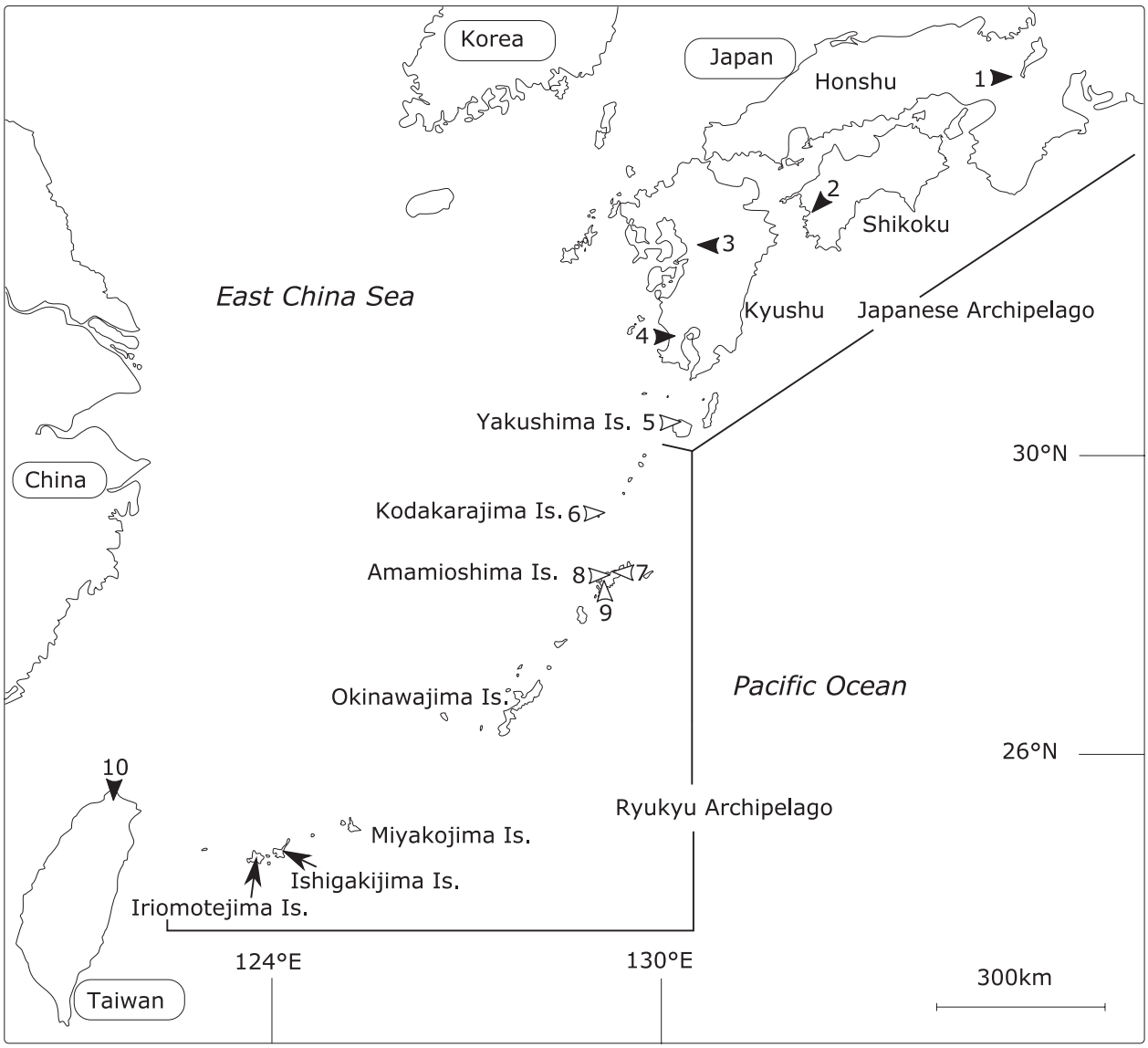


**Figure 1.** Map showing the known populations of *Meteterakis* Karve, 1930 inhabiting the Japanese Archipelago, the Ryukyu Archipelago and Taiwan: *Meteterakis occidentalis* sp. n. (sites 1–4), *M. amamiensis* Hasegawa, 1990 (sites 5–9; Hasegawa 1990, Sata 2015, 2018), and *Meteterakis formosensis* sp. n. (site 10). Solid arrows indicate the new species, and open arrows indicate *M. amamiensis*. Detailed site information can be found in Table 1.

or to nerve ring or front end of nerve ring in both sexes and ending at region near proximal end of spicule in male (never reaching region of preclocal sucker) or at region anterior to anus in female. Prevulval flap present and well developed in female. Gubernacular mass absent. Spicules with thin alae, funnel-shaped proximal ends, hyaline tips, and both proximal and distal ends bent ventrally. Right spicule, 437–510 long; left spicule 457–537 long. Each spicule with thick and long cuticular backing structures, not covered by cuticular pouch. Caudal papillae present in male, 8–13 (*N*=4) pairs with additional papillae: 12–15 (*N*=4) on right side; 9–15 (*N*=5) on left side.

**Etymology.** The specific name is an adjective, derived from the old name for Taiwan, which is the type locality of the new species.

**Description.** *General*. Body short and relatively stout with tapered extremities. Cephalic end with 3 lips, each lip with 2 minute apical papillae. Dorsal lip with a pair of cephalic papillae (each papilla with 2 minute papillae); each subventral lip with single papilla (each papilla with 2 minute papillae), 1 amphid and 1 smaller papillae. Flanges in inner edge of each lip unobservable. Esophagus comprise of pharynx, cylindrical portion and bulb. Bulb bearing three valves. lateral alae commencing from region anterior to nerve ring or front end of nerve ring in both sexes and ending at region near proximal end of spicule in male (never reaching region of preclocal sucker) or at region anterior to anus in female.

*Male* (*N*=6; KUZ Z1777–Z1779, Z1783, Z1992 and Z1993). Body length 5.03 mm (4.84–5.67 mm), maximum width 206 (151–228). Body length/body width =

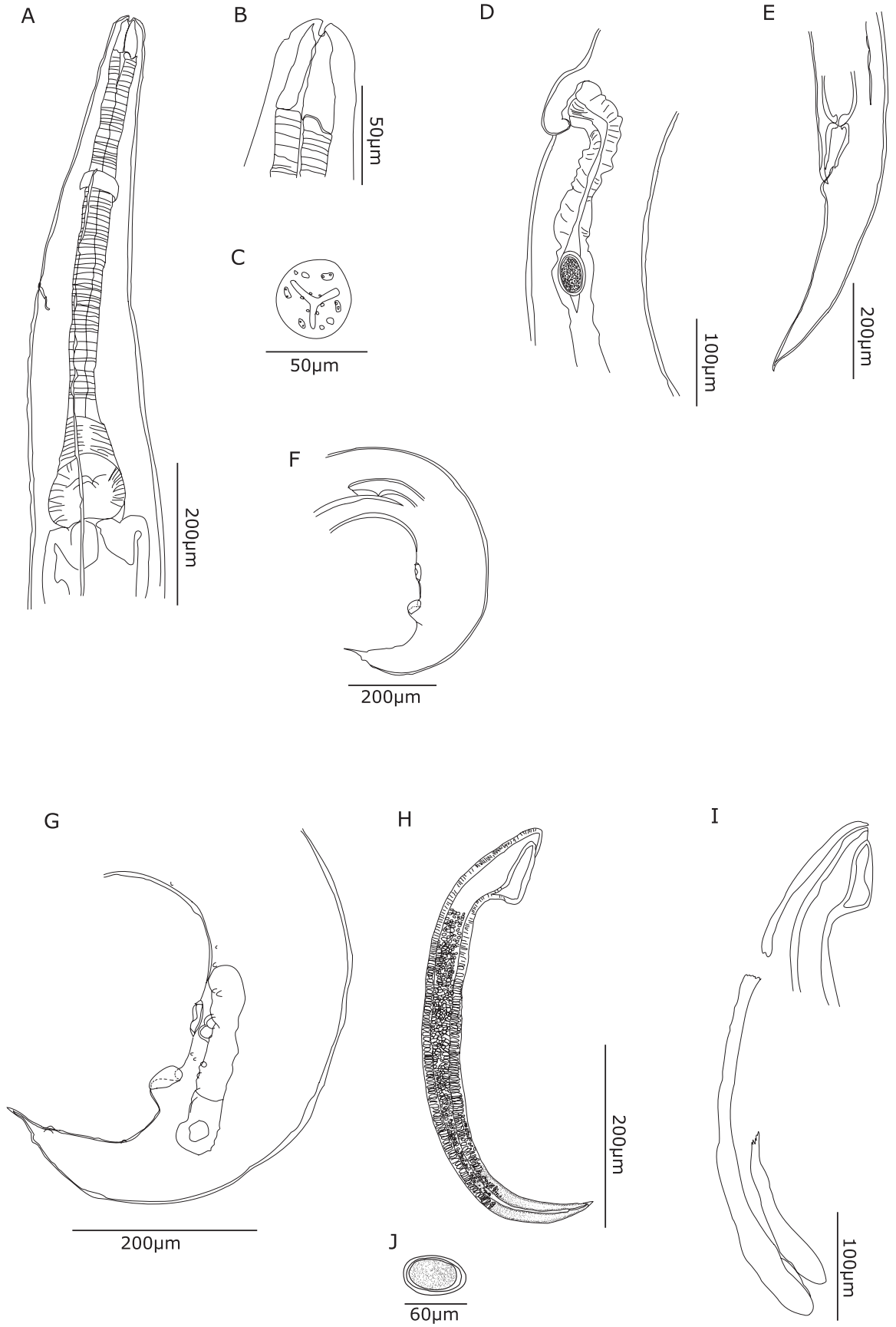


**Figure 2.** *Meteterakis formosensis* sp. n., holotype (KUZ Z1779: **A**, **B**, **F**, **G**), paratypes (KUZ Z1781: **D**, **J**; KUZ Z1782: **E**; KUZ Z1994: **H**, **I**; KUZ Z1995: **C**). **A** anterior region, lateral view; **B** pharynx, lateral view; **C** anterior end, apical view; **D** vulvar area of female, lateral view; **E** caudal region of female, lateral view; **F** caudal region of male, lateral view; **G** caudal papillae arrangement of male, lateral view; **H** spicule; **I** accessory of spicule; **J** egg.

**Table 1.** Distributions of *Meteterakis formosensis* sp. n., *Meteterakis occidentalis* sp. n., and *M. amamiensis* Hasegawa, 1990.

| Species | Site # | Locality | Geographic coordinates | References |
|---|---|---|---|---|
| *M. occidentalis* sp. n. | 1 | Mt. Yoshida, Kyoto City, Kyoto Pref., JP (Honshu) | 35°01'43.7"N, 135°47'09.4"E | Sata (2015); This study |
| *M. occidentalis* sp. n. | 2 | Uwajima City, Ehime Pref., JP (Shikoku) | 33°13'56.8"N, 132°33'13.7"E | Sata (2018); This study |
| *M. occidentalis* sp. n. | 3 | Kumamoto City, Kumamoto Pref., JP (Kyushu) | 32°47'N, 130°41'E | Sata (2015); This study |
| *M. occidentalis* sp. n. | 4 | Kagoshima City, Kagoshima Pref., JP (Kyushu) | 31°35'39.76"N, 130°27'31.55"E | Sata (2015); This study |
| *M. amamiensis* | 5 | Yakushima Town, Kagoshima Pref., JP (Yakushima Island) | 30°27'N, 130°29'E | Sata (2015); This study |
| *M. amamiensis* | 6 | Kodakarajima, Toshima Village, Kagoshima Pref., JP (Kodakarajima Is.) | 29°13'22.6"N, 129°19'39.2"N | Sata (2018); This study |
| *M. amamiensis* | 7 | Amami City, Kagoshima Pref., JP (Amamioshima Is.) | 28°24'01.3"N, 129°28'33.6"E | Sata (2015); This study |
| *M. amamiensis* | 8 | Yamato Village, Kagoshima Pref., JP (Amamioshima Is.) | 28°21'30.8"N, 129°20'11.7"E | Sata (2018); This study |
| *M. amamiensis* | 9 | Mt. Yuwan, Uken Village, Kagoshima Pref., JP (Amamioshima Is.) | N/A | Hasegawa (1990) |
| *M. formosensis* sp. n. | 10 | Mt. Guanyinshan, Bali District, New Taipei City, TW | 25°08'53.1"N; 121°25'47.3"E | Sata (2018); This study |

24.4 (24.8–36.3). Diameter of head 46 (38–50). Total length of esophagus 724 (681–774) long with width of 36 (31–44) at cylindrical portion. Body length/esophagus length = 7.0 (7.0–7.8). Pharynx 47 (34–45) long, bulb 85 (79–95) long by 110 (91–114) wide. Grooves between lips shallow and 8.2 (7.5–9.3) long. Nerve ring and excretory pore 221 (207–243) and 396 (337–400), respectively, from cephalic end. Spicules equal or slightly different, with narrow alae, strongly chitinized, tessellated from 153 (120–141) from proximal end to distal end in right spicule (i.e. corresponding to 68.1% [68.3%–75.7%] of total length), and from 66 (95–159) to distal end in left spicule (i.e. corresponding to 86.8% [65.2%–81.9%] of total length); both proximal and distal ends bent ventrally, with wide funnel-shaped proximal ends, and pointed hyaline distal ends. Right spicule 480 (437–510) long (i.e. corresponding to 9.5% [8.0%–9.8%] of body length), left spicule 500 (457–537) (i.e. corresponding to 9.9% [8.3%–10.5%] of body length). Each spicule with thick and long cuticular backing structure, not covered by cuticular pouch. Gubernacular mass absent. Narrow caudal alae present, supported by three pairs of large papillae. Caudal papillae present, 13 (8–13) (*N*=4) pairs with additional papillae: 13 (12–15) (*N*=4) on right side; 15 (9–15) (*N*=5) on left side. Occasionally, single median papilla present. Among 13 (8–13) pairs: 1–4 pairs anterior to preclocal sucker; 2 large pairs supporting caudal alae around sucher; 1–3 small pairs around sucker; 0–2 pairs between sucker and cloaca; 1 large pair supporting caudal alae at lateral to posterior cloacal lip; 0–1 pair immediately posterior to posterior cloacal lip; and 0–2 pairs in caudal region. Precloacal sucker 47 (35–47) in diameter, 54 (26–50) from cloaca. Posterior cloacal lip developed. Tail bent ventrally, conical with pointed tip, and 304 (238–315) long. Body length/tail length = 16.6 (15.4–23.0).

*Female* (*N*=3; KUZ Z1780–Z1782). Body length 6.05 mm (5.56–6.30 mm), and maximum width 224 (200–244). Body length/body width = 27.0 (25.8–27.8). Diameter of head 51 (49–52). Total length of esophagus 783 (753–806) long with width of 45 (39–50) at cylindrical portion. Body length/esophagus length = 7.7 (7.0–8.4). Pharynx 51 (38–61) long; bulb 95 (91–99) long by 108 (104–114) wide. Grooves between lips shallow and 8.5 (7.2–9.4) long. Nerve ring and excretory pore 230 (228–232) and 367 (359–372), respectively, from cephalic end. Vulva 2.71 mm (2.44–2.87 mm) from cephalic end, and located at anterior to middle of body (44.8% [43.9%–45.6%] of body length). Prevulval flap well developed. Vagina muscular running posteriorly. Tail long conical, slightly bent ventrally, and 560 (518–583) long. Body length/tail length = 10.8 (10.7–10.9). Eggs elliptical, 60 (49–68) by 41 (34–51) (*N*=29), thick shelled, containing morula stage embryos.

**Occurrence.** This new species was located on Mt. Guanyinshan, Bali District, New Taipei City, Taiwan (type locality) (site 10 in Fig. 1). Although a *Meteterakis*-like specimen (KUZ Z2021) with undeveloped spicules was obtained from the rectum of a *P. chinensis* specimen (KUZ R70948) from Miaoli County, Taiwan, its taxonomic account is unclear. *P. chinensis* is the only known host of this species.

**Comparisons.** This new species can be discriminated from almost half of the other *Meteterakis* species by the lengths of the spicules. Because *M. formosensis* sp. n. has spicules that are 437–537 µm in length, it can be distinguished from the following eight congeners, which are diagnosed by their spicules longer than 600 µm: *M. aurangabadensis* Deshmukh & Choudhari, 1980 (620–720 µm), *M. karvei* Naidu & Thakare, 1981 (660–840 µm), *M. longispiculata* (Baylis, 1929) (630–680 µm), *M. louisi* Inglis, 1958 (970–1100 µm), *M. singaporensis* (Sandosham, 1953) (740–960 µm), *M. striaturus* Oshmarin & Demshin, 1972 (680 µm), *M. vaucheri*

Adamson, 1986 (1057–1242 µm) and *M. wangi* Zhang & Zhang, 2011 (740–930 µm). This new species is also distinguishable from *M. bufonis* (Biswas & Chakravarty, 1963) (left, 270 µm; right, 310 µm), *M. gambhiri* Gambhir et al., 2006 (220–270 µm), *M. govindi* (180–270 µm; Karve 1930, Inglis 1958) and *M. mabuyi* (Chakravarty, 1944) (300 µm), because its spicules are longer than 400 µm. Moreover, the new species differs from *M. lyriocephali* (Crusz & Ching, 1975) because the spicules are similar in length on the left (457–537 µm) and right (437–510 µm) sides (in *M. lyriocephali*: left, 595–754 µm; right, 340–561 µm).

In addition to the spicule length, the new species is distinguishable from the eight congeners by the following characteristics of spicules: proximal end wide, funnel-shaped and ventrally bent vs. proximal end slightly widened in *M. ishikawanae* (Hasegawa 1987) and *M. wonosoboensis* Purwaningshi, 2015 or vs. proximal end straight in *M. guptai* Gupta & Naiyer, 1993 and *M. triaculeata* (Kreis, 1933) (Inglis 1958); surface smooth, vs. rough surface in *M. saotomensis* Junker et al., 2015; and spicule alae narrow vs. wider in *M. baylisi* Inglis, 1958, *M. crombiei* Bursey et al., 2005 and *M. sinharajensis* Crusz & Ching, 1975.

*Meteterakis formosensis* sp. n. is distinguished from *M. lombokensis* Purwaningshi et al., 2016 by the presence of a well-developed prevulval flap in the female. Additionally, this species possesses a 35–47 µm (diameter) preclocal sucker and elliptically-shaped eggs. These characteristics can be used to discriminate this new species from *M. andamanensis* Soota & Chaturvedi, 1972, which has a 55–66 µm (diameter) preclocal sucker and spherical-shaped eggs. The number of caudal papillae (9–15) on both lateral sides of the new species can distinguish it from *M. paucipapillosa* Wang, 1980 because the latter possesses only 6 caudal papillae on both lateral sides. The new taxon is clearly distinguishable form *M. japonica* and *M. hurawensis* Bursey et al., 2017 by the absence of a gubernacular mass (Wilkie 1930, Inglis 1958, Bursey et al. 2017). *M. formosensis* sp. n. bears narrow lateral alae ending at the region near the proximal end of the spicule in the male or at the region anterior to the anus in the female. Thus, this new species differs from *M. amamiensis*, which is diagnosed by possession of the wider lateral alae ending at the preclocal region in the male, and at the region near the posterior end in the female (Hasegawa 1990).

Morphologically, *M. formosensis* sp. n. most resembles *M. occidentalis* sp. n., which is described below, but it differs from the latter species by the female having a relatively stout body (body length/body wide: 25.8–27.8 in *M. formosensis* sp. n. vs. 31.8–41.2 in *M. occidentalis* sp. n.), relatively longer tail length in the female (body length/tail length: 10.7–10.9 in *M. formosensis* sp. n. vs. 13.6–17.8 in *M. occidentalis* sp. n.), spicules with hyaline tips (lacking in *M. occidentalis* sp. n.) and well-developed backing structures for spicules (undeveloped in *M. occidentalis* sp. n.).

### ***Meteterakis occidentalis* sp. n.**

http://zoobank.org/95FED6DF-22ED-486B-8AD2-B950FEDE87B1
Fig. 3

*Meteterakis amamiensis*; Sata 2015: 17 (in part); Sata 2018: figs 2, 3 (in part), table 1 (in part).

**Type materials.** Holotype: KUZ Z2000, whole specimen, adult male, obtained from the small intestine of a *P. japonicus* specimen (KUZ R69034), collected from Mt. Yoshida, Kyoto City, Kyoto Prefecture, Japan (35°01'43.7"N, 135°47'09.4"E; elevation 70 m) (site 1 in Fig. 1) on 16 May 2012. Paratypes: KUZ Z1996–Z1999 and Z2002–Z2008, whole specimens, four adult males and seven adult females, obtained from the rectum and small intestine of the same specimen of holotype's host and another *P. japonicus* specimen (KUZ R69036), data same as those from the holotype's host-specimen; KUZ Z2001, whole specimen, adult male, obtained from the small intestine of a *P. japonicus* specimen (KUZ R69575), collected from Ohura, Uwajima City, Ehime Prefecture, Japan (33°13'56.8"N, 132°33'13.7"E; elevation 4 m) (site 2 in Fig. 1) on 8 April 2013; KUZ Z2009, prepared slides of male spicules with the spicule pouch; Z2010, prepared slides of male spicules; KUZ Z2011 and Z2012, remaining body specimens of KUZ Z2009 and Z2010, respectively; KUZ Z2013, a section of the anterior end and a remaining body. KUZ Z2009, Z2010 and Z2013 were obtained from the rectum of a *P. japonicus* specimen (KUZ R69030), collected from same locality as the holotype's host-specimen on 9 May 2012.

**Additional material.** To reveal the geographic range of *M. occidentalis* sp. n., the following specimens deposited in the KUZ collection were examined: *M. amamiensis* (sensu Sata 2015): KUZ Z2014 from Kumamoto City, Kumamoto Prefecture, Japan (site 3 in Fig. 1), and KUZ Z658 and Z2022 from Kagoshima City, Kagoshima Prefecture, Japan (site 4 in Fig. 1).

**Type locality.** Japan, Kyoto: Kyoto, Mt. Yoshida.

**Type host.** *Plestiodon japonicus* (Peters, 1864) (Reptilia, Scincidae); site of infection: rectum and small intestine.

**Diagnosis.** Short and slender body, with narrow lateral and caudal alae; lateral alae commencing from region anterior to nerve ring in both sexes and ending at region near proximal end of spicule (never reaching region of preclocal sucker) in male or at region anterior to anus in female. Prevulval flap well developed in female. Male with well-developed posterior cloacal lip. Gubernacular mass absent. Spicules with narrow alae, funnel-shaped proximal end, and both proximal and distal ends bent ventrally. Right spicule 359–517 long, left spicule 368–538 long. Dorsal surfaces of each spicule covered by thin cuticular pouch. Caudal papillae present in male, 7–11 ($N$=6) pairs with additional papillae: 10–14 ($N$=6) on right side; 10–14 ($N$=6) on left side.

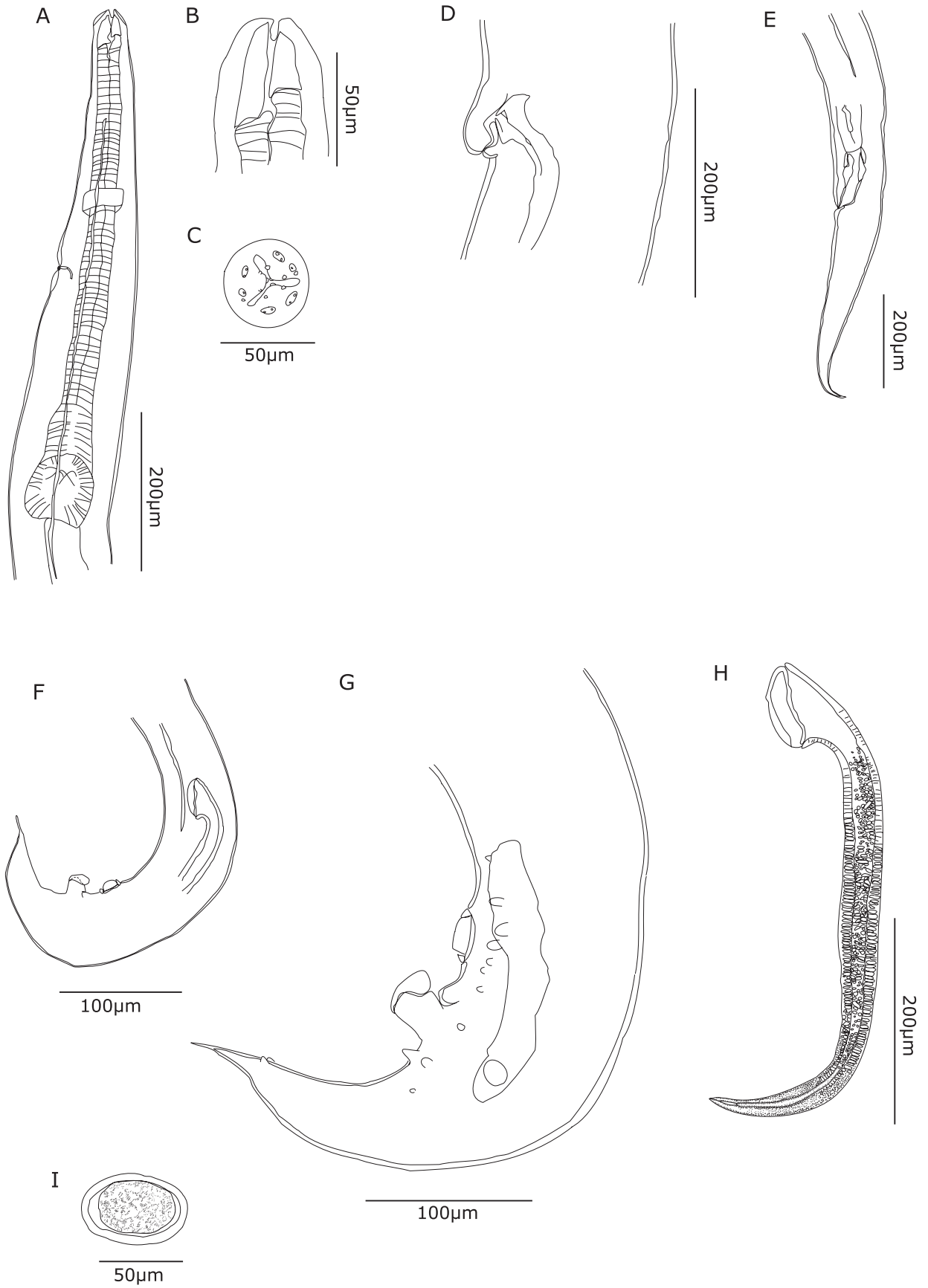


**Figure 3.** *Meteterakis occidentalis* sp. n., holotype (KUZ Z2000: **A**, **B**, **F**, **G**), paratypes (KUZ Z2005: **D**, **E**, **I**; KUZ Z2010: **H**; KUZ Z2013: **C**). **A** anterior region, lateral view; **B** pharynx, lateral view; **C** anterior end, apical view; **D** vulvar area of female, lateral view; **E** caudal region of female, lateral view; **F** caudal region of male, lateral view; **G** caudal papillae arrangement of male, lateral view; **H** spicule; **I** egg.

**Etymology.** The specific name is a Latin adjective in the nominative singular, occidentalis (western), referring to its distribution in the western Japanese Archipelago.

**Description.** *General*. Body short and slender with tapered extremities. Cephalic end with 3 lips, each lip with 2 minute apical papillae. Dorsal lip with a pair of cephalic papillae (each papilla with 2 minute papillae); each subventral lip with single papilla (each papilla with 2 minute papillae), 1 amphid and 1 papilla. Inner edge of each lips with flange. Esophagus comprise of pharynx, cylindrical portion and bulb. Bulb has three valves. Narrow lateral alae present in both sexes, commencing from region anterior to nerve ring in both sexes and ending at region near proximal end of spicule (never reaching region of preclocal sucker) in male or at region anterior to anus in female.

*Male* (*N*=6; KUZ Z1996–Z2001). Body length 4.68 mm (4.19 mm –5.75 mm), maximum width 132 (120–163). Body length/body width = 35.5 (29.2–39.7). Diameter of head 42 (42–47). Total length of esophagus 621 (621–732) long with width of 39 (32–44) wide at cylindrical portion. Body length/esophagus length = 7.5 (6.7–7.9). Pharynx 36 (35–47) long, bulb 83 (65–97) long by 83 (84–100) wide. Grooves between lips shallow and 7.3 (7.0–13.5) long. Nerve ring and excretory pore 217 (203–241) and 328 (307–350), respectively, from cephalic end. Spicules with narrow alae, equal or slightly different, strongly chitinized, tessellated from 95 (78–104) from proximal end to distal end in right spicule (i.e. corresponding to 76.7% [76%–84%] of total length), and from 101 (43–118) to distal end in left spicule (i.e. corresponding to 76% [74.7%–91.4%] of total length), both proximal and distal ends bent ventrally, wide funnel-shaped proximal end and distal end pointed. Right spicule 408 (359–517) long (i.e. corresponding to 8.7% [8.6%–10.3%] of body length), left spicule 421 (368–538) (i.e. corresponding to 9.0% [8.3%–10.5%] of body length). Dorsal surface of each spicules covered by thin cuticular pouch. Gubernacular mass absent. Narrow caudal alae present, supported by three pairs of large papillae. Caudal papillae present, 11 (7–11) (*N*=6) pairs with additional papillae: 14 (10–12) (*N*=6) on right side; 14 (10–14) (*N*=6) on left side. Occasionally, single median papilla present. Among 11 (7–11) pairs: 1–2 pairs anterior to preclocal sucker; 2 large pairs supporting caudal alae and 0–1 small pair lateral to preclocal sucker; 0–2 pairs between posterior region of sucker and cloaca; 1 large pair supporting caudal alae at lateral to posterior cloacal lip; 0–2 pair immediately posterior to posterior cloacal lip; 1–3 pairs posterior region of tail; 0–1 pair lateral to cloaca. Precloacal sucker 36 (32–36) in diameter and 33 (23–38) from cloaca. Posterior cloacal lip developed. Tail bent ventrally, conical, with pointed tip, 258 (234–282) long. Body length/tail length = 18.1 (17.9–20.4).

*Female* (*N*=7; KUZ Z2002–Z2008). Body length 5.49 mm (4.85 mm –5.98 mm), maximum width 158 (145–182). Body length/body width = 34.8 (31.8–41.2). Diameter of head 48 (41–56). Total length of esophagus 731 (648–798) long with width of 37 (31–42) at cylindrical portion. Body length/esophagus length = 7.5 (6.7–8.0). Pharynx 43 (38–52) long; bulb 94 (87–106) long by 90 (79–101) wide. Grooves between lips shallow and 10.5 (7.3–13) long. Nerve ring and excretory pore 230 (162–275) and 355 (311–388), respectively, from cephalic end. Vulva 2.36 mm (2.09–2.54 mm) from cephalic end. Vulva located at anterior to middle of body (43.0% [39.9%–47.4%] of body length). Prevulval flap well developed. Vagina muscular running posteriorly. Tail long conical, slightly bent ventrally, 353 (329–415) long, with a few specimens possessing a few small papillae. Body length/tail length = 15.6 (13.6–17.8). Eggs elliptical, 62 (52–72) by 40 (32–47) (*N*=70), thick shelled, containing morula stage embryo.

**Occurrence.** *Meteterakis occidentalis* sp. n. occurs in the following locations in Japan: Kyoto City, Kyoto Prefecture, Honshu (site 1 in Fig. 1); Uwajima City, Ehime Prefecture, Shikoku (site 2 in Fig. 1); Kumamoto City, Kumamoto Prefecture, Kyushu (site 3 in Fig. 1); and Kagoshima City, Kagoshima Prefecture, Kyushu (site 4 in Fig. 1). *P. japonicus* is the only known host of this species (Sata 2015, 2018).

**Notes on the life cycle.** Most *Meteterakis* nematodes collected from recta were adult individuals. Several larval nematodes were collected from the small intestines of the host individuals, which were inhabited by *M. occidentalis* sp. n., and a small number of ensheathed *Meteterakis* were also found from there. The rate of females having eggs in hosts' small intestines and recta were 36.2% (21/58) and 72.5% (50/69), respectively.

**Comparisons.** *M. occidentalis* sp. n. differs from *M. formosensis* sp. n. as discussed above. Because most of the morphological characteristics of *M. occidentalis* sp. n. are concordant with those of *M. formosensis* sp. n., this new species can be distinguished from the other congeners by the features mentioned in comparisons with *M. formosensis* sp. n. and the other *Meteterakis* species. Therefor, *M. occidentalis* sp. n. can receive the taxonomic status of a distinct species within the genus.

## Discussion

Heterakidae has been classified into three subfamilies, Heterakinae, Spinicaudinae, and Meteterakinae (Inglis 1957). While the life cycles of heterakine and spinicaudine nematodes have been well documented, the life cycle of meteterakine species remains unknown (Anderson 2000). During the dissection and examination of host reptile specimens, larval nematodes were collected from the small intestines of *P. japonicus*. Because those larvae often co-occurred with adult worms of *M. occidentalis* sp. n. and/or ensheathed *Meteterakis* nematodes in the host materials, they are likely to be *M. occidentalis* sp. n. larval individuals.

Most of the *M. occidentalis* sp. n. specimens of both sexes collected from the recta were at the adult stage. Moreover, mature female of *M. occidentalis* sp. n. bearing

eggs occurred less frequently in the hosts' small intestines than in the recta, suggesting that the larvae of this species may mature in the small intestine and then migrate to the rectum to oviposit. A similar life cycle has been recorded for two spinicaudine species that are parasites in the rectum of Malagasy chameleons (Anderson 2000). The present results provide new insights into the life cycle of the meteterakine nematodes and indicates that their life cycles may resemble those of spinicaudine species.

The spicule length, which has been regarded as a useful taxonomic character of the genus *Meteterakis* (e.g. Junker et al. 2015), exhibits certain intraspecific variations. Moreover, the characteristic of spicule length sometimes overlaps with those of other congeners. In contrast, the present study revealed an obvious morphological difference between the two morphologically similar species, *M. occidentalis* sp. n. and *M. amamiensis*, in the width and ending positions of the lateral alae. Because the characteristics of the lateral alae in *M. occidentalis* sp. n. showed much less intraspecific variation, lateral alae can be used as a diagnostic character among the *Meteterakis* species. The combination of the lateral alae and other traditional taxonomic character within this genus may help discriminate the species of *Meteterakis*. However, *Meteterakis* species, which were described previously, sometimes lack morphological descriptions of their lateral alae (e.g. Inglis 1958, Gambhir et al. 2006). Therefor, the characteristics of their lateral alae should be revisited by future taxonomic revisions. Additionally, it is preferable that future descriptive studies of new *Meteterakis* species contain the characteristics of lateral alae.

Because a previous phylogenetic study (Sata 2018) did not include "*M. amamiensis*" (sensu Sata 2015) specimens from Kumamoto City, Kumamoto Prefecture, Japan (Kyushu) and Yakushima Town, Kagoshima Prefecture (Yakushima Island), Japan, their taxonomical accounts remained unclear. The present morphological observations revealed that the *Meteterakis* specimens from Kumamoto Prefecture, and Yakushima Island are *M. occidentalis* sp. n., and *M. amamiensis*, respectively, based on the characteristics of their lateral alae. Although the herpetofauna of Yakushima Island is closely related to that of Kyushu, and their compositions have genetically diverged deeply from each congeneric species on the Amamioshima Islands (Tanaka-Ueno et al. 1998, Brandley et al. 2012), *M. amamiensis* is indigenous to Yakushima and Amamioshima Islands. Thus, the geographic range of this nematode species would be obviously discordant with the host biogeography.

This discordant pattern between the endoparasites and their host species was indicated by the two unidentified *Meteterakis* species on Ishigakijima and Iriomotejima Islands (Sata 2018). Therefore, the range of each *Meteterakis* species cannot always be predicted by the host's distribution pattern. Thus, the species diversity of *Meteterakis* should be revealed by geographically exhaustive faunal and taxonomic surveys. Moreover, the taxonomic account of the unidentified *Meteterakis* on Ishigakijima and Iriomotejima Islands should be clarified by a future study using appropriate specimens of the species.

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## References

Adamson ML (1986) *Meteterakis vaucheri* n. sp. (Nematoda; Heterakoidea) from *Varanus grayi* (Varanidae) in the Philippines. Canadian Journal of Zoology 64: 814–817. https://doi.org/10.1139/z86-122

Anderson RC (2000) Nematode Parasites of Vertebrates: Their Development and Transmission (2nd edn). CABI Publishing, Wallingford, 650 pp. https://doi.org/10.1079/9780851994215.0000

Baker MR (1984) The systematic and zoogeography of Spinicaudinae and Meteterakinae (Heterakoidea: Nematoda) parasitic in reptiles and amphibians. Systematic Parasitology 6: 275–287. https://doi.org/10.1007/BF00012206

Baker MR (1987) Synopsis of the Nematoda parasitic in amphibians and reptiles. Memorial University of Newfoundland Occasional Papers in Biology 11: 1–325.

Baylis HA (1929) Some new parasitic nematodes and cestodes from Java. Parasitology 21: 256–265. https://doi.org/10.1017/S0031182000022940

Biswas PK, Chakravarty GK (1963) The systematic studies of the zoo-parasitic oxyuroid nematodes. Zeitschrift Für Parasitenkunde 23: 411–428. https://doi.org/10.1007/BF00259929

Brandley MC, Ota H, Hikida T, de Oca ANM, Fería-Ortíz M, Guo X, Wang Y (2012) The phylogenetic systematics of blue-tailed skinks (*Plestiodon*) and the family Scincidae. Zoological Journal of the Linnean Society 165: 163–189. https://doi.org/10.1111/j.1096-3642.2011.00801.x

Bursey CR, Goldberg SR, Kraus F (2005) Endoparasites in *Sphenomorphus jobiensis* (Sauria: Scincidae) from Papua New Guinea with description of three new species. Journal of Parasitology 91: 1385–1394. https://doi.org/10.1645/GE-3502.1

Bursey CR, Goldberg SR, Siler CD, Brown RM (2017) New species of *Meteterakis* (Nematoda: Heterakidae) in *Brachymeles* spp. (Squamata: Scincidae) from the Philippines. Comparative Parasitology 84: 149–154. https://doi.org/10.1654/1525-2647-84.2.149

Bursey CR, Goldberg SR, Telford Jr SR (2005) *Plagiorchis taiwanensis* (Digenea: Plagiorchiidae), *Kurilonema markovi* (Nematoda: Rhabdiasidae) and other helminths in *Eumeces latiscutatus* (Scincidae) and *Takydromus tachydromoides* (Lacertidae) from Japan. Comparative Parasitology 72: 234–240. https://doi.org/10.1654/4170

Chakravarty GK (1944) On the nematode worms in the collection of the zoological laboratory, University of Calcutta, Part I. Families Heterakidae and Kathlaniidae. Journal of the Department of Science University of Calcutta 1: 70–77.

Crusz H, Ching CC (1975) Parasites of the relict fauna of Ceylon VI. More new helminths from Amphibians and Reptiles, with a new

host-record and redescription of *Acanthocephalus serendibensis* Crusz and Mills, 1970. Annales de Parasitologie 50: 531–558. https://doi.org/10.1051/parasite/1975505531

Deshmukh PG, Choudhari AC (1980) *Meteterakis aurangabadensis* n. sp. from toad, *Bufo melanostictus*. Indian Journal of Parasitology 4: 37–40.

Gambhir RK, Tarnita TH, Chinglenkhomba A, Gyaneswori I, Indrani CH (2006) A new species of the genus *Meteterakis* Karve, 1930 (Nematoda: Heterakidae) from *Bufo melanostictus* Schneider. Flora and Fauna 12: 57–59.

Goldberg SR, Bursey CR (2002) Helminths of 10 species of anurans from Honshu, Japan. Comparative Parasitology 69: 162–176. https://doi.org/10.1654/1525-2647(2002)069[0162:HOSOAF]2.0.CO;2

Gray JE (1838) Catalogue of the slender-tongued saurians, with descriptions of many new genera and species. Annals of Natural History 2: 287–293. https://doi.org/10.1080/00222933809496676

Gupta V, Naiyer N (1993) On a new nematode *Meteterakis guptai* sp. nov. from a lizard *Calotes versicolor* (Daudin) from Lucknow. Indian Journal of Helminthology 45: 184–187.

Hallowell E (1857) Notice of some new and rare species of Scincidae in the collection of the Academy of Natural Sciences of Philadelphia. Transactions of the American Philosophical Society 11: 71–82. https://doi.org/10.2307/3231927

Hasegawa H (1984) Helminth fauna of five Okinawan amphibian species. The Biological Magazine Okinawa 22: 11–22.

Hasegawa H (1987) *Meteterakis ishikawanae* sp. n. (Nematoda: Heterakidae) from the frogs, *Rana ishikawae*, on Okinawa Island, Japan. Proceedings of the Helminthological Society of Washington 54: 237–241.

Hasegawa H (1990) Helminths collected from amphibians and reptiles on Amami-oshima Island, Japan. Memoirs of the National Science Museum, Tokyo 23: 83–92.

Hasegawa H (1992) Parasitic helminthes collected from amphibians and reptiles on Kume-jima Island, Okinawa, Japan. The Biological Magazine Okinawa 30: 7–13.

Hasegawa H, Asakawa M (2004) Parasitic nematodes recorded from amphibians and reptiles in Japan. Current Herpetology 23: 27–35. https://doi.org/10.5358/hsj.23.27

Hikida T (1988) A new white-spotted subspecies of *Eumeces chinensis* (Scincidae: Lacertilia) from Lutao Island, Taiwan. Japanese Journal of Herpetology 12: 119–123. https://doi.org/10.5358/hsj1972.12.3_119

Inglis WG (1957) A review of the nematode superfamily Heterakoidea. The Annals and Magazine of Natural History, Series 12(10): 905–912. https://doi.org/10.1080/00222935708656094

Inglis WG (1958) A revision of the nematode genus *Meteterakis* Karve, 1930. Parasitology 48: 9–31. https://doi.org/10.1017/S003118200002103X

Junker K, Mariaux J, Measey GJ, Mutafchiev Y (2015) *Meteterakis saotomensis* n. sp. (Nematoda: Heterakidae) from *Schistometopum thomense* (Bocage) (Gymnophiona: Dermophiidae) on São Tomé Island. Systematic Parasitology 92: 131–139. https://doi.org/10.1007/s11230-015-9588-6

Karve JN (1930) Some parasitic nematodes of frogs and toads. Annals of Tropical Medicine and Parasitology 24: 481–491. https://doi.org/10.1080/00034983.1930.11684653

Kreis HA (1933) Ein neuer parasitischer nematode aus *Corucia zebrata* (Scincidae; Reptilia). *Ganguleterakis triaculeatus* n. sp. Zeitschrift für Parasitenkunde 6: 332–338. https://doi.org/10.1007/BF02121952

Kurita K, Nakamura Y, Okamoto T, Lin S-M, Hikida T (2017) Taxonomic reassessment of two subspecies Chinese skink in Taiwan based on morphological and molecular investigations (Squamata, Scincidae). ZooKeys 687: 131–148. https://doi.org/10.3897/zookeys.687.12742

Naidu TS, Thakare VK (1981) On two nematodes from Fisher and Amphibians of Vidarbha region, Maharashtra state, India. Rivista di parassitologia 42: 415–424.

Norval G, Goldberg SR, Bursey CR, Mao J, Slater K (2014) Internal parasites of lizards from Taiwan. Herpetological Conservation and Biology 9: 484–494.

Oshmarin PG, Demshin NI (1972) The helminths of domestic and some wild animals in Viet Nam. Trudy Biologo-Pochyennogo Instuta Vladivostok 11: 5–115.

Okamoto T, Hikida T (2012) A new cryptic species allied to *Plestiodon japonicus* (Peters, 1864) (Squamata: Scincidae) from eastern Japan, and diagnoses of the new species and two parapatric congeners based on morphology and DNA barcode. Zootaxa 3436: 1–23.

Peters WCH (1864) Über die Eidechsenfamilie der Scincoiden, insbesonbere über die Schneider'schen, Wiegmann'schen unt neue Arten des zoologischen Museums. Monatsberichte der Königlichen Preuss Akademie der Wissenschaften zu Berlin 1864: 44–58.

Purwaningsih E, Dewi K, Hasegawa H (2015) Nematodes of amphibians from Java, Indonesia, with a description of new species, *Meteterakis wonosoboensis* n. sp. (Nematoda: Heterakoidea). Zootaxa 3974: 507–516. https://doi.org/10.11646/zootaxa.3974.4.3

Purwaningsih E, Dewi K, Nugroho HA (2016) Parasitic nematodes of amphibians from Lombok Island, Indonesia with description of *Camallanus senaruensis* sp. nov. and *Meteterakis lombokensis* sp. nov. Journal of Costal Life Medicine 4: 708–713. https://doi.org/10.12980/jclm.4.2016J6-181

Sandosham AA (1953) Malaysian parasite XV. Seven new worms from miscellaneous hosts. Studies from the Institute for Medical Research, Federation of Malaya 26: 212–226.

Sata N (2015) Distribution of parasitic nematodes in Japan with host-parasite relationship of lizards of *Plestiodon* (Reptilia: Squamata: Scincidae). Comparative Parasitology 82: 17–24. https://doi.org/10.1654/4728.1

Sata N (2018) Allopatric speciation of *Meteterakis* (Heterakoidea: Heterakidae), a highly dispersible parasitic nematode, in the East Asian islands. Parasitology International 67: 493–500. https://doi.org/10.1016/j.parint.2018.04.008

Soota TD Chaturvedi Y (1972) The helminth fauna of Andaman and Nicobar. Nematoda. Record of the Zoological Survey of India 66: 287–301.

Tanaka-Ueno Y, Matsui M, Chen S-L, Takenaka O, Ota H (1998) Phylogenetic relationships of brown frogs from Taiwan and Japan assessed by mitochondrial cytochrome *b* gene sequences (*Rana*: Ranidae). Zoological Science 15: 283–288. https://doi.org/10.2108/zsj.15.283

Telford Jr SR (1997) The Ecology of a Symbiotic Community. Volume 1. Krieger Publishing Company, Malabar, 125 pp.

Thompson JC (1912) Herpetological Notics No. 2. Prodorome of Descriptions of New Species of Reptilia and Batrachia from the Far East. Privately published, San Francisco, 4 pp.

Wang PQ (1980) Studies on some new nematodes of the suborder Oxyirata from Fujian, China. Acta Zootaxonomica Sinica 5: 242–252.

Wilkie JS (1930) Some parasitic nematodes from Japanese Amphibia. Annals and Magazine of Natural History, Series 10(6): 606–614. https://doi.org/10.1080/00222933008673260

Yamaguti S (1935) Studies on the helminth fauna of Japan Part 10. Amphibian nematodes. Japanese Journal of Zoology 6: 387–392.

Yamaguti S (1941) Studies on the helminth fauna of Japan Part 34. Amphibian nematodes, II. Japanese Journal of Zoology 9: 397–409.

Zhang S, Zhang L (2011) A new species of *Meteterakis* Karve, 1930 (Nematoda: Heterakoide) form *Indotestudo elongata* (Blyth) in China with a key to the species of *Meteterakis*. Zootaxa 2869: 63–68.

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# New populations of two threatened species of *Alsodes* (Anura, Alsodidae) reveal the scarce biogeographic knowledge of the genus in the Andes of central Chile

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Vulnerable

## Abstract

High Andean environments of central Chile (32°–38°S) are inhabited by several endemic species of the genus *Alsodes*. Two of them, *A. pehuenche* and *A. hugoi*, have geographic distributions restricted to their type locality and surroundings. The Chilean government classifies *A. pehuenche* as Critically Endangered (like the IUCN) and *A. hugoi* as Vulnerable. In this study we report 16 new localities of *Alsodes*, corresponding to first order streams, located in the Andes of Chile between 35°58' and 36°32'S (1800–2470 m). In some of these sites, adults and juveniles morphologically similar to *A. pehuenche* and *A. hugoi* were observed, as well as specimens of *Alsodes* that could not be identified by their external morphology. A Bayesian phylogenetic analysis with mitochondrial sequences (cytochrome b) was performed to identify the new populations to species level. All populations around 36°S belong to *A. pehuenche*, while most of those located south of that area would be *A. hugoi*. The exception is Cajón de Plaza (36°23'S), where specimens with sequences of *A. hugoi* or *A. pehuenche* coexist, whose taxonomic status could not be determined. These findings imply not only a westward range extension of *A. pehuenche* in Chile of about 14.5 km and of *A. hugoi* about 100 km southward, but also that practically all the first order streams of the Andes of central Chile would be inhabited by populations of *Alsodes*. Both results demonstrate the scarce biogeographic knowledge of the genus in the Andes, which has important implications for its conservation at local and species levels.

## Introduction

The amphibian fauna of the Andes of southern South America (Chile and Argentina) is relatively poor in comparison to tropical high-altitude environments (Duellman 1979, 1999). In fact, only three anuran genera, *Alsodes* Bell, 1843, *Rhinella* Fitzinger, 1826 and *Pleurodema* Tschudi, 1838, are represented in the Andes south of 30°S above 1500 m. *Alsodes* is the most diversified of these genera, with five species (*A. montanus* (Lataste, 1902), *A. tumultuosus* Veloso, Iturra & Galleguillos, 1979, *A. hugoi* Cuevas & Formas, 2001, *A. pehuenche* Cei, 1976 and *A. nodosus* (Duméril & Bibron, 1841)) present in the western slopes (Chile) between 33° and 36°S (Charrier et al. 2017). Four of these species are endemic to Chile, since *A. pehuenche* is also present in Argentina in a small area around the border between these countries (Valle Pehuenche, its type locality, 36°S) (Corbalán et al. 2010, Correa et al. 2013).

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There are few published records of Andean populations of *Alsodes* in Chile and most of these were reported in the last decade. For instance, several reports since 2008 increased significantly the distribution ranges and number of known localities of *A. montanus* and *A. tumultuosus* from 33°20' to 35°S (Araya and Riveros 2008, Correa et al. 2008, Mora et al. 2015, Ramírez 2015, Correa 2017). Likewise, new records of *A. hugoi* (Araya and Cisternas 2008) and *A. pehuenche* (Corbalán et al. 2010, Correa et al. 2013) extended their distributions, but in both cases within a small area around their type localities. These last two studies are particularly relevant because they extended the distribution of *A. pehuenche* to Chile, a species previously known just from its type locality in Argentina. The fifth species, *A. nodosus*, also inhabits the western slopes of Andes, but it is not clear what altitude it reaches (e.g. Cuevas 2013). Despite this significant increase in the number of known localities of *Alsodes* in the Andes, there are still substantial distribution gaps. For example, there is a stretch of 380 linear kilometers where only *A. hugoi* and *A. pehuenche* are known, between the Tinguiririca River basin (34°55'S), the southern end of the distributions of *A. montanus* and *A. tumultuosus*, and San Ignacio de Pemehue, type locality of *A. vittatus* (Philippi, 1902) (38°05'S) (Fig. 1).

The distribution ranges of *A. pehuenche* and *A. hugoi* are extremely restricted. *Alsodes pehuenche* was first reported as *Telmatobius montanus* (now *A. montanus*) by Cei and Roig (1965) at two very close localities located on each side of the border of Chile and Argentina (36°S). The Argentinean locality (Valle Pehuenche) became the type locality when this species was formally described by Cei (1976), but the Chilean locality was subsequently ignored (see comment in Correa et al. 2013). For 45 years it was assumed that *A. pehuenche* inhabited only in its type locality, located in a drainage basin on the eastern slopes of the Andes (that ultimately flows into the Atlantic Ocean), until Corbalán et al. (2010) extended its distribution about 4 km to the southwest in Chile, in a contiguous drainage basin, but that drains to the west into the Pacific Ocean. Corbalán et al. (2010) also specified that on the Argentinian side the species only occupies five very close streams and indicated that attempts to find individuals in other nearby streams failed. Later, Correa et al. (2013) reported a second locality in Chile, which further extended the distribution approximately 3.4 km west. Similarly, *Alsodes hugoi* was known since its description only in its type locality (Altos de Vilches, 35°33'S, 900 m, Cuevas and Formas 2001) until Araya and Cisternas (2008) reported its presence in several sites around this locality. Although these new records extended the altitudinal range up to 2115 m, all sites are hydrologically connected to the Lircay River and the maximum distance between them does not exceed 10 km.

The known distribution ranges of these species have been fundamental in establishing their national and international conservation categories. The Chilean government, through its legal instrument Reglamento de Clasificación de Especies Silvestres (RCE, which applies similar criteria to those of IUCN), classifies *A. pehuenche* as Critically Endangered and *A. hugoi* as Vulnerable. These categories rest on the application of criterion B, which exclusively considers the extent of the geographical distribution. On the other hand, *A. pehuenche* is Critically Endangered and *A. hugoi* is Data Deficient according to the IUCN (2017). This last categorization of *A. pehuenche* is fundamentally based on estimates of the extent of occurrence (about 9 km$^2$) and area of occupancy (about 5 km$^2$) (IUCN SSC Amphibian Specialist Group 2013).

In this study we report new Andean localities of *Alsodes* situated in Chile between 35°58' and 36°32'S. These localities were found during three field campaigns aimed to locate new populations of *A. pehuenche*, one in the surroundings of the known localities in Chile (around 36°S) and the other two south of the known distribution range of the species. We performed a phylogenetic analysis with mitochondrial cytochrome b sequences to identify most of the populations discovered to species level. We discuss the implications of these findings for the biogeography and conservation of the genus in Chilean Andes.

## Material and methods

### Field campaigns

We carried out three field campaigns in the western slopes of the Andes Range between 35°55' and 36°35'S to locate new populations of *A. pehuenche* in Chile (Fig. 1). The three areas explored are located almost entirely in the southeast part of the Maule River basin, except for the southernmost area that covers the northeast end of the Ñuble River basin. The explorations were focused on the surroundings and to the south of the known distribution of *A. pehuenche* in Chile (Paso Pehuenche and Laguna del Maule, around 36°S and 70°25'W; Correa et al. 2013). The longest campaign was carried out in 2016–2017 in the surroundings of Paso Pehuenche and Laguna del Maule (Fig. 1B), where a total of nine sites between 2200 and 2500 m were explored (Table 1). Two of these sites had previously been described (Corbalán et al. 2010, Correa et al. 2013). The other two campaigns were carried out in 2016, the first in the surroundings of Laguna El Dial (around 36°27'S; six days, five sites explored between 1830 and 2000 m; Fig. 1D), and the second in some eastern tributaries of the Guaiquivilo River (around 36°08'S; five days, five sites explored between 1800 and 2160 m; Fig. 1C) (Table 1). The last two areas were reached on horseback with the help of a guide and a muleteer. The searches focused on first order streams fed by melting snow, which are generally located in areas with a steep slope and are associated with flooded meadows, since this is the environment described for *A. pehuenche* (Cei and Roig 1965, Corbalán et al. 2008, 2010). When possible, we chose streams with continuous flow (presumably permanent throughout the year), since it was described that this species has a larval development that lasts several years (Corbalán et al. 2014). We consider as first order streams those that constitute the sources of the hydric systems and that do not have tributaries that feed them, following a top-down topological ordering. Each site

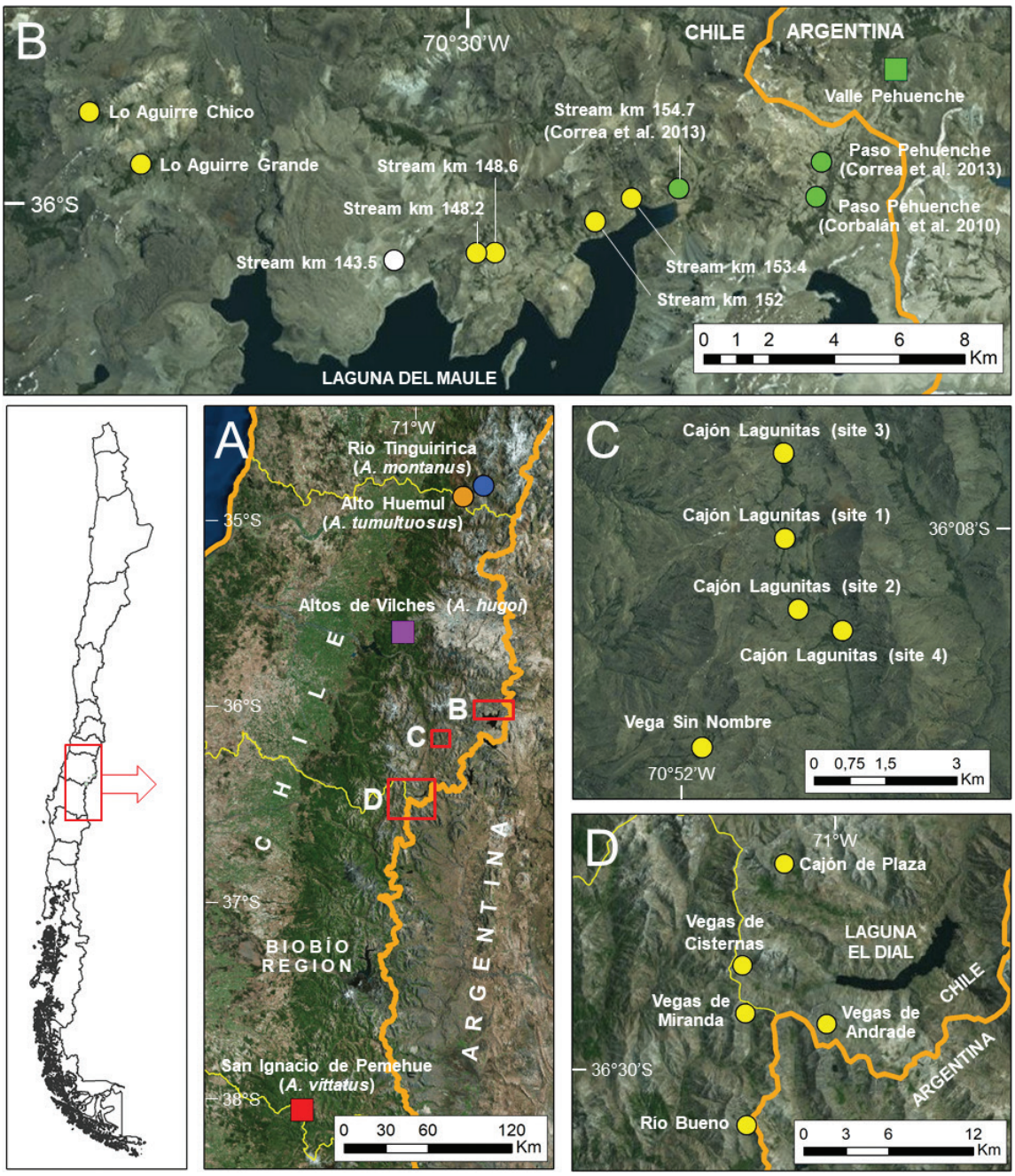


**Figure 1.** New and literature records of *Alsodes* from the Andes Range between 34°50' and 38°05'S. Yellow circles represent the new localities reported in this study; squares represent type localities. **A.** Andean localities of *Alsodes* of the literature between 34°50' and 38°05'S: the southernmost localities of *A. montanus* and *A. tumultuosus*, the type locality of *A. hugoi*, localities of *A. pehuenche* (within red box B, see map B) and the type locality of *A. vittatus*. There is a record of a putative new species related to *A. nodosus* in Pemehue (*Alsodes* sp. 1 of Blotto et al. 2013; not included in the map), presumably the same type locality of *A. vittatus*. Red boxes correspond to the three explored areas described in this study (maps B, C and D). **B.** Area explored during the first field campaign (Paso Pehuenche, Laguna del Maule and surroundings). All colored symbols correspond to localities of *A. pehuenche*: yellow circles correspond to new records; green circles and the square are all previously known localities of the species. The white circle is the place where no amphibian was found. **C.** Area and sites explored during the third field campaign (tributaries of the Guaiquivilo River). **D.** Area and sites explored during the second field campaign (surroundings of Laguna El Dial). See details of the localities and the populations discovered in Table 1. Orange lines represent the boundary between Chile and Argentina; thinner yellow lines indicate the boundaries of the administrative regions of Chile.

was visited once and was explored by three or four people for between one and three hours, using visual encounter and refugia search (cavities between rocks under water) surveys. Most of sites were explored at night, except for one small stream that flows into a tributary of the Guaiquivilo River (Cajón Lagunitas, site 4; Table 1).

**Table 1.** Geographic data and information about the specimens observed in the 19 localities of *Alsodes* surveyed in the western slopes of Andes (Chile, 35°58'–36°32'S). Localities are grouped by explored area, ordered from north to south (Fig. 1). Two of the sites explored had previously been described in the literature (citations in parentheses). The small streams that flow into the northern edge of the Laguna del Maule ("Stream km xxx") were named according to their location along the international road CH-115. Stream km 154.7 corresponds to the second locality of Correa et al. (2013). The relative number of observed tadpoles is based on a gross estimation of more (numerous) or less (a few) than 100 specimens approximately. Phenotypes refer exclusively to adults and/or juveniles (some adult males are depicted in Fig. 2). The codes of buccal mucosa or tail fin samples per locality used for the phylogenetic analysis are indicated (m: male; f: female; j: juvenil; t: tadpole).

| Locality | Latitude (S) | Longitude (W) | Altitude (m a.s.l.) | Specimens observed | Phenotypes | Sample codes |
|---|---|---|---|---|---|---|
| Paso Pehuenche (Corbalán et al. 2010) | 36°00'01" | 70°24'14" | 2463 | > 25 adults; many juveniles; numerous tadpoles of different sizes | *A. pehuenche* | - |
| Stream km 154.7 (Correa et al. 2013) | 35°59'55" | 70°26'31" | 2215 | > 20 adults; numerous tadpoles of different sizes | *A. pehuenche* | DBGUCH1203027 (sequence from GenBank) |
| Stream km 153.4 | 36°00'02" | 70°27'18" | 2225 | Eight adults, one juvenile; a few tadpoles of medium size | *A. pehuenche* | - |
| Stream km 152 | 36°00'20" | 70°27'54" | 2270 | Four adults; a few tadpoles of medium and small size | *A. pehuenche* | - |
| Stream km 148.6 | 36°00'46" | 70°29'33" | 2375 | Seven adults; a few tadpoles of medium and small size | *A. pehuenche* | - |
| Stream km 148.2 | 36°00'46" | 70°29'51" | 2418 | 10 adults, two juveniles; a few tadpoles of medium size | *A. pehuenche* | - |
| Stream km 143.5 | 36°00'52" | 70°31'12" | 2372 | No amphibian was observed | | |
| Lo Aguirre Grande | 35°59'35" | 70°35'24" | 2260 | > 25 adults; many juveniles; numerous tadpoles of different sizes | *A. pehuenche* | AgGr1m, AgGr19m |
| Lo Aguirre Chico | 35°58'53" | 70°36'14" | 2316 | > 25 adults; many juveniles; numerous tadpoles of different sizes | *A. pehuenche* (Fig. 2F) | AgCh2f |
| Cajón Lagunitas (site 1) | 36°07'58" | 70°50'52" | 2026 | Two adult males and two juveniles; numerous tadpoles of great size | similar to *A. hugoi* | - |
| Cajón Lagunitas (site 2) | 36°08'37" | 70°50'43" | 1913 | Two adult males and one female; numerous tadpoles of different sizes | similar to *A. hugoi* | CL2·1m, CL2·2m |
| Cajón Lagunitas (site 3) | 36°07'11" | 70°50'53" | 2157 | > 10 adult males and females; a few tadpoles of medium and great size | similar to *A. hugoi* (Fig. 2E) | CL3·1m, CL3·2f, CL3·3m, CL3·4m, CL3·5m, CL3·6f |
| Cajón Lagunitas (site 4) | 36°08'48" | 70°50'13" | 1805 | A few tadpoles of different sizes | Undetermined | - |
| Vega Sin Nombre | 36°09'52" | 70°51'49" | 2046 | A few tadpoles of great size | Undetermined | VSN1t |
| Vegas de Cisternas | 36°26'47" | 71°03'27" | 1967 | One adult male; numerous tadpoles of different sizes | similar to *A. hugoi* (Fig. 2A) | VC1m, VC2t |
| Cajón de Plaza | 36°23'40" | 71°01'52" | 1966 | Seven adults, four juveniles; a few tadpoles of great size | similar to *A. pehuenche* (Fig. 2B) | CP1f, CP2m, CP4m, CP5m, CP9m, CP10m, CP11f |
| Vegas de Andrade | 36°28'36" | 71°00'15" | 1998 | One adult male; numerous tadpoles of different sizes | similar to *A. hugoi* (Fig. 2C) | VA1m, VA2t |
| Vegas de Miranda | 36°28'16" | 71°03'21" | 1972 | Two adults, five juveniles; a few tadpoles of great size | Undetermined (most) (Fig. 2D) and *A. hugoi* (one juvenile) | VM2j, VM3j, VM4m, VM5m, VM6m, VM7j, VM10j |
| Río Bueno | 36°31'41" | 71°03'19" | 1836 | One juvenile; a few tadpoles of great size | similar to *A. hugoi* | RB1j, RB2t, RB3t |

## Molecular data and phylogenetic analysis

For extracting DNA, we used mainly buccal mucosa of adults and juveniles obtained with Copan 516CS01 swabs (immediately dried with silica gel). Individuals were released at the same capture site after being measured and photographed. A few unidentified tadpoles were also sampled. A small piece of the end of the tail fin from these individuals (3 mm × 3 mm, approximately) was excised for DNA extraction. For this procedure, the tadpoles were anesthetized with buffered MS222 (tricaine methanesul-

fonate, 0.2%; Mitchell 2009) and then released after being submerged in fresh water for about half an hour to remove the anesthetic. DNA was extracted with the Promega Wizard SV Genomic DNA Purification System kit. A fragment of the mitochondrial cytochrome b gene (cytb) was used for the phylogenetic analysis. Information about primers (MVZ15-L and ControlP-H) and PCR conditions for amplifying this fragment are found in Correa et al. (2013). PCR products were sequenced in both directions in an ABI3730XL automatic sequencer (Macrogen Inc., Seoul, South Korea). Sequences were edited and aligned manually with the program BIOEDIT v7.1.3 (Hall 1999). Substitution saturation of the sequences was assessed with DAMBE v6.4.29 (Xia 2017). Sequences were deposited in GenBank (MH332789–MH332821 and MH378965–MH378971).

We performed a phylogenetic analysis to assess the specific identity of the new *Alsodes* populations. In this analysis 14 of the 19 species of *Alsodes* were represented, including specimens from the type localities of *A. pehuenche* and *A. hugoi*. This set of specimens represents the maximum number of species of the genus that can be included by combining all published sequences (Blotto et al. 2013, Correa et al. 2013, Charrier et al. 2015). Most of the sequences of Blotto et al. (2013) are shorter (about one third) than those of the other sources, so we included additional specimens of some species to evaluate possible topological artifacts due to the unequal length of the sequences. We included specimens from 11 of the 17 new localities (excluding streams very close to each other or sites located in the same water system). Phylogenetic relationships were estimated using a Bayesian inference (BI) method, performed with the program MRBAYES v3.2.6 (Ronquist et al. 2012). A reversible-jump Markov Chain Monte Carlo (MCMC) method for exploring the space of all General Time Reversible sub-models, plus gamma and proportion of invariable sites parameters, was applied to the entire cytb fragment. Two independent analyses (each consisting of two groups of four chains that run independently) applying that method were run for 20 million generations, sampling every 1000 generations. The first 25% of generations was conservatively discarded as burn-in after observing the stationarity of ln-likelihoods of trees in TRACER v1.6 (Rambaut et al. 2014). Convergence and mixing of chains were assessed by examining values of average standard deviation of split frequencies (ASDSF), and expected sampling sizes (ESS) and Potential Scale Reduction Factor (PSRF) for all parameters. Trees were rooted with one specimen of *Eupsophus emiliopugini* Formas, 1989, a representative of the sister genus of *Alsodes* (Blotto et al. 2013).

## Results

Considering the three field campaigns we found tadpoles of *Alsodes* in 18 of the 19 sites explored (Fig. 1, Table 1). In 16 of those 18 sites we also observed adults and/or juveniles (see their tentative identifications in Table 1). The only site where no specimen was observed was a relatively short and narrow stream located north of Laguna del Maule (Stream km 143.5, Fig. 1), which at the sampling date (January) had an intermittent flow. Most of the other streams had a continuous flow, except Vegas de Miranda and Vega Sin Nombre. In both sites we observed large tadpoles in isolated remnant pools located in dry sectors of the streambeds. Tadpoles could not be identified to species by their external characteristics, so their identification relied exclusively on the phylogenetic analysis. Some of the adults and juveniles were tentatively identified as *A. pehuenche* or *A. hugoi* by their external characters (Table 1), but most specimens from Vegas de Miranda could not be identified to species level (Fig. 2D).

We obtained an alignment of the cytochrome b of 933 nucleotide sites, although this matrix included a few sequences of Blotto et al. (2013) of 318 base pairs. We did not observe effects of saturation or stop codons in the cytb sequences. The phylogenetic analysis shows that all the new populations are related to *A. pehuenche* or *A. hugoi* (most) (Fig. 3). All specimens from Laguna del Maule and surroundings comprise one well-supported clade together with the reference samples of *A. pehuenche* (from the type locality, Valle Pehuenche, Blotto et al. 2013, and from Paso Pehuenche and Stream km 154.7, Correa et al. 2013). The geographic distribution of this lineage implies a range extension of this species of 14.5 km to the west (Lo Aguirre Chico and Lo Aguirre Grande, Fig. 1B). Within this clade also four specimens from Cajón de Plaza (Figs 1 and 2) are included (see comment below). The genetic divergence among the specimens within this group is extremely low, including some that have identical sequences coming from localities separated by more than 60 km (Lo Aguirre Grande and Cajón de Plaza, Fig. 1). On the other hand, almost all samples coming from the ten southern sites (field campaigns two and three, Figs 1C and 1D), which are genetically and phenotypically more variable (Fig. 2), comprise two well-supported sister clades in the Bayesian consensus tree (Fig. 3). One of these clades includes the representative of the type locality of *A. hugoi* (Altos de Vilches). These clades do not exactly match the two groups of geographically closest localities, since specimens of some localities are distributed into the two clades, so we consider them as conspecific with that species. Although the specimens that constitute these two groups come from localities separated by up to 100 km they have very low genetic distances, which reinforces the hypothesis of their conspecificity. The population of Cajón de Plaza (Fig. 1D), represents a special case, since there specimens with sequences of the *A. pehuenche* and *A. hugoi* type are mixed, but they all resemble *A. pehuenche* phenotypically (Fig. 2B).

In summary, the phylogenetic analysis indicates that all sampled individuals belong to only two previously known species in the Andes Range between 35°30' and 36°S, *A. pehuenche* or *A. hugoi*. These results imply the extension of the distribution range of *A. pehuenche* 14.5 km to the west in Chile, and of *A. hugoi* of 100 km to the south. Also, the

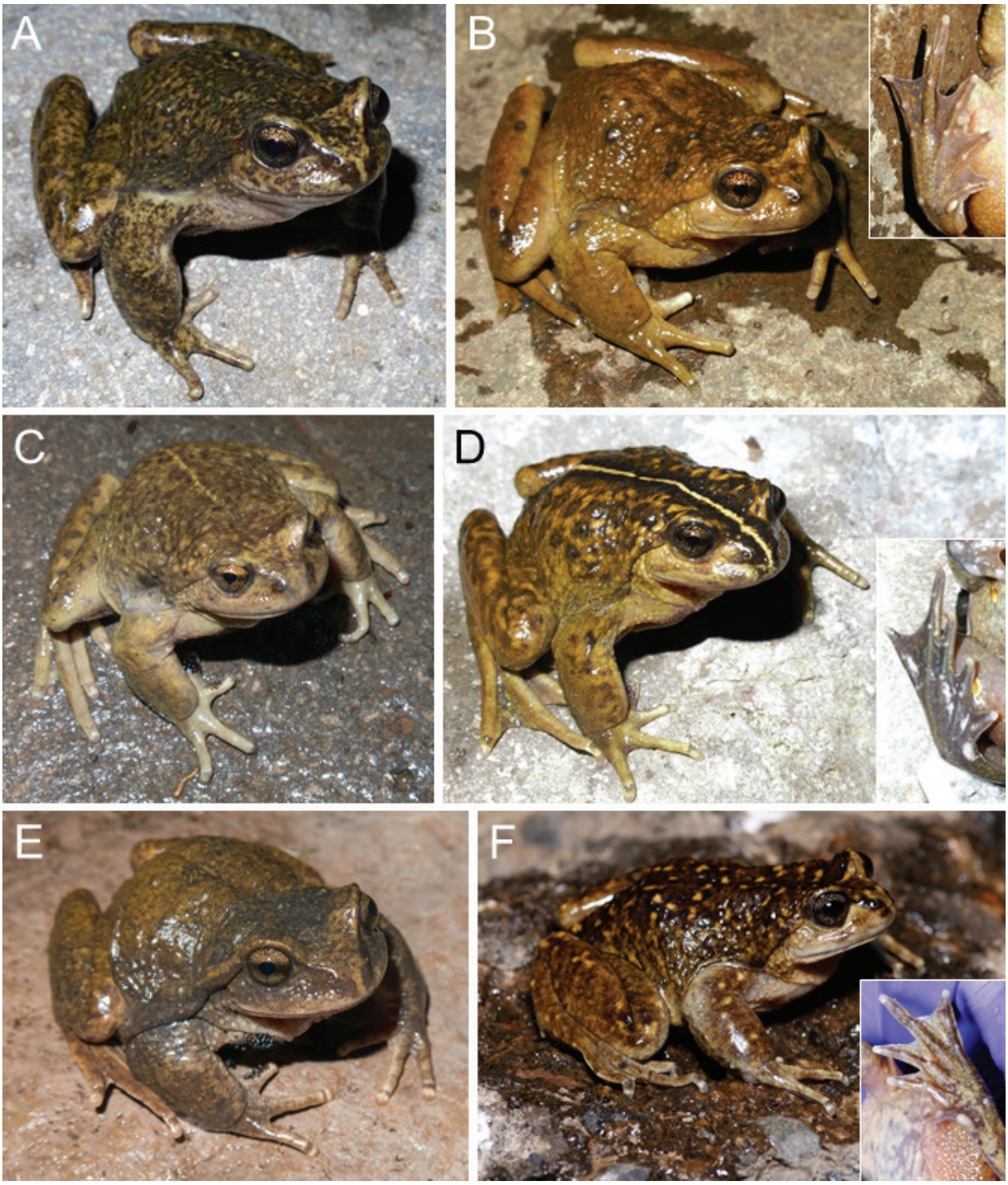


**Figure 2.** Adult males of *Alsodes* from the new discovered localities. In parentheses the specific identification according to the phylogenetic analysis (Fig. 3), the snout-vent length (SVL) and code of the respective buccal mucosa sample are indicated. **A.** Vegas de Cisternas (*A. hugoi*, SVL = 68.3 mm, VC1m). **B.** Cajón de Plaza (undetermined, SVL = 54.8 mm, CP5m). **C.** Vegas de Andrade (*A. hugoi*, SVL = 57.2 mm, VA1m). **D.** Vegas de Miranda (*A. hugoi*, SVL = 56.3 mm, VM6m). **E.** Cajón Lagunitas (site 3) (*A. hugoi*, SVL = 71.7 mm, CLP3-5m). **F.** Lo Aguirre Chico (*A. pehuenche*, SVL = 52.0 mm, AgCh4m). Some populations are characterized by well-developed interdigital webbing in the hind feet (shown in the insets).

altitudinal range of *A. hugoi* was slightly increased since 2115 m (Araya and Cisternas 2008) to 2157 m (Table 1). We do not recognize Cajón de Plaza as a new population of *A. pehuenche* because even though all the specimens we observed resemble this species externally (Fig. 1B), some of them have mitochondrial sequences of the *A. hugoi* type. More phenotypic and genetic data are needed to determine the taxonomic status of this population.

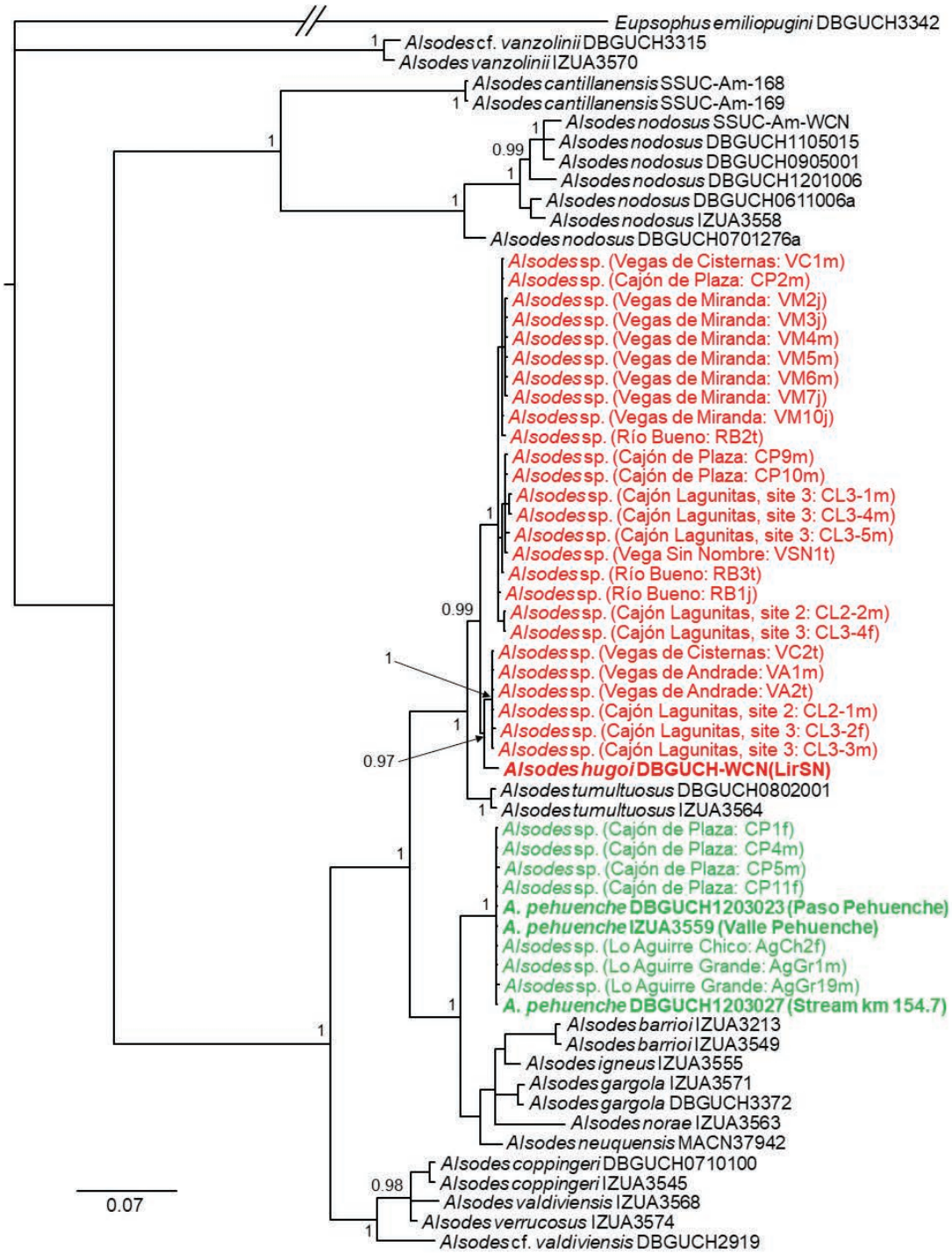


**Figure 3.** Bayesian consensus tree (50% majority-rule) showing the relationships of the new Andean populations of *Alsodes*. Representatives of the new populations are labeled in green (related to *A. pehuenche*) and red (related to *A. hugoi*) (see details of the new localities in Table 1). Note that specimens from Cajón de Plaza are distributed in both the red and green clades. Reference sequences of the type localities of *A. pehuenche* and *A. hugoi* are in bold. Numbers next to the nodes correspond to posterior probabilities (only values ≥ 0.95 of the more internal nodes are shown). The scale bar in the lower left corner represents the expected substitutions per site along the branches.

## Discussion

This study reports new localities of two species of *Alsodes* endemic to the Andes Range, whose geographic distributions were considered extremely restricted. These findings add to a series of Andean populations of *Alsodes* reported in the last decade between 33°25' and 36°S in Chile (Araya and Cisternas 2008, Araya and Riveros 2008, Correa et al.

2008, Corbalán et al. 2010, Correa et al. 2013, Mora et al. 2015, Ramírez 2015, Correa 2017), which have expanded considerably our knowledge about the geographic distribution of the genus. Below we discuss the implications of these discoveries for the biogeography and conservation of the species involved and the genus.

The localities of *A. pehuenche* reported here constitute the third and widest distribution extension since the species was described (Cei 1976), more than doubling the known distribution until 2013 (Correa et al. 2013). Moreover, the occurrence of this species is extended to another hydric system in Chile (Lo Aguirre Grande and Lo Aguirre Chico), not connected directly to Laguna del Maule (Fig. 1). However, the area of occupancy is still very small as this species is restricted to watercourses (see comment below). Although all known sites in Chile are found within the hydrographic basin of the Maule River, it is possible that currently populations from Lo Aguirre Grande and Lo Aguirre Chico are demographically disconnected from those streams that discharge directly into the Laguna del Maule. The new localities also allow us to discard the presence of *A. montanus* around Laguna del Maule mentioned by Cei and Roig (1965). We could not confirm the presence of *A. pehuenche* south of 36°S. Although in Cajón de Plaza we found specimens phenotypically similar to *A. pehuenche*, even with mitochondrial sequences identical to those found in the type locality, they coexist with individuals with sequences of *A. hugoi*. Currently, we have no additional data to identify the possible causes of this mixture of mitochondrial sequences, so we consider the taxonomic status of this population as uncertain. The distribution extension of *A. hugoi* reported here is much more significant, reaching the northeastern end of the Ñuble River basin about 100 km south of the type locality (which is in the Maule river basin). Moreover, this species presents high levels of geographical variation in attributes such as body coloration and development of interdigital webbing, which had been described so far only in another species of the genus, *A. gargola* (Blotto et al. 2013).

These findings have important implications for the biogeography of the genus at different spatial scales. The new localities discovered south of 36°S are in a stretch of the western slopes of the Andes of about 250 km where no populations of *Alsodes* were known. However, an apparent discontinuity in the distribution of the genus of approximately 180 km still persists, covering almost completely the mountainous zones of the Administrative Region of Biobío (Fig. 1A). The relief, altitude, climatic conditions and types of vegetation in this area of the Andes are similar to those found where the new populations of *A. hugoi* were discovered, so this distribution gap is probably due to a lack of exploration. The new localities of *Alsodes* recently reported in the Andes Range (see references above), several of them located in areas of difficult access, support this idea. One of the issues that remains to be clarified is the reciprocal distribution limits between *A. hugoi* and *A. pehuenche*, since *A. hugoi* is to the south and north of the Maule River, whereas *A. pehuenche* is restricted to the head of that river at an intermediate latitude. Although there seems to be an altitudinal segregation between them (*A. hugoi* is between 900 and 2157 m, *A. pehuenche* between 2215 and 2463 m; Table 1), the data are very scarce and fragmentary to establish if one species replaces the other along the water systems. The mixture of mitochondrial sequences at Cajón de Plaza is compatible with the syntopy of both species, but this issue must be assessed with more phenotypic and genetic data.

At a smaller spatial scale, the effectiveness of our searches, together with previous antecedents, suggest that the genus is present in practically all first order watercourses of the western slopes of the Andes. We found tadpoles in 18 of the 19 sites explored (adults and/or juveniles in 16), with a sampling effort of up to three hours per day per site. Similar patterns have been reported for species of the same genus in the Andes. For example, Araya and Cisternas (2008) found reproductive specimens of *A. hugoi* in the 10 sites that they explored, expanding the altitudinal range and the type of environments where this species inhabits (although they did not specify the time spent in the searches). Further north, at Potrero Grande (Metropolitan Region), Correa (2017) reported three localities of *A. montanus* and *A. tumultuosus* discovered in a three-day field campaign, two of them first order streams. Moreover, the relative ease of finding individuals in various stages of development in most localities reported here (for example, Paso Pehuenche, Lo Aguirre Grande and Lo Aguirre Chico; see Table 1) suggests high local abundance. It is important to note that these observations were made at night, when the activity of these species is greater. In many cases the tadpoles were observed immediately at the beginning of the surveys (at sunset) and the first adults or juveniles in less than half an hour. The presence of numerous tadpoles, and juveniles in some cases, indicates that first order streams are reproduction and recruitment sites that could harbor abundant populations. Although we did not quantify exactly the number of larvae, our estimates are compatible with the number of specimens and densities reported for the type locality of *A. pehuenche* (Corbalán et al. 2008, 2014). This last study showed that *A. pehuenche* has a prolonged larval development that lasts at least four years, a strategy that could also occur in other Andean species of the genus (see references in Corbalán et al. 2014). This development strategy would require hydric systems with permanent flow, so our observations of tadpoles in two intermittent watercourses suggest that adverse environmental conditions, such as a drought (Corbalán et al. 2014), could affect the recruitment.

The distribution range extensions of *A. pehuenche* and *A. hugoi* have the potential to change their conservation categories, as happened with *A. montanus* and *A. tumultuosus*, which recently were down-listed from Critically Endangered to Vulnerable due to significant range extensions (IUCN 2017). However, the known range of *A. pehuenche* remains extremely restricted and is not represented in any protected area. Moreover,

it is unclear whether more than one subpopulation (as defined by the IUCN) can be considered to exist. This species, due to its aquatic habits, only occupies streams and contiguous swamps (Corbalán et al. 2008, 2010; C. Correa, D. Vásquez, personal observations), so its area of occupancy (AOO) is probably much less than its extent of occurrence (EOO). The estimates of the IUCN (2017) reflect these observations, considering an EOO of 9 $km^2$ and an AOO of about 5 $km^2$ (although this last figure would be an overestimation as it is based on lower resolution satellite images). In addition, it remains to be assessed whether the localities of the western Andean slopes (Chile) are genetically and/or demographically separated from those located on the eastern slopes (Argentina), since they belong to different drainage basins. However, the limit of the drainage basins, corresponding to the border crossing between both countries, does currently not seem to be an important geographical barrier since the nearest streams of both basins are less than 50 m apart. Preliminary mitochondrial data are consistent with the absence of a barrier, suggesting that the localities from both sides of the border can be considered as inhabited by one population (Correa et al. 2013; unpublished data). Some of the threats identified by Corbalán et al. (2010) (habitat alteration, contamination, aquatic invasive species, livestock, natural floods, climate change) and Ghirardi et al. (2014) (chytrid fungus) also might affect the new described localities. Until now we have only been able to verify the effects of livestock and the presence of garbage, which are particularly intense at sites close to the border crossing (here called Paso Pehuenche), but all sites may be potentially threatened by environmental factors such as natural floods and climate change. In the case of *A. hugoi*, its distribution is currently composed of three highly disconnected areas separated by 60 and 40 km approximately, so they surely represent at least three populations, only one of which is present in a protected area (the type locality). This species is threatened by forest fires and tourism in its type locality (IUCN 2017), but we detected the effects of transhumant livestock activities on all discovered sites.

The observations made in this study add to a series of antecedents that show that high Andean environments where *Alsodes* species inhabit are threatened by natural and anthropogenic causes (Araya and Riveros 2008, Corbalán et al. 2010, Charrier et al. 2017). In this context, the geographic data obtained in this study acquire a special relevance since they suggest that the genus is widely distributed in the Andes, so any type of anthropic intervention (for example, mining projects, hydroelectric plants, mountain recreational centers, intensive livestock) has the potential to affect some population. Therefore, although the knowledge of the biogeography of Andean *Alsodes* species has improved, several aspects must be considered to reassess their conservation status. First, the few known populations seem to be threatened by several natural and anthropogenic factors. Second, their known distribution ranges are highly fragmented so their estimated AOOs would be extremely small. And third, there is no information on population connectivity, abundance or demographic trends, so we recommend maintaining their categories until more population and distribution data are obtained.

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## References

Araya S, Cisternas J (2008) Antecedentes sobre el hábitat, localización y anuros simpátricos a *Alsodes hugoi* (Amphibia, Cycloramphidae) en la localidad tipo. Boletín del Museo Nacional de Historia Natural 57: 127–131.

Araya S, Riveros E (2008) Ampliación del rango de distribución geográfica de *Alsodes montanus* (Amphibia, Leptodactylidae) a la Región de O'Higgins. Boletín del Museo Nacional de Historia Natural 57: 117–123.

Blotto BL, Nuñez JJ, Basso NG, Úbeda CA, Wheeler WC, Faivovich J (2013) Phylogenetic relationships of a Patagonian frog radiation, the *Alsodes* + *Eupsophus* clade (Anura: Alsodidae), with comments on the supposed paraphyly of *Eupsophus*. Cladistics 29(2): 113–131. https://doi.org/10.1111/j.1096-0031.2012.00417.x

Cei JM (1976) Remarks on some neotropical amphibians of the genus *Alsodes* from southern Argentina. Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano 117(3–4): 159–164.

Cei JM, Roig VG (1965) The systematic status and biology of *Telmatobius montanus* Lataste (Amphibia: Leptodactylidae). Copeia 1965 (4): 421–425. http://www.jstor.org/stable/1440989

Charrier A, Correa C, Castro C, Méndez MA (2015) A new species of *Alsodes* (Anura: Alsodidae) from Altos de Cantillana, central Chile. Zootaxa 3915(4): 540–550. http://dx.doi.org/10.11646/zootaxa.3915.4.5

Charrier A, Mora M, Correa C, Palma E (2017) Monitoreo y Conservación de Anfibios Alto Andinos de la Región Metropolitana. Imprenta América Ltda., Valdivia, 79 pp.

Corbalán V, Debandi G, Úbeda CA (2008) *Alsodes pehuenche* (NCN). Larval biology. Herpetological Review 39(4): 457–458.

Corbalán V, Debandi G, Martínez F (2010) *Alsodes pehuenche* (Anura: Cycloramphidae): Past, present and future. Cuadernos de Herpetología 24(1): 17–23. http://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S1852-57682010000100002

Corbalán V, Debandi G, Martínez F, Úbeda C (2014) Prolonged larval development in the Critically Endangered Pehuenche's frog *Alsodes pehuenche*: implications for conservation. Amphibia-Reptilia 35: 283–292. https://doi.org/10.1163/15685381-00002951

Correa C (2017) Refuting the revalidation of *Telmatobius laevis* Philippi 1902. Herpetological Journal 27(1): 69–78.

Correa C, Pastenes L, Iturra P, Calderón P, Vásquez D, Lam N, Salinas H, Méndez MA (2013) Confirmation of the presence of *Alsodes pehuenche* Cei, 1976 (Anura, Cycloramphidae) in Chile: morphological, chromosomal and molecular evidence. Gayana 77(2): 117–123. https://doi.org/10.4067/S0717-65382013000200006

Correa C, Sallaberry M, Iturra P, Collado G, Méndez MA (2008) Amphibia, Anura, Cycloramphidae, *Alsodes montanus*: New record and geographic distribution map. Check List 4(4): 467–471. https://doi.org/10.15560/4.4.467

Cuevas CC (2013) The identity of the Chilean frog *Alsodes laevis* (Philippi 1902) (Cycloramphidae): Synonymy and generic partitioning of the type series. Herpetological Journal 23(3): 145–152.

Cuevas CC, Formas JR (2001) A new species of *Alsodes* (Amphibia, Anura, Leptodactylidae) from Central Chile. Amphibia-Reptilia 22(2): 187–198. https://doi.org/10.1163/15685380152030418

Duellman WE (1979) The Herpetofauna of the Andes: Patterns of Distribution, Origin, Differentiation and Present Communities. In: Duellman WE (Ed.). The South American herpetofauna: its origin, evolution, and dispersal. Monograph of the Museum of Natural History, The University of Kansas 7: 371–459.

Duellman WE (1999) Patterns of Distribution of Amphibians: A Global Perspective. The Johns Hopkins University Press, Baltimore, 633 pp.

Ghirardi R, Levy MG, López JA, Corbalán V, Steciow MM, Perotti MG (2014) Endangered amphibians infected with the chytrid fungus *Batrachochytrium dendrobatidis* in Austral temperate wetlands from Argentina. Herpetological Journal 24(2): 129–133.

Hall TA (1999) BioEdit: an user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

IUCN (2017) The IUCN Red List of Threatened Species. Version 2017-3. http://www.iucnredlist.org [accessed: March 10, 2018]

IUCN SSC Amphibian Specialist Group (2013) *Alsodes pehuenche*. The IUCN Red List of Threatened Species 2013: e. T56319A18136036. http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T56319A18136036.en [Downloaded on 16 October 2017]

Mitchell MA (2009) Anesthetic considerations for amphibians. Journal of Exotic Pet Medicine 18(1): 40–49. https://doi.org/10.1053/j.jepm.2008.11.006

Mora M, Horta I, Soffia F, Charrier A, Palma E, Correa C (2015) Estudios para la conservación de los anfibios altoandinos de Chile central. Boletín Chileno de Herpetología 2: 8–11.

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. http://beast.bio.ed.ac.uk/Tracer

Ramírez D (2015) Nueva localidad para *Alsodes tumultuosus* Veloso, Iturra & Galleguillos, 1979 (Amphibia, Alsodidae) en la Cordillera de los Andes, Región de O'Higgins, Chile. Biodiversity and Natural History 1: 1–5.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Xia X (2017) DAMBE6: New tools for microbial genomics, phylogenetics and molecular evolution. Journal of Heredity 108 (4): 431–437. https://doi.org/10.1093/jhered/esx033

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# A new miniature cryptic species of the seasonal killifish genus *Spectrolebias* from the Tocantins River basin, central Brazil (Cyprinodontiformes, Aplocheilidae)

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## Abstract

The miniature seasonal killifish *Spectrolebias costae*, first described for the middle Araguaia River basin, has been also recorded from two areas in the middle Tocantins River basin, from where male specimens exhibit some differences in their colour pattern. Analyses directed to species delineation (GMYC and bPTP), using a fragment of the mitochondrial gene COI, strongly support two species, *S. costae* from the Araguaia River basin and a new species from the Tocantins River basin. *Spectrolebias gracilis* **sp. n.** is described on the basis of specimens collected from two localities separated by about 530 km, Canabrava River floodplains near Alvorada do Tocantins and Tocantins River floodplains near Palmeirante. Field inventories were unsuccessful in finding additional populations in the region, which is attributed to the high environmental degradation, including several large dams that have permanently inundated typical killifish habitats. *Spectrolebias gracilis* is member of a clade also including *S. costae*, *S. inaequipinnatus*, and *S. semiocellatus*, diagnosed by having the dorsal and anal fins in males with iridescent dots restricted to their basal portion, caudal fin in males hyaline, and caudal-fin base with two pairs of neuromasts. Within this clade, a single miniaturisation event is supported for the most recent common ancestor of the subclade comprising *S. costae* and *S. gracilis*, which differ from other congeners by reaching only about 20 mm standard length as maximum adult size.

## Introduction

The great species diversity, striking colour patterns and the broad array of unique biological specializations make aplocheiloid killifishes important members of the tropical biota of Americas, Africa, and southern Asia (Costa 2008). The subequatorial South American area comprising the largest southern tributaries of the Amazonas River (Tocantins, Araguaia, Xingu and Tapajós River drainages), is remarkable by concentrating numerous endemic aplocheiloid fishes (Costa 1990, 2007a, b, 2011, 2016), many of them consisting of miniatures not surpassing 30 mm of standard length (SL) when adults (Costa 1998). Some endemic groups, such as the genera *Maratecoara* Costa, 1995, *Pituna* Costa, 1989, *Plesiolebias* Costa, 1989, and part of the genus *Spectrolebias* Costa & Nielsen, 1997 are members of the aplocheiloid clades known as seasonal or annual killifishes (Myers 1942; Costa 2002a), that comprise species completing their whole life cycle in seasonal pools formed during the rainy seasons.

*Spectrolebias* is the most basal lineage and the only genus of the seasonal killifish tribe Cynolebiini that is geographically widespread along southern Amazon tributaries (Costa 2007a; Costa et al. 2017). *Spectrolebias costae* (Baker 1990) is the smallest member of the Cynolebiini, with its maximum adult size not surpassing about 20 mm SL (Costa 2007a). This species was first discovered in the early 1980's, near the central Brazilian town of Aruanã, in the middle Araguaia River floodplains, by local fishermen (L. Costa, pers. comm. to WJEMC, January 1986;

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Costa 2002b). It was soon exported as an aquarium fish species and quickly became a worldwide popular species, but its formal description was only published about ten years later, in an American aquarium association journal (Lazara 1991). It was then named *Cynolebias costai* Lazara, 1991, in honour of the Brazilian fisherman L. C. Costa from Aruanã. However, one year before Lazara's description, Baker (1990) reported his experience with this species in aquarium referring to it as *Cynolebias costae* and providing a brief description of the colour pattern of males and females. Therefore, following the International Code of Zoological Nomenclature (ICZN 1999), Baker (1990) should be considered the author of *C. costae*, of which *Cynolebias costai* Lazara, 1991 is the junior synonym (Costa 2008). More recently, this species was transferred to *Spectrolebias* (Costa 2010).

Between 1986 and 1994, in addition to the type locality region around Aruanã, *S. costae* was also found in other localities of the Araguaia River basin in the Bananal Island and adjacent areas in the Formoso River basin, as well as in the das Mortes River floodplains, which is a main tributary of the Araguaia River (Costa 1995b). However, some years later new populations morphologically similar to *S. costae* were found in the middle section of the Tocantins River basin (Costa 2007a). More detailed comparison revealed that populations from the Tocantins basin differ from populations from the Araguaia basin in some characters of the male colour pattern, suggesting that it might be a distinct cryptic species (*sensu* Bickford et al. 2007). That hypothesis is herein corroborated by mitochondrial-DNA species delimitation analyses, and so we provide a formal description of this new species.

## Materials and methods

### Specimens

Field studies failed to find specimens of *S. costae* in the type locality region or in the das Mortes River basin, areas that were drastically modified in recent years (see Discussion). Consequently, for molecular studies, field collections were made only in the floodplains of the Formoso River, middle Araguaia River basin, where populations of *S. costae* are still abundant, and in two localities of the middle Tocantins River, in the Canabrava River floodplains and in the Tocantins River floodplains near the town of Palmeirante. For morphology, both recent and older collections deposited in the ichthyological collection of the Institute of Biology, Federal University of Rio de Janeiro (UFRJ), were analysed. Specimens were captured with small dip nets (40 × 30 cm) and euthanized soon after collection. Euthanasia was conducted in a buffered solution of tricaine methanesulfonate (MS-222) at a concentration of 250 mg/l, for a period of about 10 minutes, i.e., until opercular movements ceased. Collections were made with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit number 20618-1 to WJEMC) and methods for euthanasia were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 01200.001568/2013-87). See Costa (2007a) for a list of comparative material of *Spectrolebias*.

### DNA extraction, PCR, and sequencing

Specimens were fixed in absolute ethanol and preserved in the same fixative. We used a fragment of the mitochondrial gene cytochrome oxidase c subunit I (COI), which is the single-locus marker most used for species delimitation. Total genomic DNA was extracted from muscle tissue of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer's instructions. To amplify the fragments of the DNA were used the primers LCO1490, HCO2198 (Folmer et al. 1994) and Cox1R (Costa and Amorim 2011). Polymerase chain reactions (PCR) were performed in 30µl reaction mixtures containing 5x Green GoTaq Reaction Buffer (Promega), 3.2 mM $MgCl_2$, 1 µM of each primer, 75 ng of total genomic DNA, 0.2 mM of each dNTP and 1U of Taq polymerase. The thermocycling profile was: (1) 1 cycle of 4 minutes at 94 °C; (2) 35 cycles of 1 minute at 92 °C, 1 minute at 47 °C and 1 minute at 72 °C; and (3) 1 cycle of 4 minutes at 72 °C. In all PCR reactions, negative controls without DNA were used to check contaminations. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 10 µl reaction volumes containing 1 µl BigDye 2.5, 1.55 µl 5x sequencing buffer (Applied Biosystems), 2 µl of the amplified products (10–40ng), and 2 µl primer. The thermocycling profile was: (1) 35 cycles of 10 seconds at 96 °C, 5 seconds at 54 °C and 4 minutes at 60 °C. The sequencing reactions were purified and denatured and the samples were run on an ABI 3130 Genetic Analyzer. Sequences were edited using MEGA 6 (Tamura et al. 2013) and aligned using ClustalW (Chenna et al. 2003). The DNA sequences were translated into amino acids residues to test for the absence of premature stop codons or indels using the program MEGA 6.0. A list of specimens with respective catalogue numbers, locality coordinates and GenBank accession numbers are given in Table 1.

### Morphological studies

Specimens were fixed in formalin for a period of 10 days, and then transferred to 70% ethanol. Material is deposited in the ichthyological collections of Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro (UFRJ) and Centre of Agrarian and Environmental Sciences, Federal University of Maranhão, Chapadinha (CICCAA). Measurements and counts follow Costa (1988). Measurements are presented as percentages of standard length (SL), except for those related to head morphology, which are expressed as percentages of head length. Fin-ray counts include all elements. Specimens were cleared and stained for osteological preparations following Taylor

**Table 1.** List of specimens used in molecular analysis, with respective catalogue numbers, locality coordinates and GenBank accession numbers.

| Species | Catalogue number | Coordinates | GenBank |
|---|---|---|---|
| *Nematolebias whitei* | UFRJ 6841.1 | 22°34'34"S, 41°59'10"W | KF311352 |
| *Spectrolebias semiocellatus* | UFRJ 9297.1 | 11°47'30"S, 49°45'56"W | MF441496 |
| *Spectrolebias costae* | UFRJ 9298.1 | 11°47'30"S, 49°45'56"W | MF441497 |
| | UFRJ 9298.2 | 11°47'30"S, 49°45'56"W | MF441498 |
| | UFRJ 9298.3 | 11°47'30"S, 49°45'56"W | MF441499 |
| *Spectrolebias gracilis* sp. n. | UFRJ 9299.1 | 07°53'02"S, 47°55'45"W | MF441500 |
| | UFRJ 9299.2 | 07°53'02"S, 47°55'45"W | MF441501 |
| | UFRJ 9299.3 | 07°53'02"S, 47°55'45"W | MF441502 |
| | UFRJ 9299.4 | 07°53'02"S, 47°55'45"W | MF441503 |
| | UFRJ 9300.1 | 12°29'45"S, 49°00'28"W | MF441504 |
| | UFRJ 9300.2 | 12°29'45"S, 49°00'28"W | MF441505 |
| | UFRJ 9300.3 | 12°29'45"S, 49°00'28"W | MF441506 |
| | UFRJ 9300.4 | 12°29'45"S, 49°00'28"W | MF441507 |

and Van Dyke (1985); the abbreviation C&S in lists of material indicates those specimens prepared for osteological examination. Terminology for osteological structures followed Costa (2006), for frontal squamation Hoedeman (1958), and for cephalic neuromast series Costa (2001). Osteological characters used in species description are those showing informative variability among the Cynolebiini (Costa 2006). Characters involving colouration of living specimens were based on photographs taken in small aquaria between 12 and 48 hours after collections. Characters informative for distinguishing the cryptic species were formatted according to Sereno (2007).

### Phylogenetic analysis and species delimitation

Phylogenetic analyses were performed in the programs Garli 2.0 (Zwickl 2006), for maximum likelyhood (ML) and MrBayes 3.2.6 (Ronquist et al. 2012), for Bayesian inference (BI). Ingroup terminal taxa were three specimens of *S. costae* and four specimens of each population of *S.* cf. *costae*; outgroups were *Spectrolebias semiocellatus* Costa & Nielsen, 1997, a member of the sister group of *S. costae* (Costa, 2010) and *Nematolebias whitei* (Myers, 1942), a member of the basal-most lineage of the Cynolebiini (Costa et al. 2017). The best fitting evolutionary model of each codon position was determined using Akaike information criteria (AIC), with jModeltest version 2.1.7 (Darriba et al. 2012); the best model found for each position was GTR+G. Support values of the ML analysis were calculated by 1000 bootstrap replications (Felsenstein 1985). For BI analysis, two independent Markov-Chain Monte Carlo (MCMC) runs were performed with 3 million generations each, sampling one of every 1,000 trees. The quality of the MCMC chains was evaluated in Tracer 1.6 (Rambaut et al. 2013). Posterior probabilities were obtained after applying a burn-in of the first 25% of the trees.

For unilocus species delimitations, the dataset was reduced to include unique haplotypes, comprising two haplotypes of *S. costae* and two of each population of *S.* cf. *costae*. These analyses were conducted using the generalized mixed Yule coalescent (GMYC) using both single and multiple threshold analyses (Pons et al. 2006; Fujisawa and Barraclough 2013) and the Bayesian implementation of the Poisson tree processes (bPTP; Zhang et al. 2013), which were performed in the Exelixis Lab's web server (http://species.h-its.org/gmyc/ and http://species.h-its.org/ptp/, respectively). The ultrametric tree was generated with BEAST v1.8.4 and its user interface BEAUti 1.8.4 (Drummond et al. 2012). The clock model was set to the uncorrelated lognormal, the tree prior was set to a Coalescent Constant Population prior, the number of generations for MCMC was 10 million, with sampling of the trees every 1000 generations. Convergence was evaluated with Tracer v1.6. The value of parameters of the analyses, convergence of the MCMC chains, sample size and the stationary phase of the chains were evaluated using Tracer v. 1.5; a burn-in discarding the first 20% of the trees was applied using Tree-Annotator v1.8.4 (as for BEAST).

## Results

### Molecular data

The phylogenetic analyses supported the two populations of the Tocantins River basin as a single exclusive lineage with high support in both ML and BI analyses (Fig. 1). However, the two populations of the Tocantins River basin, although separated by about 530 km were not individually supported as exclusive lineages; the Canabrava population was supported by low bootstrap value for the ML analysis, and the Palmeirante population as a basal non exclusive lineage, which may be an indicative of persisting genetic flow (Wiens and Penkrot 2002). Concordantly, both the GMYC and bPTP models delimited the Tocantins River basin populations as two distinct specific entities, whereas haplotypes of the Canabrava and Palmeirante populations were clustered in a single species (Fig. 2).

### Morphological characters

Morphometric and meristic data obtained from specimens representing the populations of the Tocantins River basin (see description below) were similar to data recorded for specimens collected along the Araguaia River basin (Costa 2007a). Females were nearly identical in all characters examined, including colour pattern. Only two characters

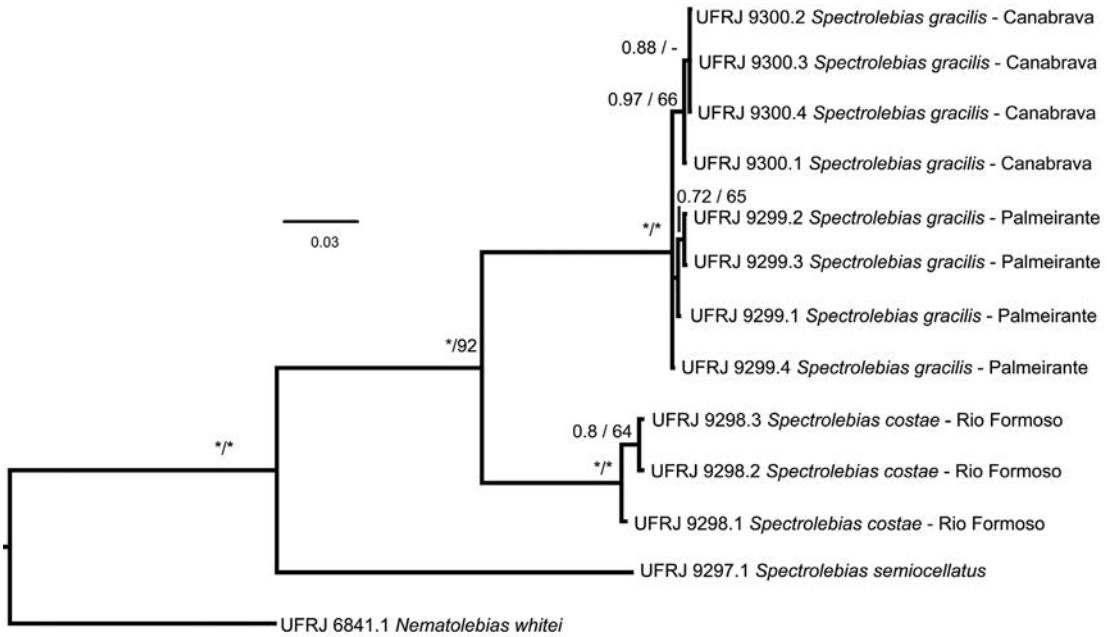


**Figure 1.** Bayesian phylogenetic tree for *Spectrolebias costae* sp. n., *S. gracilis*, *S. semiocellatus* and *Nematolebias whitei*. Numbers above nodes are support values, including posterior probabilities for the Bayesian analysis, followed by bootstrap percentages for the maximum likelihood analysis; *, means maximum support value and, bootstrap value below 50.

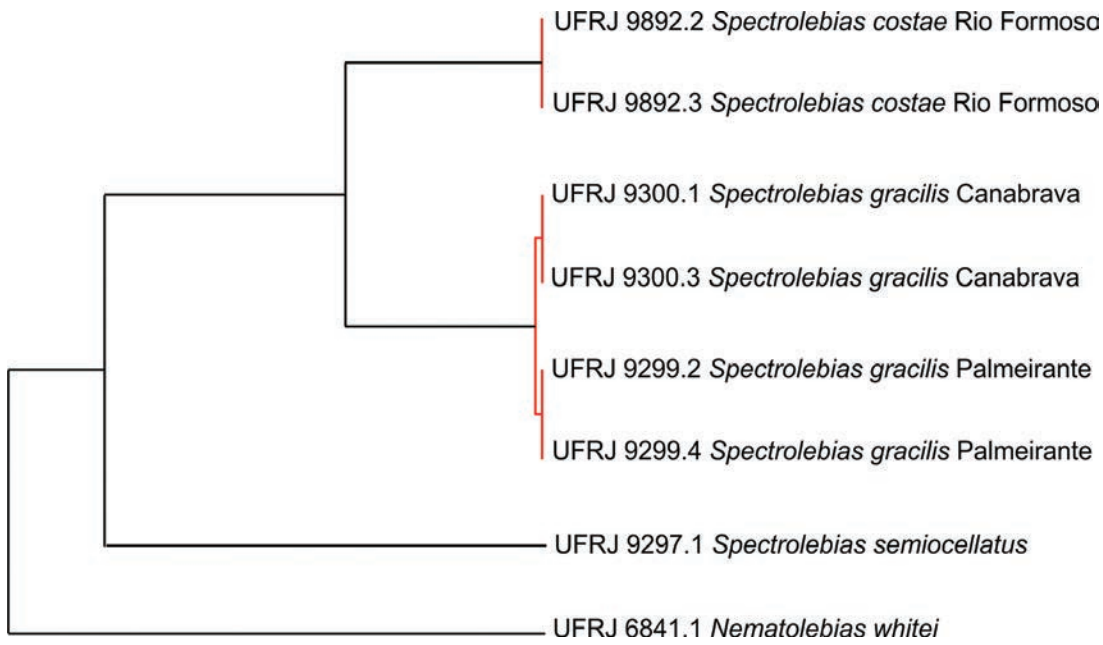


**Figure 2.** Species delimitation tree generated by the General Mixed Yulle Coalescent (GMYC) and the Bayesian Poisson Tree Processes (bPTP) models, using a fragment of the gene COI. Black lines indicate branching processes among species, red lines indicate branching processes within species.

of the colour pattern of males were consistently effective to distinguish specimens of different populations:

1. Opercle in males, iridescent pattern: (0) 6–8 small blue dots usually arranged in three vertical rows over dark ground colour (in all populations of the Araguaia River basin; Fig. 3); (1) 10–12 blue spots in close proximity, irregularly arranged and surrounded by diffuse blue iridescence (in all populations of the Tocantins River basin; Fig. 4).
2. Dorsal in males, basal portion, iridescent pattern: (0) blue dots regularly arranged in single longitudinal row (in all populations of the Araguaia River basin; Fig. 3); (1) blue dots irregularly arranged, often forming two rows (in all populations of the Tocantins River basin; Fig. 4).

**Figure 3.** *Specrolebias costae*, UFRJ 3549, male, 18.8 mm SL; das Mortes River floodplains.

## Taxonomic accounts

### *Spectrolebias gracilis* sp. n.

http://zoobank.org/D920DB7C-E3EA-477E-A744-3904651C2F8A
Figs. 4–5; Table 2

**Holotype.** UFRJ 6440, male, 19.2 SL; Brazil: Tocantins state: Alvorada do Tocantins municipality: temporary lagoons close to the Canabrava River, a tributary of the Santa Teresa River, middle Tocantins River basin, road TO-373, 12°29'46"S, 49°00'51"W, altitude about 290 m asl; W. J. E. M. Costa et al., 16 Apr. 2006.

**Paratypes.** UFRJ 6441, 5 males, 18.9–20.8 mm SL, 3 females, 16.1–17.8 mm SL; UFRJ 6442, 3 males, 18.7–19.1 mm , 1 female, 16.8 mm SL (C&S); collected with holotype. UFRJ 9300, 2 males, 19.6–19.7 mm SL, 2 females, 18.4–18.5 mm SL; type locality area, 12°29'45"S, 49°00'28"W, altitude about 290 m; W. J. E. M. Costa et al., 26 Feb. 2013. – UFRJ 9593, 2 males, 19.9–20.0 mm SL, 2 females, 16.2–16.8 mm SL; UFRJ 9299, 4 males, 18.5–20.5 mm SL, 1 female, 19.2 mm SL; Goiatins municipality, temporary pool in the floodplains of the right bank of the Tocantins River, near Palmeirante, 07°53'02"S, 47°55'45"W, altitude about 170 m asl; W. J. E. M. Costa et al., 28 Feb. 2013. – UFRJ 10802, 9 males, 15.8–17.6 mm SL, 22 females, 13.0–17.4 mm SL; UFRJ 10803, 3 males, 16.3–20.7 mm SL, 3 females, 13.9–15.3 mm SL (C&S); CICCAA 00692, 5 males, 16.2–17.2 mm SL, 5 females, 14.6–16.0 mm SL; same locality; A.C. de Luca, 2012.

**Diagnosis.** *Spectrolebias gracilis* is member of a clade endemic to the Araguaia-Tocantins River System, also including *S. costae*, *S. semiocellatus* Costa & Nielsen, 1997 and *S. inaequipinnatus* Costa & Brasil, 2008, and morphologically diagnosed by: dorsal and anal fins in males with iridescent dots restricted to the basal portion of fins (vs. scattered over the whole fin), caudal fin in males hyaline (vs. variably coloured, usually dark red or grey), caudal-fin base with two pairs of neuromasts (vs. one). *Spectrolebias gracilis* is similar to *S. costae* and distinguished from *S. semiocellatus* and *S. inaequipinnatus* by having dorsal fin rounded in males (vs. pointed), dark brown to black pigmentation on the flank in males (vs. light brownish grey), and a subdistal bright blue stripe on the dorsal and anal fins in males (vs. subdistal bright blue absent). *Spectrolebias gracilis* differs from *S. costae* by the iridescent light blue colour pattern in males, comprising the presence of 10–12 small blue spots irregularly arranged on opercle, surrounded by diffuse blue iridescence (Fig. 4; vs. 6–8 small blue spots, usually arranged in three vertical series, contrasting with dark brown colour ground, Fig. 3) and one or two series of dots irregularly arranged on the basal portion of the dorsal fin (Fig. 4; vs. blue dots arranged in single longitudinal row close to fin base, Fig. 3).

**Description.** Morphometric data is given in Table 2. Largest male examined 20.8 mm SL; largest female examined 18.5 mm SL. Body relatively deep, compressed. Greatest body depth in vertical through pelvic-fin insertion. Dorsal profile convex between snout and posterior end of dorsal fin, nearly straight and horizontal on caudal peduncle; ventral profile convex between lower jaw and pectoral-fin base, approximately straight and moderately steep between pelvic-fin base and posterior end of anal fin, nearly straight and horizontal on caudal peduncle. Urogenital papilla short and cylindrical in males, globular in females. Head moderately wide, sub-triangular in lateral view. Jaws short, teeth numerous, conical, irregularly arranged; outer teeth hypertrophied, inner teeth small and numerous. Vomerine teeth 13. Gill-rakers on first branchial arch 2 + 7, gill-rakers short, straight, without denticles. Head narrow, sub-triangular in lateral view.

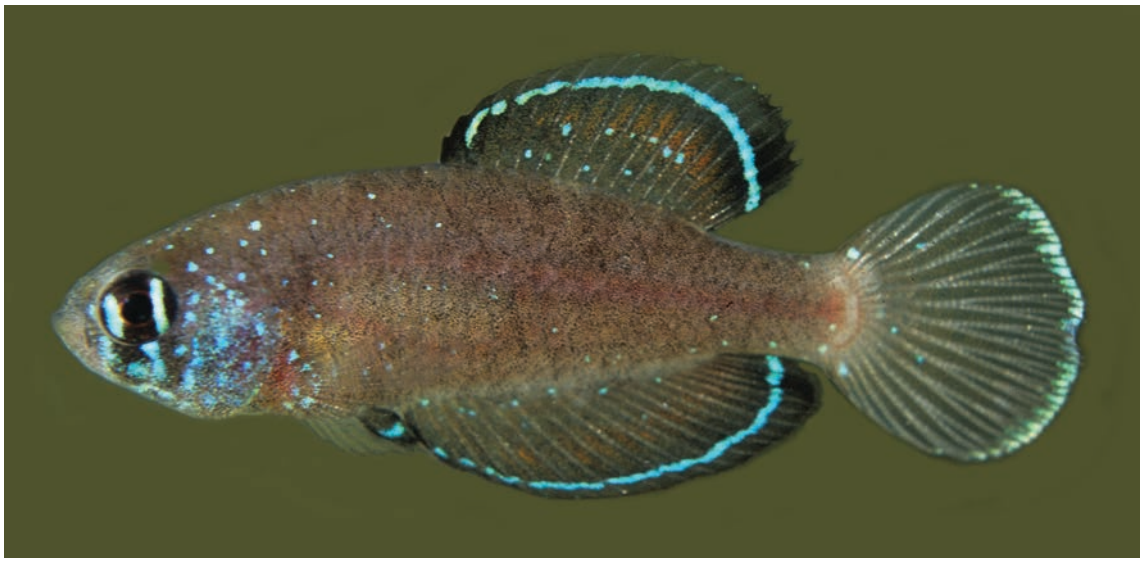

**Figure 4.** *Spectrolebias gracilis* sp. n., UFRJ 6440, holotype, male, 19.2 mm SL; Canabrava floodplains.

**Figure 5.** *Spectrolebias gracilis* sp. n., UFRJ 6441, paratype, female, 17.8 mm SL; Canabrava floodplains.

Snout short, blunt. Jaws short, premaxilla and dentary teeth conical, small, numerous, irregularly arranged, except for external series with longer fang-like teeth. Vomerine teeth absent. Dermosphenotic absent. Gill-rakers on first branchial arch 2 + 8–9. Six branchiostegal rays. Total vertebrae 26–27.

Dorsal and anal fins rounded, broader and fan-shaped in males, without filamentous rays. Caudal fin subtruncate, dorsal and ventral margins nearly straight, posterior margin gently convex. Pectoral fin elliptical, posterior margin reaching vertical between base of fifth and sixth anal-fin rays in males, reaching urogenital papilla in females; in males, minute contact organs on two uppermost pectoral-fin rays. Pelvic-fin small, tip reaching between second and third anus anal-fin ray in males, between first and second anal-fin ray in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin in vertical between base of 3rd and 5th anal-fin rays in males, between base of 4th and 6th anal-fin rays in females. Dorsal-fin origin between neural spines of vertebrae 7 and 8 in males, between neural spines of vertebrae 9 and 10 in females; anal-fin origin between pleural ribs of vertebrae 6 and 7 in males, between pleural ribs of vertebrae 7 and 8 in females. Hypurals ankylosed, forming single hypural plate. Ventral process of posttemporal absent. Dorsal-fin rays 21–23 in males, 15–18 in females; anal-fin rays 23–25 in males, 19–21 in females; caudal-fin rays 21–23; pectoral-fin rays 12–13; pelvic-fin rays 5–6.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 20% of caudal-fin base; no scales on dorsal, anal and pectoral-fin bases. Longitudinal series

**Table 2.** Morphometric data of *Spectrolebias gracilis* (sp. n.).

| | Holotype | Paratypes | |
|---|---|---|---|
| | male | males (10) | females (6) |
| Standard length (mm) | 19.2 | 17.3–20.8 | 16.1–17.8 |
| Percent of standard length | | | |
| Body depth | 33.6 | 31.3–36.7 | 32.1–33.6 |
| Caudal peduncle depth | 14.2 | 13.2–15.7 | 12.9–13.7 |
| Pre-dorsal length | 50.6 | 46.8–51.2 | 56.4–61.8 |
| Pre-pelvic length | 43.7 | 39.6–45.6 | 45.3–47.9 |
| Length of dorsal-fin base | 36.8 | 36.3–40.9 | 21.1–26.4 |
| Length of anal-fin base | 43.9 | 39.6–44.2 | 26.7–30.3 |
| Caudal-fin length | 35.7 | 33.8–38.1 | 33.0–37.1 |
| Pectoral-fin length | 24.8 | 21.6–24.8 | 19.0–21.3 |
| Pelvic-fin length | 11.1 | 9.8–11.7 | 9.9–11.8 |
| Head length | 30.5 | 29.8–33.7 | 31.7–33.5 |
| Percent of head length | | | |
| Head depth | 95.6 | 94.4–103.3 | 84.7–93.3 |
| Head width | 58.6 | 55.7–62.8 | 56.5–60.7 |
| Snout length | 12.1 | 11.9–14.9 | 9.4–13.4 |
| Lower jaw length | 17.4 | 15.2–19.2 | 14.8–17.2 |
| Eye diameter | 38.5 | 35.1–39.6 | 35.9–39.7 |

of scales 24–25; transverse series of scales 9–10; scale rows around caudal peduncle 12. No contact organs on scales. Total vertebrae 26–27. Frontal squamation E-patterned; E-scales overlapping medially; anterior-most frontal G-scale.

Latero-sensory canals absent. Cephalic neuromasts: supraorbital 11–13, parietal 3–4, anterior rostral 1, posterior rostral 1, infraorbital 1 + 16–20, preorbital 3, otic 2, post-otic 2, supratemporal 1, pre-opercular 11–14, median opercular 1, ventral opercular 1, mandibular 6–7, lateral mandibular 3–5, paramandibular 1. One or two neuromasts per scale of trunk lateral line. Two pairs of neuromasts on caudal-fin base.

**Colouration in life.** ***Males*** (Fig. 4). Body dark purplish brown to black; posterior-most extremity of caudal peduncle light pinkish brown; minute bright blue dots irregularly scattered over flank, more concentrated on its anterior portion. Head brown to black, with 10–12 small bright blue spots irregularly arranged on opercle, surrounded by diffuse blue iridescence; two bright blue bars on suborbital region. Iris dark brown, with two bright blue bars. Dorsal and anal fins dark reddish grey to black, with sub-distal bright blue line; light blue dots irregularly scattered over basal portion of both fins. Caudal fin hyaline, with light blue dots irregularly scattered over its basal portion; posterior margin bluish white. Pectoral fin hyaline with bright blue dots on basal portion. Pelvic fin dark grey to black, with subdistal bright blue stripe.

***Females*** (Fig. 5). Body pale brown, with irregularly arranged, vertically elongated dark brown blotches, more concentrated on its anterior portion. Opercular region and venter with greenish golden iridescence. Two black bars on suborbital region. Iris dark brown, with two brownish yellow bars. Dorsal and anal fins hyaline, with small dark brown spots. Caudal and paired fins hyaline.

**Etymology.** From the Latin *gracilis*, meaning thin, referring to the thin body of the small-sized new species.

**Distribution and habitat.** *Spectrolebias gracilis* is known from temporary pools of two localities of the middle Tocantins River basin, central Brazil (Fig. 6). In both localities pools were shallow, about 80 cm in deeper places, and densely occupied by aquatic vegetation.

# Discussion

*Spectrolebias gracilis* has been collected in two localities of the middle section of the Tocantins River basin, separated by about 530 km (Fig. 6). Our field inventories in temporary pools of middle, lower and upper parts of the basin indicated that this species is not present. Collecting trips in the last three decades in the middle Tocantins River region have shown that the typical habitat of seasonal killifishes, consisting of temporary pools with dense aquatic vegetation, is relatively rare, when compared to similar areas in the middle Araguaia River region, probably as result of the Araguaia River basin occupying vast plain areas not present on the Tocantins River basin. However, the scarcity of suitable habitats for the occurrence of seasonal killifishes in the entire region is due to major impacting environmental factors resulted from intensive anthropic activities.

In the Araguaia River basin, the region around Aruanã, an important regional touristic site, has been highly deforested and the temporary swamps have been extirpated. The same occurred in the das Mortes floodplains, where original vegetation was substituted by plantations and swampy areas were drained. In the Tocantins River basin, the dense forest previously present at the river floodplains was drastically removed in recent years, and large dams have inundated floodplain areas (Akama 2017). For example, in recent field studies we found that the Lajeado Dam, which shut off river flow in 2002, forming a reservoir that now occupies an area of 630 km$^2$ along 172 km of the middle section of the Tocantins River (Agostinho et al. 2007). As a result, the river has permanently inundated all seasonal killifish habitats around the dam, including the type locality of rare killifishes endemic to this region, such as *Hypsolebias multiradiatus* (Costa & Brasil, 1994), *Maratecoara formosa* Costa & Brasil in Costa, 1995, *Plesiolebias xavantei* (Costa, Lacerda & Tanizaki, 1988), and *Trigonectes strigabundus* Myers, 1925. The Tocantins River basin has been considered as the most impacted Amazon tributary, with a dense concentration of dams (Winemiller et al. 2016; Akama 2017). The five presently operating dams in the middle section of the Tocantins River probably promote wide distribution gaps for fish living in temporary pools situated close to rivers, putting in risk their existence.

*Spectrolebias costae* and *S. gracilis* are members of a species group endemic to central Brazil, also including *S. semiocellatus* and *S. inaequipinnatus*, diagnosed by some

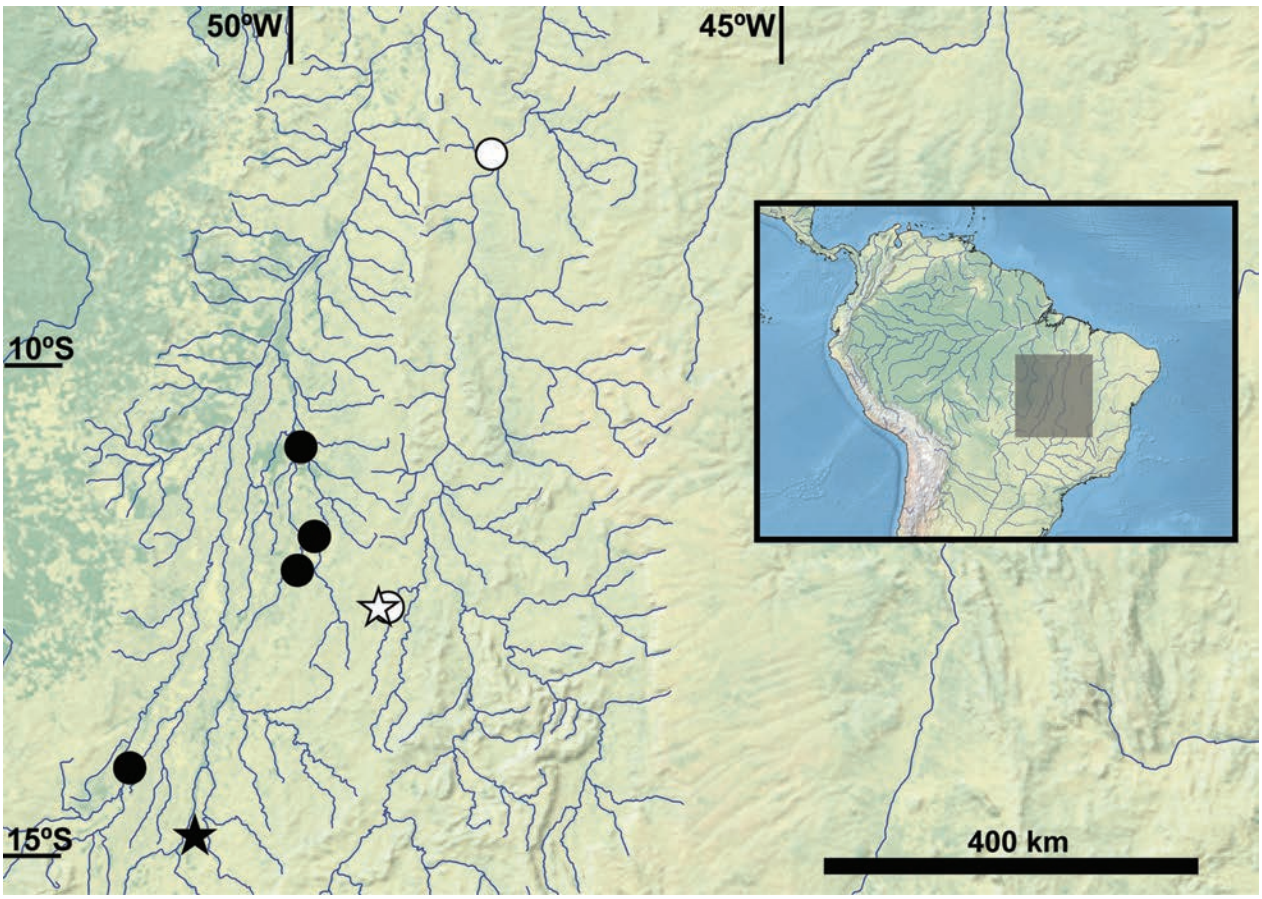


**Figure 6.** Geographical distribution of *Spectrolebias costae* (black symbols) and *S. gracilis* sp. n. (white symbols). Stars indicate type localities.

derived character states: dorsal and anal fins in males with iridescent dots restricted to the basal portion of fins (vs. scattered over the whole fin), caudal fin in males hyaline (vs. variably coloured, usually dark red or grey), caudal-fin base with two pairs of neuromasts (vs. one) (Costa 2010). *Spectrolebias costae* and *S. gracilis* are unique among species of this clade by possessing rounded dorsal fin in males, dark brown to black pigmentation on flank in males, and subdistal bright blue stripes on dorsal and anal fins in males. *Spectrolebias semiocellatus*, endemic to the Araguaia River basin, and *S. inaequipinnatus*, endemic to the Tocantins River basin, are closely related species, sharing the presence of a subtriangular dorsal fin in males with a long filamentous ray on its distal tip and frontal squamation F-patterned (vs. E-patterned), two derived conditions not occurring in other members of the tribe Cynolebiini (Costa & Brasil, 2008).

Weitzman and Vari (1988) reported a high incidence of events of miniaturization in Neotropical freshwater fishes, establishing an arbitrary standard of 26 mm SL as maximum adult size to recognise miniatures species. Costa (1998) argued that this standard value could be mostly useful in a phylogenetic context, particularly when detecting decreasing size gaps in sister lineages. *Spectrolebias costae* and *S. gracilis* reach about 20 mm SL as maximum adult size, thus contrasting with other closely related (*S. semiocellatus* and *S. inaequipinnatus*) and basal (*S. chacoensis* (Amato, 1986) congeners that reach about 30 mm SL or more (Costa et al. 1997; Costa 2007a; Costa and Brasil 2008). This abrupt size gap suggests that a unique event of miniaturization occurred in the most recent common ancestor of the clade comprising *S. costae* and *S. gracilis*, which are also the smallest species among the about 100 species included in the tribe Cynolebiini

As discussed by Weitzman and Vari (1988), in addition to reduction in body size, miniaturization processes may also result in other morphological changes, more notoriously reduction of serial structures or structural simplification, which often occur in parallel in not closely related miniaturized taxa. The clade comprising *S. costae* and *S. gracilis* exhibits low counts of scales of the longitudinal (22–25) series and vertebrae (25–27), thus contrasting with higher values (usually 27 or more scales in the longitudinal series and 29 or more vertebrae) in congeners and species of closely related genera that reach 30 mm SL or more (Costa 2007). However, similar low counts are also found in *Spectrolebias reticulatus* (Costa & Nielsen, 2003) a species endemic to the Xingu River basin, Brazilian Amazon, as well as in the smallest species of *Simpsonichthys* Carvalho, 1959, all of them barely reaching 25 mm SL (Costa 2007). These data suggest that low scale and vertebra counts have independently arisen in at least three unrelated lineages of miniature cynolebiines.

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## References

Agostinho AA, Marques EE, Agostinho CS, Almeida DA, Oliveira RJ, Melo RB (2007) Fish ladder of Lajeado Dam: migrations on one-way routes? Neotropical Ichthyology 5: 121–130. http://dx.doi.org/10.1590/S1679-62252007000200005

Akama A (2017) Impacts of the hydroelectric power generation over the fish fauna of the Tocantins River: Marabá Dam, the final blow. Oecologia Australis 21: 222–231. https://doi.org/10.4257/oeco.2017.2103.01

Baker P (1990) *Cynolebias costae*. Killi News 299: 10–12.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution 22: 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31: 3497–3500. https://doi.org/10.1093/nar/gkg500

Costa WJEM (1988) Sistemática e distribuição do complexo de espécies *Cynolebias minimus* (Cyprinodontiformes, Rivulidae), com a descrição de duas espécies novas. Revista Brasileira de Zoolgia 5: 557–570. http://www.scielo.br/pdf/rbzool/v5n4/v5n4a04.pdf

Costa WJEM (1989a) Descrição e relações filogenéticas de dois gêneros novos e três espécies novas de peixes anuais neotropicais (Cyprinodontiformes, Rivulidae). Revista Brasileira de Biologia 49: 221–230.

Costa WJEM (1989b) Descrição de um gênero e duas espécies novas de peixes anuais do Centro da América do Sul (Cyprinodontiformes, Rivulidae). Comunicações do Museu de Ciências da PUCRS serie Zoologia 2: 191–202.

Costa WJEM (1990) Systematics and distribution of the neotropical annual fish genus *Trigonectes* (Cyprinodontiformes, Rivulidae), with description of two new species. Ichthyological Exploration of Freshwaters 1: 135–150.

Costa WJEM (1995a) Two new genera and two new species of the neotropical annual fishes Plesiolebiatini (Cyprinodontiformes : Rivulidae), with studies on the relationships of the tribe. Revue Française d'Aquariologie et Herpetologie 21: 65–74.

Costa WJEM (1995b) Pearl killifishes, the Cynolebiatinae: a revision of the neotropical subfamily of annual fishes. TFH Publications, Neptune City, 128 pp.

Costa WJEM (1998) Phylogeny and classification of Rivulidae revisited: evolution of annualism and miniaturization in rivulid fishes (Cyprinodontiformes: Aplocheiloidei). Journal of Comparative Biology 3: 33–92.

Costa WJEM (2001) The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. Ichthyological Exploration of Freshwaters 12: 333–383.

Costa WJEM (2002a) The seasonal fish genus *Nematolebias* (Cyprinodontiformes: Rivulidae: Cynolebiatinae): taxonomic revision with description of a new species. Ichthyological Exploration of Freshwaters 13: 41–52.

Costa WJEM (2002b) Peixes anuais brasileiros: diversidade e conservação. Editora da Universidade Federal do Paraná, Curitiba, 238 pp.

Costa WJEM (2006) Descriptive morphology and phylogenetic relationships among species of the Neotropical annual killifish genera *Nematolebias* and *Simpsonichthys* (Cyprinodontiformes: Aplocheiloidei: Rivulidae). Neotropical Ichthyology 4: 1–26. http://dx.doi.org/10.1590/S1679-62252006000100001

Costa WJEM (2007a) Taxonomic revision of the seasonal South American killifish genus *Simpsonichthys* (Teleostei: Cyprinodontiformes: Aplocheiloidei). Zootaxa 1669: 1–134.

Costa WJEM (2007b) Taxonomy of the plesiolebiatine killifish genera *Pituna, Plesiolebias* and *Maratecoara* (Teleostei: Cyprinodontiformes: Rivulidae), with descriptions of nine new species. Zootaxa 1410: 1–41.

Costa WJEM (2008) Catalog of aplocheiloid killifishes of the world. Reproarte, Rio de Janeiro, 127 pp.

Costa WJEM (2010) Historical biogeography of cynolebiasine annual killifishes inferred from dispersal-vicariance analysis. Journal of Biogeography 37: 1995–2004. https://doi.org/10.1111/j.1365-2699.2010.02339.x

Costa WJEM (2011) Comparative morphology, phylogenetic relationships and historical biogeography of plesiolebiasine seasonal killifishes (Teleostei: Cyprinodontiformes: Rivulidae). Zoological Journal of the Linnean Society 162: 131–148. https://doi.org/10.1111/j.1096-3642.2010.00672.x

Costa WJEM (2016) Comparative morphology, phylogenetic relationships, and taxonomic revision of South American killifishes of the *Melanorivulus zygonectes* species group (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 27: 107–152.

Costa WJEM, Amorim PA (2011) A new annual killifish species of the *Hypsolebias flavicaudatus* complex from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes: Rivulidae). Vertebrate Zoology 61: 99–104.

Costa WJEM, Amorim PF, Mattos JLO (2017) Molecular phylogeny and timing of diversification in South American Cynolebiini seasonal killifishes. Molecular Phylogenetics and Evolution 116: 61–68. doi http://dx.doi.org/10.1016/j.ympev.2017.07.020

Costa WJEM, Barrera S, Sarmiento J (1997) *Simpsonichthys filamentosus*, une nouvelle espèce des Llanuras Benianas, bassin du Rio Mamoré basin, Bolivia. Revue Française d'Aquariologie et Herpetologie 24: 83–86.

Costa WJEM, Brasil GC (1994) Un nouveau Poisson annuel du genre *Cynolebias* (Cyprinodontiformes: Rivulidae) du bassin du rio Tocantins, Brésil. Revue Française d'Aquariologie et Herpetologie 21: 1–4.

Costa WJEM, Brasil GC (2008) *Simpsonichthys inaequipinnatus,* a new seasonal killifish from the Tocantins River basin, Brazil (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 19: 245–248.

Costa WJEM, Lacerda MTC, Tanizaki K (1988) Description d'une nouvelle espèce de *Cynolebias* du Brésil central (Cyprinodontiformes,

Rivulidae). Revue Française d'Aquariologie et Herpetologie 14: 123–126.

Costa WJEM, Nielsen DTB (1997) A new genus and species of annual fish (Cyprinodontiformes: Rivulidae) from the Araguaia basin, central Brazil. Ichthyological Exploration of Freshwaters 7: 257–265.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. https://doi.org/10.1093/molbev/mss075

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294.

Fujisawa T, Barraclough TG (2013) Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. Systematic Biology 62: 702–724. https://doi.org/10.1093/sysbio/syt033

Hoedeman JJ (1958) The frontal scalation pattern in some groups of toothcarps (Pisces, Cyprinodontiformes). Bulletin of Aquatic Biology 1: 23–28.

ICNZ [International Commission on Zoological Nomenclature] (1999) International Code of Zoological Nomenclature, fourth edition. http://www.iczn.org/iczn/index.jsp

Lazara KJ (1991) *Cynolebias lacortei*, *Cynolebias costai*, and *Cynolebias aruana*. Three new species of cloud fish from Brazil (Teleostei, Cyprinodontiformes, Rivulidae). Journal of the American Killifish Association 23: 139–152.

Myers GS (1925) Results of some recent studies on the American killifishes. The Fish Culturist 4: 370–371.

Myers GS (1942) Studies on South American fresh-water fishes. Stanford Ichthyological Bulletin 2: 89–114.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55: 595–609. https://doi.org/10.1080/10635150600852011

Rambaut A, Suchard MA, Xie D, Drummond AJ (2013) Tracer v1.5. http://beast.bio.ed.ac.uk/Trace

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sereno PC (2007) Logical basis for morphological characters in phylogenetics. Cladistics 23: 565–587. https://doi.org/10.1111/j.1096-0031.2007.00161.x

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–109. http://sfi.mnhn.fr/cybium/numeros/1985/92/01-Taylor%5b92%5d107-119.pdf

Weitzman SH, Vari RP (1988) Miniaturization in South American freshwater fishes: An overview and discussion. Proceedings of the Biological Society of Washington 101: 444–465.

Wiens JJ, Penkrot TA (2002) Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (Sceloporus). Systematic Biology 51: 69–91. https://doi.org/10.1080/106351502753475880

Winemiller KO, McIntyre PB, Castello L, Fluet-Chouinard E, Giarrizzo T, Nam S, Baird IG, Darwall W, Lujan NK, Harrison I, Stiassny MLJ, Silvano RAM, Fitzgerald DB, Pelicice FM, Agostinho AA, Gomes LC, Albert JS, Baran E, Petrere-Jr M, Zarfl C, Mulligan M, Sullivan JP, Arantes CC, Sousa LM, Koning AA, Hoeinghaus DJ, Sabaj M, Lundberg JG, Armbruster J, Thieme ML, Petry P, Zuanon J, Torrente Vilara G, Snoeks J, Ou C, Rainboth W, Pavanelli CS, Akama A, van Soesbergen A, Sáenz L (2016) Balancing hydropower and biodiversity in the Amazon, Congo and Mekong. Science 351(6269): 128–129. https://doi.org/10.1126/science.aac7082

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29: 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Dissertation, University of Texas, Austin.

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# Taxonomic review of the planthopper genus *Orthopagus* (Hemiptera, Fulgoromorpha, Dictyopharidae), with descriptions of two new species

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## Abstract

The Oriental and eastern Palaearctic planthopper genus *Orthopagus* Uhler, 1897 (Hemiptera, Fulgoromorpha, Dictyopharidae, Dictyopharinae, Orthopagini) is revised. Six species are included: *O. bartletti* Song, Malenovský & Deckert, **sp. n.** (described from India), *O. exoletus* (Melichar, 1903), **comb. n.**, **stat. rev.** (material studied from India and Sri Lanka), *O. hainanensis* Song, Chen & Liang, **sp. n.** (described from China: Hainan island), *O. lunulifer* Uhler, 1897 (the type species of the genus; confirmed from Japan, China, Vietnam, Laos, India, and Nepal), *O. philippinus* Melichar, 1914 (Philippines), and *O. splendens* (Germar, 1830) (confirmed from China, Vietnam, Thailand, India, Malaysia, and Indonesia). *Orthopagus helios* Melichar, 1912 is newly synonymized with *O. lunulifer*. Lectotypes are designated for *O. helios*, *O. helios* var. *diffusus* Melichar, 1912, *O. elegans* Melichar, 1912, and *O. philippinus*. *Dictyophara indiana* Walker, 1851 is considered a nomen dubium. All species are redescribed, including habitus photographs and detailed illustrations of the male genitalia. Female genitalia are described for the genus for the first time. A key for identification of the species of *Orthopagus* and a distribution map are given.

## Introduction

The family Dictyopharidae is one of twenty currently recognized extant families of planthoppers (Hemiptera, Fulgoromorpha) (Bourgoin 2018). With more than 720 species in 155 extant and extinct genera, this family is currently divided into two subfamilies Dictyopharinae Spinola, 1839 and Orgeriinae Fieber, 1872 (Muir 1923, Metcalf 1946, Song et al. 2016c, 2018). The dictyopharid species are widely distributed in all biogeographic regions, being most numerous in tropical and subtropical zones, e.g. in South America, the Oriental region and the East Indies (Metcalf 1946, Bourgoin 2018). Both adults and nymphs of Dictyopharidae are phytophagous and suck phloem sap from above-ground portions of plants. Their associations with host-plants are generally poorly known. Most species are probably dicot feeders, perhaps often with narrow trophic niches (monophagous), but there are also polyphagous and monocot-feeding taxa (Wilson et al. 1994, Krstić et al. 2016). A few species are economically important agricultural pests, e.g. on rice, sugarcane and cranberry (Wilson and O'Brien 1987), with the potential of acquiring and spreading phytoplasma pathogens (Krstić et al. 2016).

The larger nominotypic subfamily Dictyopharinae is further divided in twelve extant tribes (Song et al. 2018).

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One of them is Orthopagini first recognized by Emeljanov (1983) based on the type genus *Orthopagus* Uhler, 1897 and six other genera, and later extended to include a total of 23 genera (Emeljanov 2011, Song et al. 2014, 2016d). The Orthopagini taxa are mainly distributed in the Old World tropics and subtropics, including sub-Saharan Africa, India, Sri Lanka, southern China, Indochina, Malaya, the Greater Sunda Islands, the Philippines, the Moluccas, and northern Australia (Song et al. 2016d, Bourgoin 2018). A few species of *Orthopagus* and *Saigona* Matsumura, 1910 extend into the eastern Palaearctic region (Liang and Song 2006). Recently, most Orthopagini genera have been revised (Liang and Song 2006, Song and Liang 2006a, b, 2007, 2011, 2012a, b, Song et al. 2012, 2014, 2016a, b, d, 2017). The monophyly of the tribe was tested and phylogenetic relationships among most genera were analysed by Song et al. (2014, 2016d, 2018). Morphological characters support Orthopagini as a sister-group to Dictyopharini (Song et al. 2016b, d, 2018).

The genus *Orthopagus* has been known to include five valid species distributed in the Oriental and eastern Palaearctic regions (Bourgoin 2018). Its complicated nomenclatorial and taxonomic history can be summarised as follows. The type species, *Orthopagus lunulifer* Uhler, 1897 was described from Japan. *Orthopagus* is congeneric with *Anagnia* erected earlier by Stål (1861) for *Flata splendens* Germar, 1830 from Java, which Stål considered a senior synonym of *Dictyophara indiana* Walker, 1851 described from India, but *Anagnia* had been preoccupied by Walker (1854) for a genus of moths in the Erebidae (Lepidoptera). Melichar (1903) described *Udugama* based on *Udugama exoleta* Melichar, 1903 from Sri Lanka. Kirkaldy (1904) proposed a new generic name *Kareol* Kirkaldy to replace *Anagnia* Stål (nec Walker). *Kareol* was later synonymized with *Udugama* by Distant (1906), and the latter was synonymized with *Orthopagus* by Oshanin (1908). Distant (1906) also proposed that the species names *U. exoleta* and *F. splendens* were synonyms. Melichar (1912) redescribed *Orthopagus* and added two species names based on specimens from Taiwan, China, *O. elegans* Melichar, 1912 and *O. helios* Melichar, 1912. *Udugama fletcheri* Kirkaldy, 1908 also listed by Melichar (1912) in *Orthopagus* is currently considered a junior synonym of *Truncatomeria viridistigma* (Kirby, 1891) (see Song and Liang 2011). The last species described so far and still placed in *Orthopagus* was *O. philippinus* Melichar, 1914 from Luzon, the Philippines (Melichar 1914).

Based on examination of most *Orthopagus* types and a critical review of the literature, *Orthopagus* is here revised. We redescribe all previously known taxa and add two new species, *O. bartletti* Song, Malenovský & Deckert, sp. n. from India and *O. hainanensis* Song, Chen & Liang, sp. n. from China. We provide an identification key and photographic illustrations for each species, showing also the structures of the male and female genitalia, described and illustrated in detail.

## Material and methods

The specimens studied in the course of this work are deposited in the following institutions, which are subsequently referred to by their acronyms: **BMNH**, Natural History Museum, London, UK; **BPBM**, Bernice Pahaui Bishop Museum, Honolulu, Hawaii, USA; **HNHM**, Magyar Természet-Tudományi Múzeum (Hungarian Natural History Museum), Budapest, Hungary; **IZCAS**, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; **JSSNU**, Jiangsu Second Normal University, Nanjing, China; **LBOB**, personal collection of Lois B. O'Brien, Tucson, Arizona, USA; **MFNB**, Museum für Naturkunde, Berlin, Germany; **MMBC**, Moravské zemské muzeum (Moravian Museum), Brno, Czech Republic; **MZPW**, Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland; **SDEI**, Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany; **SNSD**, Senckenberg Naturhistorische Sammlungen Dresden, Dresden, Germany; **UDCC**, Department of Entomology and Wildlife Ecology Collection, University of Delaware, Newark, Delaware, USA; and **USNM**, National Museum of Natural History, Smithsonian Institution, Washington D. C., USA.

The post-abdomina of the specimens used for dissections were cleared in 10% KOH at room temperature for ca. 6–12 hours, rinsed and examined in distilled $H_2O$ and then transferred to 10% glycerol and enclosed in microvials to be preserved with the specimens. Observations were conducted under a stereomicroscope, measurements and photography under Zeiss Discovery V12 or Leica M205 C stereomicroscopes equipped with a Nikon D7000 digital camera in IZCAS. Some final images were compiled from multiple photographs using CombineZM image stacking software and improved with the Adobe Photoshop CS5 software.

The morphological terminology and measurements used in this study follow Song et al. (2016c, d, 2018) for most characters, Bourgoin (1993) for the female genitalia, and Bourgoin et al. (2015) for the forewing.

## Results

### *Orthopagus* Uhler, 1897

*Anagnia* Stål, 1861: 149. Type species: *Flata splendens* Germar, 1830; by original designation and monotypy. Preoccupied by *Anagnia* Walker, 1854: 446 (Lepidoptera: Erebidae).

*Orthopagus* Uhler, 1897: 278; Melichar 1912: 57. Type species: *Orthopagus lunulifer* Uhler, 1897; by original designation and monotypy.

*Udugama* Melichar, 1903: 27; Distant 1906: 249. Type species: *Udugama exoleta* Melichar, 1903; by original designation and monotypy. Synonymized with *Orthopagus* by Oshanin 1908: 444.

*Kareol* Kirkaldy, 1904: 279. Replacement name for *Anagnia* Stål. Synonymized with *Udugama* by Distant 1906: 249.

**Diagnosis.** *Orthopagus* can be distinguished from other genera in the Orthopagini by the following combination of characters: cephalic process short, truncated in front in dorsal view; vertex with lateral carinae strongly ridged and sub-parallel in basal half, slightly constricted at an-

terior margin of eyes, median carina sharp and complete; frons with intermediate carinae approaching frontoclypeal suture, median carina complete; pronotum with intermediate carinae distinct in basal half; mesonotum with lateral carinae curving anteriad towards median carina; forewings with a wide sublunate streak on distal half of wing, transverse veins sparse, pterostigmal area with 2–4 cells; fore femora flattened and dilated, with a large and blunt spine near apex; hind tibiae with seven apical teeth; phallobase with inflated membranous paired lobes, with or without numerous small superficial spines.

**Description.** Adult. General colour of body brownish ochraceous to dark brown marbled, with pale green and reddish ochraceous streaks on dorsum (Figs 1A–B, 2A–L). Females distinctly darker than males. Head pale ochraceous with dark brown markings on vertex and frons the extent of which varies among species. Clypeus pale ochraceous basally, with two small dark spots at frontoclypeal suture on each side of median carina, apical half dark brown. Pronotum brownish ochraceous to dark brown, median carina and spots on lateral marginal areas and paranotal lobes pale ochraceous. Mesonotum dark brown, median and lateral carinae, and lateral marginal areas flavescent or greenish. Forewing veins light ochraceous, membrane hyaline to translucent with three dark brown markings: (i) a large sublunate streak extending along the posterio–apical margin from basal portion of areola postica across apical portions of cells of the medial area to the apex of RP vein; (ii) triangular patch on pterostigma, extending also into radial area (C1 cell) and rarely more mesiad as a dark streak along nodal line; (iii) streak along postclaval wing margin filling out whole inner claval cell (Figs 2A–L, 3A–F). Hind wing veins ochraceous, membrane clear, with a more or less developed dark brown marking along the apical portion of $CuA_1$ vein. Legs pale to dark brown: femora dull ochraceous to fuscous, banded and marked with ivory white; fore and mid tibiae yellowish green to ochraceous with two dark brown transverse bands medially; hind tibiae yellowish green (pale ochraceous in old dry-mounted specimens), with base and apex including lateral and apical spines fuscous; fore and mid tarsi fuscous, hind tarsi ochraceous. Abdomen dorsally and ventrally ochraceous to dark brown, with dark brown or pale ochraceous spots and stripes of various sizes and shape.

Head (Figs 4A–C, 5A–C, 6A–C, 8A–C, 9A–C, 10A–C) usually produced in a short and stout cephalic process. Vertex (Figs 4A, 5A, 6A, 8A, 9A, 10A) moderately broad, basal width slightly greater than transverse diameter of eyes in dorsal view, posterior plane elevated above pronotum; lateral carinae strongly ridged, foliaceous, and sub-parallel in basal half, slightly constricted at anterior margin of eyes, broadly convex at apex; posterior margin ridged, concave, forming angle of 80–90°; median carina sharp and complete. Frons (Figs 4C, 5C, 6C, 8C, 9C, 10C) with lateral carinae ridged, nearly parallel, slightly expanded outward below antennae; intermediate carinae slightly converging posteriad and approaching frontoclypeal suture; median carina distinct and complete; basal margin of frons projecting anteriad of apex of vertex. Postclypeus and anteclypeus (Figs 4C, 5C, 6C, 8C, 9C, 10C) convex medially, with distinct median carina. Rostrum long, surpassing base of hind femora; basal segment nearly equal to distal one. Compound eyes large and globose. Ocelli relatively large, reddish. Antennae with very small scape; pedicel large and subglobular, with more than 50 distinct sensory plaque organs distributed over entire surface; flagellum long, setuliform.

Pronotum (Figs 4A, 5A, 6A, 8A, 9A, 10A) distinctly shorter than mesonotum at midline, anterior margin angularly convex medially, lateral marginal areas straight and sloping down with two long longitudinal carinae on each side, posterior margin concave, forming obtuse angle (100–120°); intermediate carinae distinct in basal half, strongly diverging laterad; median carina sharp and elevated, with a large lateral pit on each side. Mesonotum (Figs 4A, 5A, 6A, 8A, 9A, 10A) tricarinate on disc, lateral carinae converging anteriad towards median carina. Forewings (Fig. 3A–F) hyaline, ratio of length to width about 3:1; venation with sparse transverse veins; MP bifurcating $MP_{1+2}$ and $MP_{3+4}$ near middle and beyond CuA; number of apical cells between R and CuA equal to 14; Pcu and $A_1$ veins fused into a long Pcu+A1 vein at apical 1/5 in clavus; pterostigmal area clear, with 2–4 cells. Legs moderately long; fore femora flattened and dilated, with a large and blunt spine near apex; hind tibiae with 5–7 (mostly six) lateral spines and seven apical teeth; hind tarsomeres I with 18–20 and tarsomeres II with 12–14 apical teeth, respectively.

Male genitalia. Pygofer (Figs 4D–F, 5D–F, 6D–F, 8D–F, 9D–F, 10D–F) in lateral view distinctly wider ventrally than dorsally, dorsal margin slightly excavated to accommodate segment X, dorso-posterior margins angular, produced into a distinct lobe which is short and broad or larger and tooth-like. Gonostyles (Figs 4E–F, 5E–F, 6E–F, 8E–F, 9G, 10E–F) symmetrical, with narrow base, expanded toward apex, broadest at apical fourth; dorsal margin with a claw-like, apically sclerotised process directed dorsad, outer dorsal edge with a spiny hook-like sclerotised process near middle directed ventrad. Aedeagus (Figs 4G–I, 5G–I, 6G–I, 8G–I, 9H–J, 10G–I) with one pair of elongate endosomal processes extended from phallobase posteriad and strongly curved dorso-anteriad or laterad; these processes are membranous, acute apically and smooth or bearing numerous minute spines over their entire surface; phallobase sclerotised and pigmented basally, membranous and inflated apically, with paired lobes. Segment X (Figs 4D–E, 5D–E, 6D–E, 8D–E, 9D–E, 10D–E) large, in dorsal view with apex deeply excavated to accommodate anal style; anal style elongate and large.

Female genitalia. Gonocoxae VIII (Fig. 7D) with two membranous and flattened endogonocoxal processes (Gxp) on endogonocoxal lobe: Gxp1 large and elongate, with a long sclerotized plate in it; Gxp2 smaller and shorter. Gonapophyses VIII (Fig. 7D) with anterior connective lamina large and sclerotized, with seven teeth of varying sizes and shapes. Gonapophyses IX (Fig. 7E–F) with posterior connective lamina triangular, symmetrical, fused with intergonocoxal plate at base; intergonocoxal

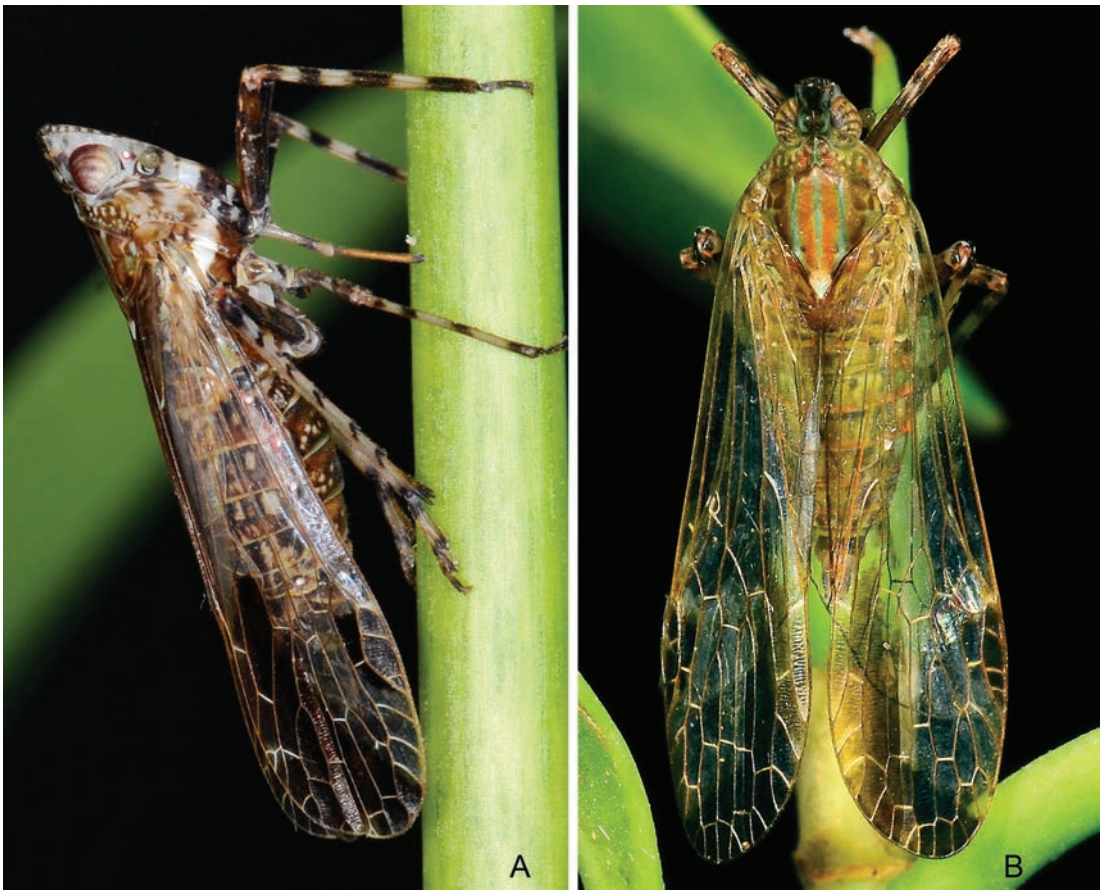


**Figure 1.** (**A**) *Orthopagus lunulifer* Uhler, female, Henan, China; (**B**) *Orthopagus splendens* (Germar), male, Yunnan, China. Photographed by Z.-S. Song.

plate extended cephalad into genital cavity, forming wall of gonospiculum. Gonoplacs (Fig. 7G) with two lobes homologous; lateral lobe large and moderately sclerotized, with long setae at apex; the posterior lobe membranous, containing long sclerotized plate. Segment X (Fig. 7A) large and broad in dorsal view, apex deeply excavated to accommodate anal style; anal style large and elongate. Female ectodermal genital ducts ditrysian. Bursa copulatrix (Fig. 7A–C) superficially membranous, regularly gridded, without sclerotized ornamentations. A pair of large digitiform glands (Fig. 7B) branched at anterior extremity of the anterior vagina on each side of the spermatheca. Spermatheca (Fig. 7B) divided clearly into five parts: orificium receptaculi, ductus receptaculi, diverticulum ductus, pars intermedialis, and glandula apicalis.

Fifth instar nymph. See Yang and Yeh (1994) for a detailed description.

**Diversity and distribution.** *Orthopagus* is revised here to include six valid species (see below). The species of the genus are widely distributed in the Oriental and eastern Palaearctic regions from India in the southwest to Japan in the northeast (Fig. 11).

**Nomenclatorial remark on *Dictyophara indiana* Walker, 1851.** The identity of one more available species name belonging to *Orthopagus* could not be sufficiently cleared during this study: *Dictyophora* [sic] *indiana* Walker, 1851: 310 described from India (without more precise locality data). This name was synonymized under *Anagnia splendens* (Germar) (now *Orthopagus splendens*) by Stål (1861): 149. However, this synonymy is considered doubtful here because the original description of *D. indiana* lacks diagnostic information and illustrations which would enable recognition of its species identity and the single available type specimen of *D. indiana* (deposited in BMNH) could not be directly examined during this study due to its very poor condition which did not allow its sending out for a loan. Based on a photograph kindly provided by M. D. Webb (BMNH), the type specimen belongs to an *Orthopagus* species but it lacks the abdomen and its head has been partly damaged. As the details of the male genitalia and the coloration and proportions of the head, i.e. the characters which are missing or damaged in the type, are the most reliable diagnostic morphological characters of *Orthopagus* species, it is not certain that even a direct examination of the type would help to solve the identity of *D. indiana*. Therefore, it is proposed here to treat *Dictyophara indiana* as a nomen dubium. Currently, four *Orthopagus* species are known from the Indian subcontinent: *O. bartletti* sp. n., *O. exoletus*, *O. lunulifer* and *O. splendens*, of which *O. exoletus* is the most widespread (Fig. 11).

## Key to the species of *Orthopagus*

1 Forewings with a dull brownish streak along nodal line connecting pterostigmal area and distal sublunate streak (Fig. 3A); head very short, hardly produced in front of eyes, in lateral view, broadly rounded (Fig. 4A–B); male pygofer, in lateral view, with dorso-posterior margin on each side produced into a large, broadly truncate, biangular lobe (Fig. 4E); lobes of phallobase muricate apically (Fig. 4G–I) ..... *O. bartletti* Song, Malenovský & Deckert, sp. n.

– Forewings without a brownish streak along nodal line (Fig. 3B–F); head longer, distinctly produced in front of eyes (Figs 5A–B, 6A–B, 8A–B, 9A–B, 10A–B); male pygofer, in lateral view, with dorso-posterior margin on each side produced either into a large but narrow, simply tooth-like lobe (Figs 5E, 9E, 10E) or into a broadly truncate but short and blunt lobe (Figs 6E, 8E); lobes of phallobase smooth apically (Figs 5G, 6G, 8G, 9H, 10G) ..... 2

2 Male pygofer, in lateral view, with dorso-posterior margin produced into a large and narrow tooth-like lobe (Figs 5E, 9E, 10E) ..... 3

– Male pygofer, in lateral view, with dorso-posterior margin produced into a short and broad lobe (Figs 6E, 8E)..... 5

3 Vertex ivory white to pale ochraceous, with 3–4 pairs of small dark brown markings (Fig. 5A); aedeagus with endosomal processes lacking minute superficial spines; phallobase with three pairs of membranous lobes, of which dorsal lobes thumb-like, directed dorso-laterad (Fig. 5G–I) ..... *O. exoletus* (Melichar, 1903)

– Vertex largely dark brown from base to apex (Figs 9A, 10A); aedeagus with endosomal processes covered with numerous minute spines; phallobase with two pairs of membranous lobes, of which dorsolateral lobes relatively large, bladder-like (Figs 9H–J, 10G–I) ..... 4

4 Head, in lateral view, distinctly inflated and bulbous apically (Fig. 9B); frons with a large roundish dark brown spot at base (Fig. 9C); aedeagus with endosomal processes relatively slender, weakly curved dorso-laterad (Fig. 9H–I)..... ..... *O. philippinus* Melichar, 1914

– Head, in lateral view, not inflated, narrow apically (Fig. 10B); frons light, without roundish dark brown spot at base (Fig. 10C); aedeagus with endosomal processes relatively robust, strongly curved cephalad (Fig. 10G–H)..... ..... *O. splendens* (Germar, 1830)

5 Vertex light ochraceous, with a pair of small dark brown patches on each side of midline in basal third (Fig. 6A); transition of vertex to frons, in lateral view, broadly rounded (Fig. 6B); male segment X elongate in dorsal view, with ratio of length to width near middle 1.9–2.0 (Fig. 6D) ..... *O. hainanensis* Song, Chen & Liang, sp. n.

– Vertex nearly dark brown from base to apex (Fig. 8A); transition of vertex to frons, in lateral view, almost angular (Fig. 8B); male segment X relatively short and broad in dorsal view, with ratio of length to width near middle 1.2–1.3 (Fig. 8D) ..... *O. lunulifer* Uhler, 1897

**Species descriptions** (character states shared with the generic description are not repeated)

### *Orthopagus bartletti* Song, Malenovský & Deckert, sp. n.

http://zoobank.org/7CA90D40-0E5F-47CF-B85B-04321A516EED
Figs 2A–B, 3A, 4A–I

**Type material.** Holotype male, **INDIA:** Karnataka: Shimoga district, Someshwari Wildlife Sanctuary, 10 km W Agumbe, 13°28′24″N; 75°00′40″ E, alt. 372 ft, early successional, wet evergreen forest, 22.ix.2005, C. R. Bartlett leg. (UDCC).

**Diagnosis.** *Orthopagus bartletti* sp. n. can be separated from all other *Orthopagus* species by the very short head, hardly produced in front of eyes; the forewings with a brownish streak along nodal line connecting the pterostigmal area with the distal sublunate streak; the dorso-posterior margin of the male pygofer with a large and broad process forming two distinct angles; the robust male segment X; and the lobes of the phallobase muricate apically.

**Description.** Measurements (1 male). Body length (from apex of head to tip of forewings): 11.5 mm; head length (from apex of cephalic process to base of eyes): 1.2 mm; head width (including eyes): 1.5 mm; forewing length: 9.6 mm.

Coloration (Fig. 2A–B). General colour brownish ochraceous marked with dark brown on dorsum. Head greenish ochraceous, vertex with basal corners, a pair of round patches in basal third, and apical diamond-shaped spot dark brown (Fig. 4A); frons with median area between intermediate carinae extensively dark brown anteriorly and with series of small pale fuscous spots along intermediate and lateral carinae and narrow ivory white band basally (Fig. 4C). Clypeus ivory white, with two small spots at base and apex dark brown. Compound eyes fuscous with posterior margin ochraceous; ocelli purplish-red. Pronotum brownish ochraceous, median carina, apical marginal areas of ventral lobes, and posterior lateral angles ivory white. Mesonotum brownish ochraceous (Fig. 4A). Forewings hyaline, veins ochraceous, pterostigmal area, a streak along nodal line, and a wide sublunate streak on distal third dull ochraceous; posterior (claval) margin broadly faintly brown (Fig. 3A). Hind wings hyaline, veins and an apical spot dull ochraceous. Legs pale brown; fore femora subapically and hind tibiae at base and apex (including lateral and apical spines) blackish. Abdomen dorsally and ventrally brownish ochraceous.

Structure. Head (Fig. 4A–C) very short, cephalic process practically absent. Vertex (Fig. 4A) with ratio of length at midline to width between eyes 1.4. Frons with base slightly inflated anteriad in dorsal view (Fig. 4A),

**Figure 2.** Habitus of *Orthopagus* species. (**A, B**) *O. bartletti* sp. n., male, holotype, Karnataka, India; (**C, D**) *O. exoletus* (Melichar), male and female, Sri Lanka; (**E, F**) *O. hainanensis* sp. n., male and female, paratypes, Hainan, China; (**G, H**) *O. lunulifer* Uhler, male and female, China; (**I, J**) *O. philippinus* Melichar, male and female, Philippines; (**K, L**) *O. splendens* (Germar), male and female, China.

with transition to vertex broadly rounded in lateral view (Fig. 4B); in ventral view, frons with ratio of length at midline to maximum width 2.2; median carina more or less obscure at base (Fig. 4C).

Male genitalia. Pygofer, in lateral view, with dorso-posterior margin forming a large, broad, biangular lobe (Fig. 4E); in ventral view (Fig. 4F) much longer than in dorsal view (Fig. 4D) with ratio of ventral to dorsal

width about 3.4. Gonostyles (Fig. 4E, F) elongate, relatively narrow in basal half, with strongly sinuate dorsal margin medially. Aedeagus (Fig. 4G–I) with endosomal processes relatively short and robust, without distinct minute superficial spines, and directed laterad; phallobase with a pair of large, elongate, thumb-like ventral lobes, curved dorso-posteriad and muricate apically (Fig. 4G–H); and a pair of shorter lateral lobes, directed posteriad (Fig. 4H). Segment X, in lateral view, relatively short and robust, with ventral margin gradually widening from base to broadly truncate apex (Fig. 4E); in dorsal view broad, broadest medially, with ratio of length to maximum width 1.1 (Fig. 4D).

Female genitalia unknown.

**Etymology.** The new species is named after Dr. Charles R. Bartlett, collector of the type specimen and curator of the insect collection at the Department of Entomology and Wildlife Ecology, University of Delaware, USA, in recognition of his kindest help and support to the first author when he visited UDCC in 2017. The species name is to be treated as a noun in genitive case.

**Distribution.** So far only known from southwestern India (Fig. 11).

### *Orthopagus exoletus* (Melichar, 1903), comb. n., stat. rev.

Figs 2C–D, 3B, 5A–I

*Udugama exoleta* Melichar, 1903: 28, Pl. I, figs 7, 7a–b. Syntypes: 5 females, Moruwale, Sri Lanka (not examined). Synonymized under *Udugama splendens* (Germar, 1830) by Distant 1906: 249.

*Udugama exoleta*: Kirkaldy 1908: 14.

**Material examined. INDIA**: 2 females, [no state indicated], 1934-394, T. R. Bell leg. (BMNH); West Bengal: 1 female, Calcutta [= Kolkata], 3.x.1907 (coll. Distant, BMNH); Maharashtra: 1 male, Sindhudurg district, roadside on ridge 2 km W Amboli, 15°58'04"N, 73°59'23"E, alt. 2394 ft, pasture and successional, moist deciduous forest, 28.ix.2005, C. R. Bartlett leg. (UDCC); 1 male, Pune district, 5 km E Mulshi Lake near Tamini village, 18°26'37"N, 73°25'46"E, alt. 2047 ft, deciduous forest and open areas, 2.x.2005, C. R. Bartlett (UDCC); Goa: 2 females, Sanguem district, near Bhagwan Mahaveer Sanctuary 100 m E Molem, 15°22'43"N, 74°13'52"E, alt. 342 ft, moist deciduous forest, 24–25. ix.2005, C. R. Bartlett leg. (UDCC); Kerala: 2 males, Malabar, Nadungayam [forest near Nilambur], 200 ft, 16.–22.ix.1938 (BMNH); 1 female, Parambikulam, alt. 1700–3200 ft, 16.–24.ix.1914, F. H. Gravely leg. (coll. Distant, BMNH); 2 females, Tenmalai [= Thenmala], 12.–15.v.1937 (BMNH); Tamil Nadu: 1 female, Nilgiri Hills, 11 km SE Kotagiri, Kunchappanai, 11°24′N 76°56′E, alt. 1100 ± 100 m, 3.–15.v.2002, L. Dembický leg. (MMBC). **SRI LANKA**: North Western Province: 1 female, Puttalam district, Puttalam, "12." [= ?1912] (coll. Melichar, MMBC); North Central Province: 1 male, Anuradnapura district, Wilpattu National Wildlife Park, Hunuwilagama, Wildlife Soc. Bungalow, 200 ft, 10–19.iii.1970, D. Davis & B. Rowe leg. (USNM); Central Province: 1 female, Kandy district, Talwatte, 29.xi.1995, M. Schaffer leg. (BMNH); Samaragamuwa: 1 male, Ratnapura district, Uggalkaltota, Irrigation Bungalow, alt. 350 ft, 31.i–8.ii.1970, D. Davis & B. Rowe leg. (USNM).

**Redescription.** Measurements (2 males, 8 females). Body length (from apex of head to tip of forewings): male 11.1 mm, female 12.3–14.7 mm; head length (from apex of cephalic process to base of eyes): male 1.28–1.30 mm, female 1.28–1.40 mm; head width (including eyes): male 1.40–1.45 mm, female 1.48–1.60 mm; forewing length: male 9.0–9.4 mm, female 10.2–12.2 mm.

Coloration. General coloration as in generic description (Fig. 2A). Vertex predominantly light ochraceous, with 3–4 pairs of small dark brown markings: an elongate patch on each side of midline apically, a small spot at each lateral keel medially, a roundish spot on each side of midline at basal third, and a small spot in each postero-lateral corner (Fig. 5A). Frons light ochraceous with small dark brown spots along intermediate and lateral carinae, frons base slightly infuscated (Fig. 5C). Forewing membrane pattern as in Fig. 3B. Hind wing membrane with a relatively narrow dark brown streak along the apical portion of $CuA_1$ vein, extending along hind wing apical margin in some specimens.

Structure. Head with cephalic process very short, not inflated (Figs 5A–C). Vertex (Fig. 5A) with ratio of length at midline to width between eyes 1.2–1.5. Transition of vertex to frons narrowly rounded in lateral view (Fig. 5B). Frons relatively broad, with ratio of length at midline to maximum width 2.3–2.7.

Male genitalia. Pygofer, in lateral view, with dorso-posterior margin produced into a relatively large but narrow, tooth-like, apically obtuse process (Fig. 5E); in ventral view (Fig. 5F) much longer than in dorsal view (Fig. 5D) with ratio of ventral to dorsal width about 2.6. Gonostyles (Fig. 5E) large and broad medially, with dorsal margin weakly sinuate medially. Aedeagus (Fig. 5G–I) relatively small and slender, endosomal processes curved laterad and slightly ventro- or dorsoanteriad, membranous, without distinct minute superficial spines; phallobase with three pairs of relatively small (not conspicuously inflated) membranous lobes: a pair of elongate lateral lobes with their apices gradually convergent and tapering posteriad (Fig. 5H–I), a pair of elongate thumb-like ventral lobes, directed ventroposteriad (Fig. 5H), and a pair of small thumb-like dorsal lobes, directed dorsolaterad (Fig. 5G). Segment X, in lateral view, elongate, basal half narrow, gradually widening to apex beyond middle (Fig. 5E); in dorsal view, widest medially, with ratio of length to maximum width 1.1 (Fig. 5D).

Female genitalia as in generic description.

**Distribution.** India (southwestern part and West Bengal), Sri Lanka (Fig. 11).

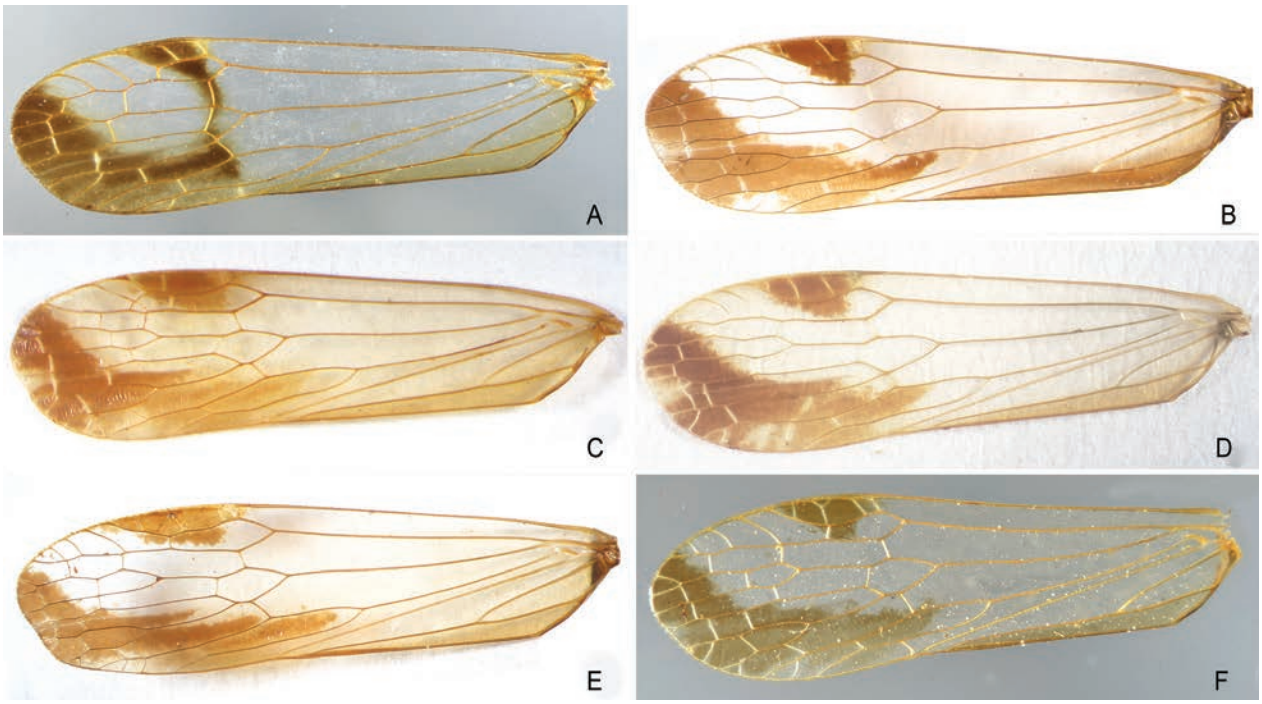


**Figure 3.** Forewing of *Orthopagus* species. (**A**) *O. bartletti* sp. n.; (**B**) *O. exoletus* (Melichar); (**C**) *O. hainanensis* sp. n.; (**D**) *O. lunulifer* Uhler; (**E**) *O. philippinus* Melichar; (**F**) *O. splendens* (Germar).

**Remarks.** *Udugama exoleta* was described from Sri Lanka as the type species of *Udugama* (Melichar 1903). Distant (1906) synonymized this species name with *Udugama splendens* described from Java, Indonesia. However, Kirkaldy (1908), probably based on comparisons of figures in Melichar (1903) and Distant (1906), commented that *U. exoleta* was "very different" from *U. splendens* in having a much longer face. Nevertheless, the synonymy of *U. exoleta* with *U. splendens* was accepted by Melichar (1912), and later included in Metcalf's (1946) catalogue of world Dictyopharidae.

Based on our critical review of the published information and examination of *Orthopagus* material from Sri Lanka which agrees with the original description of *U. exoleta*, we propose here to resurrect *Orthopagus exoletus* comb. n. from the synonymy with *O. splendens* and to restore it as a valid species. *Orthopagus exoletus* can be distinguished from *O. splendens* by the coloration of the vertex and the structure of the male genitalia, particularly the structure of the endosomal processes of aedeagus, lobes of the phallobase and the shape of the segment X. The relative length of frons mentioned by Kirkaldy (1908) is probably not a relevant diagnostic character because the length of the head in *Orthopagus* species varies within a certain range.

According to Melichar (1903), *U. exoleta* was described based on five female specimens from "Moruwale", deposited in the collection of the museum in Colombo, Sri Lanka. This material was not available to our study. Nevertheless, we have studied one female from Sri Lanka, "Puttalam" preserved in Melichar's personal collection in MMBC. Even though this specimen bears original identification labels handwritten by Melichar as "*Udugama*" and "*exoleta* det. Melichar" and a dark red label "Typus" originally also attached to the specimen by Melichar, it probably cannot be considered as a syntype because it differs in its locality and deposition from the information published in the original description and probably it was also collected later than the original species description had been published. Melichar did not use type labels in the modern sense. He had rather adopted the practice of placing a 'type' label on one or more specimens of the most taxa (even on species previously described by other authors and identified by Melichar), specimens presumably which he himself used for comparison (Young and Soós 1964, Wilson and Malenovský 2007).

### *Orthopagus hainanensis* Song, Chen & Liang, sp. n.

http://zoobank.org/3809F64E-8BDD-47F3-83B8-97FEAB5692C4

Figs 2E–F, 3C, 6A–I, 7A–G

**Type material.** Holotype male, **CHINA**: Hainan: Baoting, 80 m, 21.vii.1960, S. F. Li leg. (IZCAS).

Paratypes. **CHINA**: Hainan: 6 males, 24 females, Baoting, 80 m, 23., 24. and 27.vii.1960, S. F. Li, X. Z. Zhang & C. Q. Li leg.; 11 males, 15 females, Tongshi, 340 m, 23., 24. and 25.vi., 31.vii., 1., 4. and 6.viii.1960, S. F. Li, X. Z. Zhang & C. Q. Li leg.; 1 male, 1 female, Yinggen, 200 m, 4.v. and 5.vii.1960, S. F. Li leg.; 1 female, Shuiman, 640 m, 25.v.1960, C. Q. Li leg.; 2 males, Wanning, 10 m, 12., 13.iv.1960, S. F. Li & C. Q. Li leg.; 3 females, Qiongzhong, 15–17.vii.1960, X. Z. Zhang & C. Q. Li leg.; 2 males, 3 females, Kwangtung, 3., 4. and 5.iv., 13. and 26.viii.1934, C. Ho leg. (all IZ-

**Figure 4.** *Orthopagus bartletti* sp. n. (**A**) Head, pronotum and mesonotum, dorsal view. (**B**) Head and pronotum, lateral view. (**C**) Head and pronotum, ventral view. (**D**) Male segment X and pygofer, dorsal view. (**E**) Male pygofer, gonostyles, and segment X, lateral view. (**F**) Male pygofer and gonostyles, ventral view. (**G**) Aedeagus, dorsal view. (**H**) Aedeagus, lateral view. (**I**) Aedeagus, ventral view.

CAS); 1 male, Mangrin, 9.vi.1904; 1 female, Mon Boi, 29.v.1904 (both BMNH).

**Diagnosis.** *Orthopagus hainanensis* sp. n. is similar to *O. lunulifer* in most characters, but can be differentiated from

the latter by the ivory white to pale ochraceous vertex with a pair of dark brown spots on each side of midline in basal third (in *O. lunulifer*, the vertex is nearly dark brown from base to apex) and the male segment X being elongate in dorsal view, with ratio of length to width near middle 1.9–2.0 (in *O. lunulifer*, the male segment X is shorter and wider in dorsal view, with ratio of length to width near middle 1.2–1.3). *Orthopagus hainanensis* sp. n. is also similar to *O. exoletus* in the predominantly light vertex but it differs from the latter by a slightly longer head, the male pygofer with dorso-posterior margin produced into a broad and short lobe in lateral view (the pygofer bears a larger tooth-like process dorso-posteriorly in *O. exoletus*) and the aedeagus with two pairs of larger (more strongly inflated) dorsolateral membranous lobes and endosomal processes covered with minute spines (indistinct in *O. exoletus*).

**Description.** Measurements (5 males, 10 females). Body length (from apex of head to tip of forewings): male 11.7–12.9 mm, female 13.1–14.6 mm; head length (from apex of cephalic process to base of eyes): male 1.4–1.5 mm, female 1.5–1.6 mm; head width (including eyes): male 1.6–1.7 mm, female 1.6–1.7 mm; forewing length: male 9.3–10.3 mm, female 10.6–11.8 mm.

Coloration. General coloration as in generic description (Fig. 2E–F). Head ivory white to pale ochraceous, vertex with basal corners castaneous, a pair of large fuscous patches on each side of midline at basal third, and pale fuscous apical diamond-shaped spot (Fig. 6A); frons pale with series of small pale fuscous spots along intermediate and lateral carinae, base without distinct dark spot (Fig. 6C). Forewing pattern as in Fig. 3C. Hind wing membrane with a relatively narrow dark brown streak along the apical portion of $CuA_1$ vein, extending along hind wing apical margin.

Structure. Cephalic process (Fig. 6A–C) relatively elongate. Vertex (Fig. 6A) with ratio of length at midline to width between eyes 1.65–1.75. Transition of vertex to frons narrowly rounded in lateral view (Fig. 6B). Frons (Fig. 6C) relatively narrow, with ratio of length at midline to maximum width 2.7–2.9.

Male genitalia. Pygofer in lateral view (Fig. 6E) with dorso-posterior margin produced into a broad and relatively short blunt lobe; in ventral view (Fig. 6F) much longer than in dorsal view (Fig. 6D) with ratio of ventral to dorsal length about 4.2. Gonostyles (Fig. 6E–F) large, broad medially, with dorsal margin weakly sinuate. Aedeagus (Fig. 6G–I) with endosomal processes covered with minute spines, extended posteriad and strongly curved dorso-anteriad; phallobase with one pair of large, strongly inflated dorsolateral lobes, their apices gradually convergent and tapering posteriad (Fig. 6G–I), and one pair of small, thumb-like ventral lobes (Fig. 6I). Segment X relatively narrow and elongate, in lateral view, narrow basally, widening beyond middle, apex subacute (Fig. 6E), in dorsal view, widest medially, with ratio of length to width 1.9–2.0 (Fig. 6D).

Female genitalia as in generic description (Fig. 7A–G).

**Etymology.** The new species is named for its occurrence in Hainan Island, China. The specific epithet *hainanensis* is to be treated as a latinized adjective in nominative singular.

**Distribution.** So far only known from Hainan Island, China.

### *Orthopagus lunulifer* Uhler, 1897

Figs 1A, 2G,H, 3D, 8A–I

*Orthopagus lunulifer* Uhler, 1897: 279. Lectotype (designated by Liang 1996: 47): male, Gifu, Japan (USNM, examined).

*Orthopagus splendens*: Matsumura 1905a: 61, Pl. 21, fig. 14; Matsumura 1905b: 19; nec Germar 1830: 48.

*Orthopagus helios* Melichar, 1912: 60. Lectotype (here designated): female, Ku Sia, Taiwan, China (MMBC, examined). **Syn. n.**

*Orthopagus helios* var. *diffusus* Melichar, 1912: 61. Lectotype (here designated), female, Taihanroku, Taiwan, China (HNHM, examined). Synonymized under *Orthopagus helios* Melichar by Schumacher (1915): 130.

*Orthopagus elegans* Melichar, 1912: 61. Lectotype (here designated), female, Taihanroku, Taiwan, China (MMBC, examined). Synonymized under *Orthopagus helios* Melichar by Schumacher 1915: 130.

*Orthopagus lunulifer*: Melichar 1912: 60; Liang 1996: 47, fig. 4; Song et al. 2016d: 36–39, figs 3I, 4A–G, 5A–H, 6A–F; Song et al. 2018: figs 5A–B, D–G, 6A–C.

**Type material examined.** *Orthopagus lunulifer*: Lectotype, male, (1) 25,18,0., Gifu, male; (2) Type, No. 3123, U.S.N.M. [red label]; (3) Cotype No. U.S.N.M. [red label] (USNM). Paralectotypes: 1 male, (1) 25,18,0., Gifu, male; (2) Type, No. 3123, U.S.N.M. [red label]; (3) Cotype No. U.S.N.M. [red label]; 1 male, 1 female, (1) 25,8,0., Gifu; (2) Type, No. 3123, U.S.N.M. [red label]; (3) Cotype No. U.S.N.M. [red label]; 1 female, (1) 20,4,27., Gifu; (2) Type, No. 3123, U.S.N.M. [red label]; (3) Cotype No. U.S.N.M. [red label]; (4) Orthopagus lunulifer Uhler [Uhler's handwriting]; (5) 1164 [Uhler's handwriting] (all USNM).

*Orthopagus helios*: Lectotype (here designated), female, (1) Formosa, Ku Sia [handwriting, yellow label]; (2) helios Mel. [Melichar's handwriting], det. Melichar; (3) Typus [dark red label]; (4) Transcriptio, Orthopagus helios sp.n. female [P. Lauterer's handwriting], L. Melichar det 1912; (5) Collectio Dr. L. Melichar, Moravské museum Brno; (6) Syn-typus [red label]; (7) Invent. č. 4947/Ent., Mor. muzeum, Brno; (8) Lectotypus female, Orthopagus helios Melichar, 1912, designated by Z. S. Song & I. Malenovský, 2018 [newly added red label] (MMBC). Paralectotypes, 2 females, (1) Formosa, Ku Sia [handwriting, yellow label]; (2) Paralectotypus female, Orthopagus helios Melichar, 1912, designated by Z. S. Song & I. Malenovský, 2018 [newly added red label] (SNSD).

*Orthopagus helios* var. *diffusus*: Lectotype (here designated), male, (1) Formosa, Sauter; (2) Taihanroku, 908.; (3) v. diffusus M. [handwriting, underlined with red], det. Melichar; (4) typus [label with red frame]; (5) Hung. Nat. Hist. Museum Budapest, coll. Hemiptera [yel-

**Figure 5.** *Orthopagus exoletus* (Melichar). (**A**) Head, pronotum and mesonotum, dorsal view. (**B**) Head and pronotum, lateral view. (**C**) Head and pronotum, ventral view. (**D**) Male segment X and pygofer, dorsal view. (**E**) Male pygofer, gonostyles, and segment X, lateral view. (**F**) Male pygofer and gonostyles, ventral view. (**G**) Aedeagus, dorsal view. (**H**) Aedeagus, lateral view. (**I**) Aedeagus, ventral view.

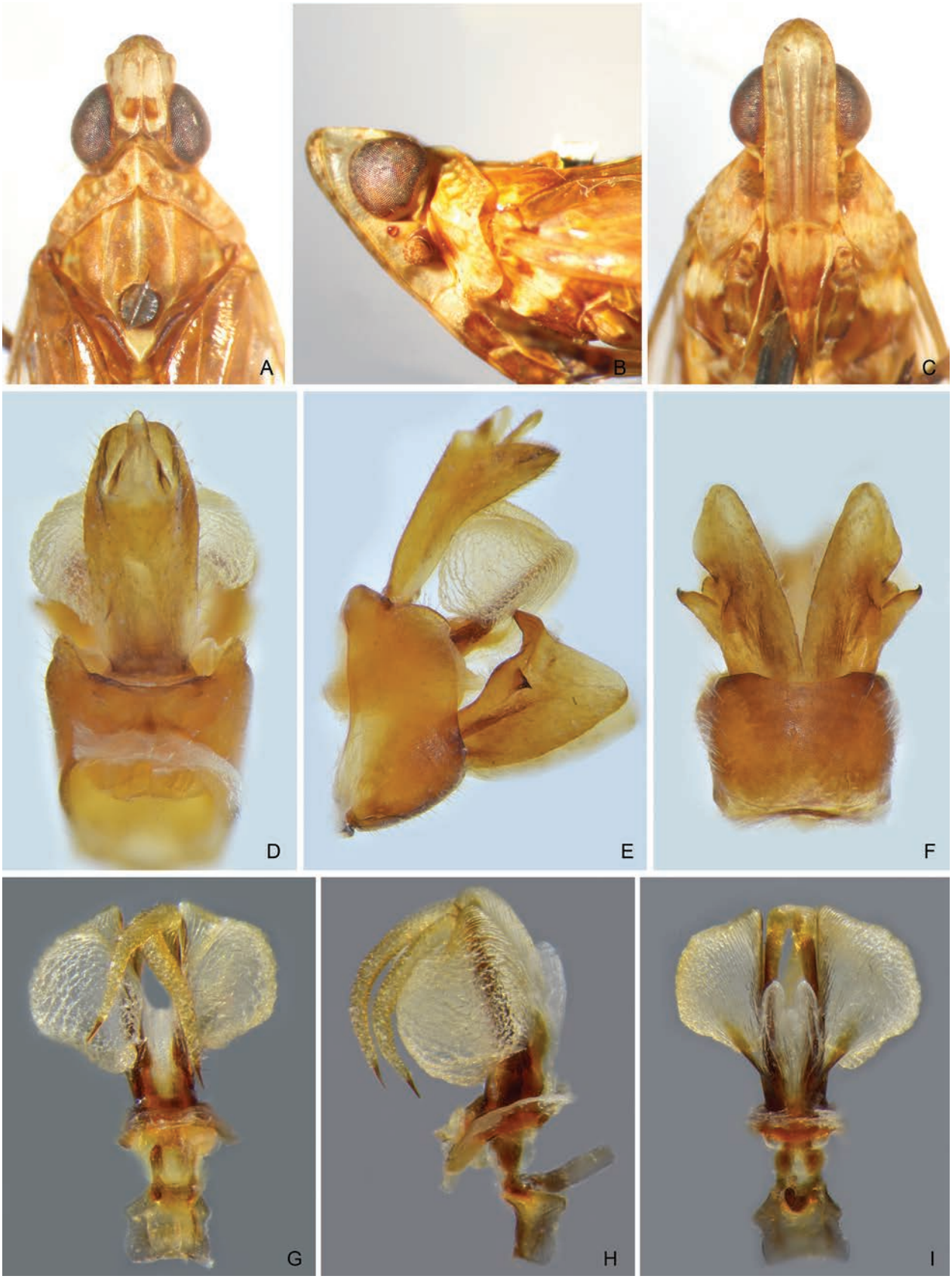


**Figure 6.** *Orthopagus hainanensis* sp. n. (**A**) Head, pronotum and mesonotum, dorsal view. (**B**) Head and pronotum, lateral view. (**C**) Head and pronotum, ventral view. (**D**) Male segment X and pygofer, dorsal view. (**E**) Male pygofer, gonostyles, and segment X, lateral view. (**F**) Male pygofer and gonostyles, ventral view. (**G**) Aedeagus, dorsal view. (**H**) Aedeagus, lateral view. (**I**) Aedeagus, ventral view.

**Figure 7.** *Orthopagus hainanensis* sp. n. (**A**) Female terminalia and ectodermal genital ducts, dorsal view. (**B**) Female terminalia and ectodermal genital ducts, lateral view. (**C**) Female terminalia and ectodermal genital ducts, ventral view. (**D**) Gonapophysis VIII, dorsolateral view. (**E**) Gonapophysis IX, ventral view. (**F**) Gonapophysis IX, lateral view. (**G**) Gonoplacs, lateral view.

low label] (6) Lectotypus male, Orthopagus helios var. diffusus Melichar, 1912, designated by I. Malenovský in Song et al. 2018 [newly added red label] (HNHM). Paralectotype, male, (1) Formosa, Sauter; (2) Kosempo, 908.; (3) v. diffusus M. [handwriting, underlined with red], det. Melichar; (4) typus [label with red frame and a hindwing glued to it]; (5) Hung. Nat. Hist. Museum Budapest, coll. Hemiptera [yellow label] (6) Paralectotypus male, Orthopagus helios var. diffusus Melichar, 1912, designated by I. Malenovský in Song et al. 2018 [newly added red label] (HNHM, abdomen detached and glued to a separate label attached to the same pin).

*Orthopagus elegans*: Lectotype (here designated), female, (1) Formosa, Sauter; (2) Taihanroku, 908.; (3) elegantulus [Melichar's handwriting], det. Melichar.; (4) Typus [dark red label]; (5) Collectio Dr. L. Melichar,

Moravské museum Brno; (6) Orthopagus female elegans sp. n. female, L. Melichar det. 1912 [Lauterer's handwriting], P. Lauterer's det. 1991; (7) Syn-typus [red label]; (8) Invent. č. 4948/Ent., Mor. muzeum, Brno; (9) Lectotypus female, Orthopagus elegans Melichar, 1912, designated by Z. S. Song & I. Malenovský, 2018 [newly added red label] (MMBC). Paralectotype, female, (1) Formosa, Sauter; (2) Taihanroku, 908.; (3) elegans M. [handwriting, underlined with red], det. Melichar; (4) typus [label with red frame]; (5) Hung. Nat. Hist. Museum Budapest, coll. Hemiptera [yellow label] (6) Paralectotypus female, Orthopagus elegans Melichar, 1912, designated by I. Malenovský in Song et al. 2018 [newly added red label] (HNHM).

**Other material examined. JAPAN**: Honshu island: 1 female, Tokyo, Matsumura leg. (IZCAS); 1 male, Kamakura, ix.1913, F. Muir leg. (BPBM); 1 female, Mie prefecture, Matagari lke, 24.x.1989, C. W. O'Brien & L. B. O'Brien leg. (LBOB). **CHINA**: Beijing municipality: 1 female, Peiping; 9 males, 15 females, Peiping, 9., 10. and 21.vii.1938, 4., 13., 15. and 28.viii.1938, 11., 16., 19., and 24.ix.1938, T. P. Chang leg.; 1 male, 1 female, Juyongguan, 250–280 m, 3. and 6.viii.1961, S. Y. Wang & X. Z. Zhang leg.; 1 male, 1 female, Zhongguancun, 4.ix.1962, S. Y. Wang leg.; 3 males, 2 females, Shisanling, 12.ix.1962, R. Z. Xie leg. (all IZCAS); 2 males, 2 females, Changping, 8.vii.2007, Z. S. Song leg. (JSSNU); Tianjin municipality: Jixian, 4.ix.1988, K. H. Zhang leg. (IZCAS); Shandong province: 2 males, 1 female, Tsingtao [Musée Heude]; 1 male, Laoshan, 800 m [Musée Heude] (all IZCAS); Henan province: 1 male, 1 female, Henan, 8.viii.2013, D. J. Zhang (JSSNU); Anhui province: 2 males, Huang Mountain, 6.viii.1936 (IZCAS); Shangai municipality: 1 male, 1 female, 27.vii.1932, O. Piel leg. [Musée Heude] (IZCAS); Zhejiang province: 18 males, 10 females, T'ienmo Shan, 22–28.viii.1936; 2 females, Hangzhou, 24. and 25.viii.1942; 8 males, 8 females, Chusan, 7., 8., 10., 18., 20., 28. and 29.viii.1931, O. Piel leg. [Musée Heude] (all IZCAS); 1♀, Fujian province: 1 female, Jianyang, Chengguan, 90–120 m, 12.viii.1960, Y. R. Zhang leg.; 1 male, 1 female, Chongan, Xingcun, Sangang, 740 m, 12. and 20.viii.1960, Y. Zuo & C. L. Ma leg.; 1 male, 2 females, Jiangle, Longqishan, 500–700 m, 12., 13. and 19.viii.1991, S. M. Song leg. (all IZCAS); Hunan province: 1 female, Hoeng-Shan, 900 m, 1933, H. Höne leg. (MFNB); Guizhou province: 1 female, Libo, 21.viii.2000, F. M. Shi leg. (IZCAS); Sichuan province: 5 males, 3 females, Emei Mountain, Baoguosi, 550–750 m, 7., 9., 10. and 14.ix.1957, F. X. Zhu & Z. Y. Wang leg. (IZCAS); Tibet (Xizang) autonomous region: 1 female, Chayu, Xiachayu, 1900 m, 21.viii.2005, Z. S. Song leg. (IZCAS); Guangxi autonomous region: 4 males, 5 females, Guilin, Yanshan, 2., 7. and 23.viii.1952, 20., 22. and 24.vii.1953; 2 females, Pingxiang, 12. and 16.vi.1976, B. L. Zhang leg.; 5 males, 5 females, Nandan, Luofu, 350 m, 27.vii.2006, J. Liu leg. (all IZCAS); 2 males, 2 females, Guangnan, Bamei, Shiw. Taoyuan, 24°18'51"N, 105°02'08"E, 811 m, 12.viii.2012, D. Rédei leg.; 2 males, same but 24°19'08"N, 105°02'57"E, 891 m; 1 male 1 female, same but 24°19'11"N, 105°01'49"E, 834 m, 13.viii.2012 (all HNHM); Yunnan province: 1 male, 5 females, Hekou, 80 m, 5. and 7.vi.1956, K. R. Huang leg.; 3 females, Hekou, Nanxi, 200 m, 8. and 12.vi.1956, K. R. Huang leg.; 1 male, 1 female, Pingbian, 1400 m, 15.vi.1956, K. R. Huang leg. (all IZCAS); Taiwan: 1 male, Tainan, Formasa, vi.1912, H. Sauter, Orthopagus helios Mel. F. Schumacher det. [Schumacher's handwriting] (SDEI); 2 males, 1 female, Taihanroku, vii. and 10.xi, H. Sauter leg. (MFNB); 1 male, 1 female, same data (MZPW); 5 males, 2 females, Taihanroku, 1908, Sauter leg. (HNHM); 1 male, 1 female, Hoozan, 10.vii. and 10.ix., H. Sauter leg. (MFNB); 1 female, Kotobuki, 11.vi.1935, (IZCAS); 2 females, Tainan county, ca. 350 m, 2–3 km S Kwantzuling, bamboo, shrub, 26–28.vi.1980, D. R. Davis leg. (USNM); 1 female, Chi Pen, 10.vi.1997, B. Herczig & L. Ronkay leg. (HNHM). **VIETNAM**: 1 female, "Indo China", R. V. de Salvaza leg. (BMNH); 1 female, Chapa [= Sa Pa], v.–vi.1916, R. V. de Salvaza leg. (BMNH); 2 males, 1 female, Hoa Binh, vii.1939, A. de Cooma leg.; 5 males, 2 females Hoa Binh; 1 male, 1 female, Hoa Binh, Thanh-ha district, 12. and 13.vi.1966, R. Bielawski & B. Pisarski leg.; 2 males, 1 female, Hanoi, 24.vi.1959, B. Pisarski & J. Prószyński leg.; 4 males, 2 females Ninh Binh, Cuc Phuong, 5., 7. and 8.vi.1966, R. Bielawski & B. Pisarski leg.; 1 female, Nghe An district, Phu Quy, 17.vi.1959, B. Pisarski & J. Prószyński leg. (all MZPW); 1 female, Cuc Phuong, 400 m, at light, 17.x.1986, Vásárhelyi leg. (HNHM). **LAOS**: 1 male, Borikhane Prov., Pakkading, 31.vii.1965, native collector leg. (BPBM). **INDIA**: Assam: 2 males, Chabua, 10.x.1943, D. E. Hardy leg. (USNM); 1 female, Tocklai, light trap, ix.1983, 943/6, C.I.E.A. 15663 (BMNH). **NEPAL**: 1 male, Chitwan National Park, Island Jungle reserve, 29–30.x.1995, L. Peregovits leg. (HNHM).

**Redescription.** Measurements (10 males, 9 females). Body length (from apex of head to tip of forewings): male 11.7–13.4 mm, female 13.0–14.9 mm; head length (from apex of cephalic process to base of eyes): male 1.33–1.50 mm, female 1.50–1.65 mm; head width (including eyes): male 1.30–1.60 mm, female 1.48–1.75 mm; forewing length: male 9.4–10.8 mm, female 10.2–11.9 mm.

Coloration. General coloration as in generic description (Figs 1A, 2G–H). Vertex dark brown with five light ochraceous streaks: along median carina in anterior third (in some specimens, the whole median carina is light), along each lateral carina subapically and along each lateral carina basally, the latter streaks being sickle-shaped and curved to median carina at the base (Fig. 8A). Frons light ochraceous with small dark brown spots along intermediate and lateral carinae, frons base slightly infuscated (Fig. 8C). Forewing membrane pattern as in Fig. 3D. Hind wing membrane with a dark brown streak along the apical portion of $CuA_1$ vein, extending along hind wing apical margin.

Structure. Head with cephalic process moderately long, not inflated (Figs 8A–C). Vertex (Fig. 8A) with ratio of length at midline to width between eyes 1.6–2.0. Transition of vertex to frons relatively sharp, almost angular

**Figure 8.** *Orthopagus lunulifer* Uhler. (**A**) Head, pronotum and mesonotum, dorsal view. (**B**) Head and pronotum, lateral view. (**C**) Head and pronotum, ventral view. (**D**) Male segment X and pygofer, dorsal view. (**E**) Male pygofer, gonostyles, and segment X, lateral view. (**F**) Male pygofer and gonostyles, ventral view. (**G**) Aedeagus, dorsal view. (**H**) Aedeagus, lateral view. (**I**) Aedeagus, ventral view.

in lateral view (Fig. 8B). Frons relatively narrow, with ratio of length at midline to maximum width 2.6–3.0.

Male genitalia. Pygofer, in lateral view (Fig. 8E), with dorso-posterior margin produced into a short and broad lobe; in ventral view (Fig. 8F) much longer than in dorsal view (Fig. 8D) with ratio of ventral to dorsal length about 4.0. Gonostyles (Fig. 8E–F) large, broad medially, with dorsal margin weakly sinuate. Aedeagus (Fig. 8G–I) with endosomal processes extended posteriad and strongly curved dorso-anteriad, relatively short, their apices not reaching the base of phallobase; phallobase with one pair of large, strongly inflated dorsolateral lobes (Fig. 8H), their apices blunt, and one pair of small, thumb-like ventral lobes, directed posteriad (Fig. 8I). Segment X, in lateral view, narrow basally, widening to apex beyond middle, apex subacute (Fig. 8E); in dorsal view, relatively short and broad in dorsal view, widest medially, with ratio of length to maximum width 1.2–1.3 (Fig. 8D).

Female genitalia as in generic description.

**Distribution.** Widely distributed in tropical, subtropical and temperate eastern Asia (Japan, China, Korea, Vietnam, Laos, northeastern India and Nepal).

**Ecology and economic importance.** Adult *O. lunulifer* was reported as a minor pest of leaves of *Morus alba* L. (Pu and Mao 2012) and *Camellia oleifera* Abel in southeastern China (Zhao et al. 2013). Matsumura (1910) listed "*Anagnia splendens*" among pests of sugarcane in Taiwan; this record perhaps also refers to *O. lunulifer*.

**Remarks.** Erroneously according to Metcalf (1946), Matsumura (1905a, b) considered *O. lunulifer* to be a junior synonym of *Anagnia splendens* (Germar). As he probably studied material from Japan, the description and illustration of "*Anagnia splendens*" in Matsumura (1905a) probably refer to *O. lunulifer* (i.e., the only *Orthopagus* species currently confirmed from Japan). The same is probable for the records and a figure of "*Anagnia splendens*" from Okinawa and Taiwan published in Matsumura (1905b and 1910, respectively).

Melichar (1912) differentiated *O. elegans*, *O. helios* and *O. lunulifer* based on slight differences in the transparency of the forewing membrane, extent of the dark brown apical band on the forewing and the shape of frons. Based on a study of material from Taiwan, Schumacher (1915) suggested that *Orthopagus helios* and *O. elegans* described by Melichar (1912) belong to the same species. However, his synonymisation of *O. helios* var. *diffusus* Melichar and *O. elegans* Melichar under *O. helios* Melichar was not widely accepted (Metcalf 1946). We have examined the corresponding type specimens and additional specimens from the same series collected by H. Sauter in Taiwan and currently deposited in HNHM, MFNB, MMBC, SDEI, and SNSD, and confirm here Schumacher's conclusion. Simultaneously, we suggest that *O. helios* should be treated as a junior synonym of *O. lunulifer* because we consider the differences among these taxa listed by Melichar (1912) to represent intraspecific variation. We designate here the lectotypes for *O. elegans*, *O. helios* and *O. helios* var. *diffusus* to stabilize the nomenclature according to Article 74 of ICZN (1999).

Liang (1996) designated the lectotype for *O. lunulifer*, and provided a left lateral view of male genitalia for this species. Detailed illustrations of the male and female genitalia (but no detailed description) were also provided for *O. lunulifer* by Song et al. (2016d, 2018).

The single male specimen examined from Nepal (Chitwan National Park) is identical in external characters to specimens of *O. lunulifer* from Japan, China, Taiwan and Vietnam. However, it differs in the shape of the lobe on the dorso-posterior margin of the pygofer which is smaller (shorter and simply angular) than in the rest of *O. lunulifer* males studied. The phallobase of this specimen could not be sufficiently compared as its membranous lobes failed to inflate during the preparation. More specimens and data are needed to confirm the identification.

### *Orthopagus philippinus* Melichar, 1914

Figs 2I–J, 3E, 9A–J

*Orthopagus philippinus* Melichar, 1914: 173, Pl. I, figs 1, 2. Lectotype (here designated) male, Los Baños, Philippines (MMBC, examined).

**Type material examined.** Lectotype male (here designated), (1) Los Banos, P.I. Baker; (2) 1311; (3) *philippinus* [Melichar's handwriting] det. Melichar; (4) Collectio Dr. L. Melichar, Moravské museum Brno; (5) Orthopagus male philippinus sp.n., L. Melichar, 1914 [Lauterer's handwriting], P. Lauterer det. 1991; (6) Syn-typus [red label]; (7) Invent. č. 4954/Ent., Mor. muzeum, Brno; (8) Lectotypus male, *Orthopagus philippinus* Melichar, 1914, designated by Z. S. Song & I. Malenovský, 2018 [newly added red label] (MMBC).

Paralectotypes, 4 females, same locality labels as holotype but Inv. nos 4949–4951, 4953;1 female, Mt. Makiling, Luzon, Baker (MMBC, Inv. no. 4952, all paralectotypes bearing the following label: Paralectotypus female, Orthopagus philippinus Melichar, 1914, designated by Z. S. Song & I. Malenovský, 2018).

**Other material examined. PHILIPPINES:** Luzon island: 1 male, Mt. Makiling, Baker leg. (USNM); 1 male, Los Baños, 11.xii.1913, D. T. Fullaway leg. (BPBM); 1 male, Los Baños, i.1913, P. Ledyard leg (LBOB); 2 males, Mt. Montalban, Rizal, Wa-wa Dam, 150–200 m, 6. and 17.iii.1965, H. M. Torrevillas leg. (BPBM); 1 male, 4 females, Manila, G. Boettcher leg. (MMBC); 1 male, 2 females, "B. M. 1925-491", E. M. Ledyard leg.; 1 female, "Acc. No. 6625, Lot, Bu. of Sci., P. I.,1908-228", C. S. Banks leg.; 1 male, "Acc. No. 5364, Lot, Govt. Lab. Coll., 1908-228", C. S. Banks leg. (all BMNH).

**Redescription.** Measurements (4 males, 9 females). Body length (from apex of head to tip of forewings): male 10.6–11.4 mm, female 12.9–13.9 mm; head length (from

**Figure 9.** *Orthopagus philippinus* Melichar. (**A**) Head, pronotum and mesonotum, dorsal view. (**B**) Head and pronotum, lateral view. (**C**) Head and pronotum, ventral view. (**D**) Male segment X and pygofer, dorsal view. (**E**) Male pygofer, gonostyles, and segment X, lateral view. (**F**) Male pygofer and gonostyles, caudal view. (**G**) Gonostyle; (**H**) Aedeagus, dorsal view. (**I**) Aedeagus, lateral view. (**J**) Aedeagus, ventral view.

apex of cephalic process to base of eyes): male 1.25–1.33 mm, female 1.35–1.45 mm; head width (including eyes): male 1.30–1.43 mm, female 1.50–1.60 mm; forewing length: male 8.5–9.1 mm, female 10.3–11.4 mm.

Coloration. General coloration as in generic description (Figs 2I–J). Vertex predominantly dark brown, lateral margins with a pale ochraceous S-shaped streak in posterior three quarters (Fig. 9A). Frons light ochraceous with small dark brown spots along intermediate and lateral carinae and a large roundish dark brown spot at base (Fig. 9C). Forewing membrane pattern as in Fig. 3E. Hind wing membrane with a narrow dark brown infuscation along the apical portion of $CuA_1$ vein, not extending along hind wing apical margin.

Structure. Head with cephalic process moderately elongate (Figs 9A–B). Vertex (Fig. 9A) with ratio of length at midline to width between eyes 1.6–1.8. Transition of vertex to frons blunt, broadly rounded in lateral view, cephalic process thus appearing inflated and bulbous apically (Fig. 9B). Frons relatively narrow, with ratio of length at midline to maximum width 2.7–3.1.

Male genitalia. Pygofer, in lateral view, with dorso-posterior margin produced into a relatively large but narrow, tooth-like, apically obtuse process (Fig. 9E); in ventral view (Fig. 9F) much longer than in dorsal view (Fig. 9D) with ratio of ventral to dorsal width about 2.5. Gonostyles (Fig. 9G) large, broad medially, with dorsal margin weakly sinuate. Aedeagus (Fig. 9H–J) with endosomal processes covered with minute superficial spines, extended posteriad and strongly curved laterad and dorso-anteriad but not reaching the base of phallobase; phallobase with one pair of large, strongly inflated dorsolateral lobes, their apex convergent and tapering posteriad (Fig. 9I), and one pair of smaller, thumb-like ventral lobes, directed ventro-posteriad (Fig. 9I–J). Segment X, in lateral view, relatively narrow basally, widening to apex beyond middle, apex blunt (Fig. 9E); in dorsal view, relatively large and broad, widest in apical third, ratio of length to maximum width 1.0–1.1 (Fig. 9D).

Female genitalia as in generic description.

**Distribution.** So far only known from the Luzon island, Philippines.

**Remarks.** *Orthopagus philippinus* can be easily recognized from other species of the genus by the shape of the head and the presence of a relatively large roundish dark spot at base of frons which is present in all specimens studied.

Melichar (1914) indicated that he described this species based on five (male and female) specimens from "Luzon, Los Baños, Mt. Maquiling (C. F. Baker)". Six specimens (1 male, 5 females) preserved in Melichar personal collection in MMBC and labelled as either from Los Baños or Mt. Makiling and collected by Baker are considered here as the original syntypes. The male specimen is designated here as the lectotype for *O. philippinus* to stabilize the nomenclature according to Article 74 of ICZN (1999).

### *Orthopagus splendens* (Germar, 1830)

Figs 1B, 2K–L, 3F, 10A–I

*Flata splendens* Germar, 1830: 48. Syntype(s) (number of specimens and sex unknown), Java, Indonesia (not examined).
*Pseudophana splendens*: Westwood 1839: 151.
*Dictyophora* [sic] *splendens*: Walker 1851: 310.
*Anagnia splendens*: Stål 1861: 149.
*Udugama splendens*: Distant 1906): 249.
*Udugama flavocarinata* Bierman, 1907: 161; Bierman (1908): 151, Pl. 3, fig. 1. Syntypes 2 males, 1 female, Semarang, Java, Indonesia (not examined). Synonymized under *Orthopagus splendens* (Germar) by Melichar 1912: 59.
*Orthopagus splendens*: Oshanin 1908: 444; Melichar 1912: 59; Yang and Yeh 1994: 108, 116, figs 71, 76.
*Orthopagus splendens* var. *tibialis* Kirkaldy in Kirkaldy & Muir, 1913: 12. Syntypes (number of specimens and sex unknown), Macao, China (not examined).

**Material examined. CHINA**: Hainan island: 1 male, Hainan, 5.v.1934, C. Ho leg.; 2 females, Shuiman, 640 m, 25.v.1960, C. Q. Li leg.; 2 males, 5 females, Tongshi, 340 m, 23. and 25.vi.1960, C. Q. Li leg.; 1 female, Yinggen, 200 m, 6.vii.1960, S. F. Li leg.; Yunnan province: 1 male, 1 female, Hekou, 80 m, 7.vi.1956, K. R. Huang leg.; 3 females, Hekou, Xioananxi, 200 m, 8.vi.1956, K. R. Huang leg.; 1 male, Gaoligong Mountain, 1000 m, 20.viii.1958, C. L. Li leg.; 1 male, Malipo, 20.vii.1958; 1 female, Xishuangbanna, Menga, 1050 m, 18.ix.1957, F. J. Pu leg.; 1 male, 1 female, Xishuangbanna, Menglun, 600 m, 9.ix.1993, H. L. Xu & L. L. Yang leg.; 1 male, Xishuangbanna, Menglun, 10.ix.1993, X. Y. Cheng leg. (all IZCAS). **VIETNAM**: 1 female, Lao Kay, 31.v.1960, at light, K. Galewski leg. (MZPW); 1 male, 1 female, Ninh Binh, Cuo-phuong, 5., 7, and 8.vi.1966, R. Bielawski & B. Pisarski leg. (MZPW); 2 males, 22 km S of Nha Trang, 20–26.xi.1960, C. M. Yoshimoto leg. (BPBM); 1 male, DaiLanh, N of Nha Trang, 30.xi.–5.xii.1960, C. M. Yoshimoto leg. (BPBM). **THAILAND**: Trang province: 1 male, Khaophappha Khaochang, 200–400 m, 3.i.1964, G. A. Samuelson leg. (BPBM). **INDIA**: Assam: 1 female, Mazbat near Mangaldai, 11–15.x.1910 (coll. Distant, BMNH). **MALAYSIA**: Penang: 1 male, Island of Penang, Baker leg. (USNM). **INDONESIA**: Java: 1 male, Java (West), Djasinga, 5.i.1966, J. Stusak (BPBM); 1 female, Samarang, iv.1909, E. Jacobson; 1 female, same but vi.1909 (both coll. Melichar, MMBC); 1 female, Wied. in CW. Java, GW [handwriting] (coll. Zool. Mus. Leipzig Übernahme 1971, SNSD); 1 male, Java [handwriting] (MFNB).

**Redescription.** Measurements (3 males, 11 females). Body length (from apex of head to tip of forewings): male 10.3–11.8 mm, female 12.7–13.6 mm; head length (from apex of cephalic process to base of eyes): male 1.28–1.36 mm, female 1.35–1.48 mm; head width (including eyes): male 1.30–1.49 mm, female 1.50–1.59 mm; forewing length: male 8.5–9.1 mm, female 10.4–11.3 mm.

Coloration. General coloration as in generic description (Figs 1B, 2K–L). Vertex predominantly dark brown, lateral margins with a pale greenish or ochraceous S-shaped streak in posterior three quarters, median ca-

**Figure 10.** *Orthopagus splendens* (Germar). (**A**) Head, pronotum and mesonotum, dorsal view. (**B**) Head and pronotum, lateral view. (**C**) Head and pronotum, ventral view. (**D**) Male segment X and pygofer, dorsal view. (**E**) Male pygofer, gonostyles, and segment X, lateral view. (**F**) Male pygofer and gonostyles, ventral view. (**G**) Aedeagus, dorsal view. (**H**) Aedeagus, lateral view. (**I**) Aedeagus, ventral view.

rina anteriorly light in some specimens (Figs 1B, 10A). Frons light ochraceous with small dark brown spots along intermediate and lateral carinae and indistinct infuscation at base (Fig. 10C). Forewing membrane pattern as in Fig. 3F. Hind wing membrane with a narrow dark brown infuscation along the apical portion of $CuA_1$ vein, extending into a narrow infuscation along hind wing apical margin.

Structure. Head with cephalic process relatively short (Fig. 10A–B). Vertex (Fig. 10A) with ratio of length at midline to width between eyes 1.5–1.6. Transition of vertex to frons narrowly rounded in lateral view, cephalic process not inflated apically (Fig. 10B). Frons relatively narrow, with ratio of length at midline to maximum width 2.8–3.1.

Male genitalia. Pygofer, in lateral view, similar to *O. philippinus*, with dorso-posterior margin with a large and relatively narrow, tooth-like, apically obtuse process (Fig. 10E); pygofer with ratio of ventral to dorsal width about 2.6 (Fig. 10D, F). Gonostyles (Fig. 10E) large, broad medially, with dorsal margin weakly sinuate. Aedeagus (Fig. 10G–I) with endosomal processes very long, extended posteriad and strongly curved dorso-anteriad, reaching the base of phallobase; phallobase with one pair of relatively small, inflated dorsolateral lobes, their apex elongate, thumb-like, directed posteriad (Fig. 10H), and one pair of relatively large ventral lobes, convergent and tapering posteriad (Fig. 10H–I). Segment X, in lateral view, narrow basally, widening to apex beyond middle (Fig. 10E); in dorsal view, elongate, widest at apical third, ratio of length to maximum width 1.5–1.6 (Fig. 10D).

Female genitalia as in generic description.

**Distribution.** Confirmed records are from Indonesia (Java), West Malaysia, Vietnam, Thailand, southern China (Hainan, Yunnnan) and north-eastern India (Assam). Based on the description and illustrations in Yang and Yeh (1994), *O. splendens* probably also occurs in Taiwan (see also Tsaur 2005). Published records from the Philippines (Luzon; Stål 1861, Distant 1906) probably refer to *O. philippinus*, while the records from Sri Lanka (Melichar 1903, 1912; Distant 1906) concern *O. exoletus* and the ones from Japan (Matsumura 1905a, b, 1910) refer to *O. lunulifer*. Records from western India (Stål 1861), Myanmar (Distant 1906), Singapore, and Indonesia: Sumatra (Bierman 1908) still need to be checked.

**Remarks.** *Flata splendens* was described by Germar (1830) from Java, and was designated as the type species of *Anagnia* by Stål (1861). *Udugama flavocarinata* Bierman, 1907 from Java was considered as a junior synonym of *O. splendens* by Melichar (1912). *Orthopagus splendens* var. *tibialis* Kirkaldy was differentiated from the typical form by having "the fore and middle tibiae distinctly bi- or tri-angulate with brownish" (Kirkaldy and Muir 1913). However, this pattern is typical for all *Orthopagus* species.

The synonymy of *Dictyophara indiana* Walker, 1851 with *Orthopagus splendens* proposed by Stål (1861) is considered doubtful (see above).

*Orthopagus splendens* has been frequently confused in literature and museum collections with other species of the genus. It can be differentiated from them by the combination of the following characters: a relatively small body size, a moderately short head, an extensive dark pattern on vertex, the pygofer with dorso-posterior margin bearing a relatively large but narrow, simply tooth-like process, the endosomal processes of the aedeagus relatively long and robust, covered with minute superficial spines and phallotheca bearing two pairs of membranous lobes.

## Discussion and conclusions

The six currently recognised species of *Orthopagus* are very similar in external morphology and female genitalia and also the differences in the male genitalia between some pairs of species are subtle. This is probably the reason for the relatively complicated synonymy and frequent misidentifications by previous authors. The individual species can be recognised mainly by the differences in extent of the dark pattern on the vertex and frons, the size and shape of the cephalic process, the extent of the dark brown infuscation on the hind wing, the shape of the male pygofer, the structure of the aedeagus (the length of the endosomal processes and the shape and size of the membranous lobes of the phallobase), and the shape of the male segment X.

Three species, *Orthopagus bartletti* sp. n., *O. hainanensis* sp. n., and *O. philippinus*, have, as far as known, very restricted distributions, being endemic to south-western India and the islands of Hainan (China) and Luzon (Philippines), respectively. The distribution of *O. exoletus* is, as far as known, restricted to the Indian subcontinent (southern India, West Bengal and Sri Lanka). *Orthopagus lunulifer* is widespread in the tropical, subtropical and temperate eastern Asia from Nepal and northeastern India in the southwest to Korea and Japan in the northeast, while the distribution of *O. splendens* is probably restricted to the tropical zone slightly more south. The areas of distribution of the latter two species, however, overlap in northeastern India (Assam), northern Vietnam, and southern China (Yunnan, Hainan and Taiwan) (Fig. 11). *Orthopagus splendens* is also sympatric with *O. hainanensis* in the Hainan island, and *O. exoletus* is sympatric with *O. bartletti* in southwestern India. More data, e.g. from molecular markers, are needed to infer a phylogeny of *Orthopagus* and propose some evolutionary scenario which would also help to explain these distributions and mechanisms of speciation involved. More studies are also needed to understand the ecology and economic importance of *Orthopagus* species since the currently available data are scarce and insufficient (Matsumura 1910, Pu and Mao 2012, Zhao et al. 2013).

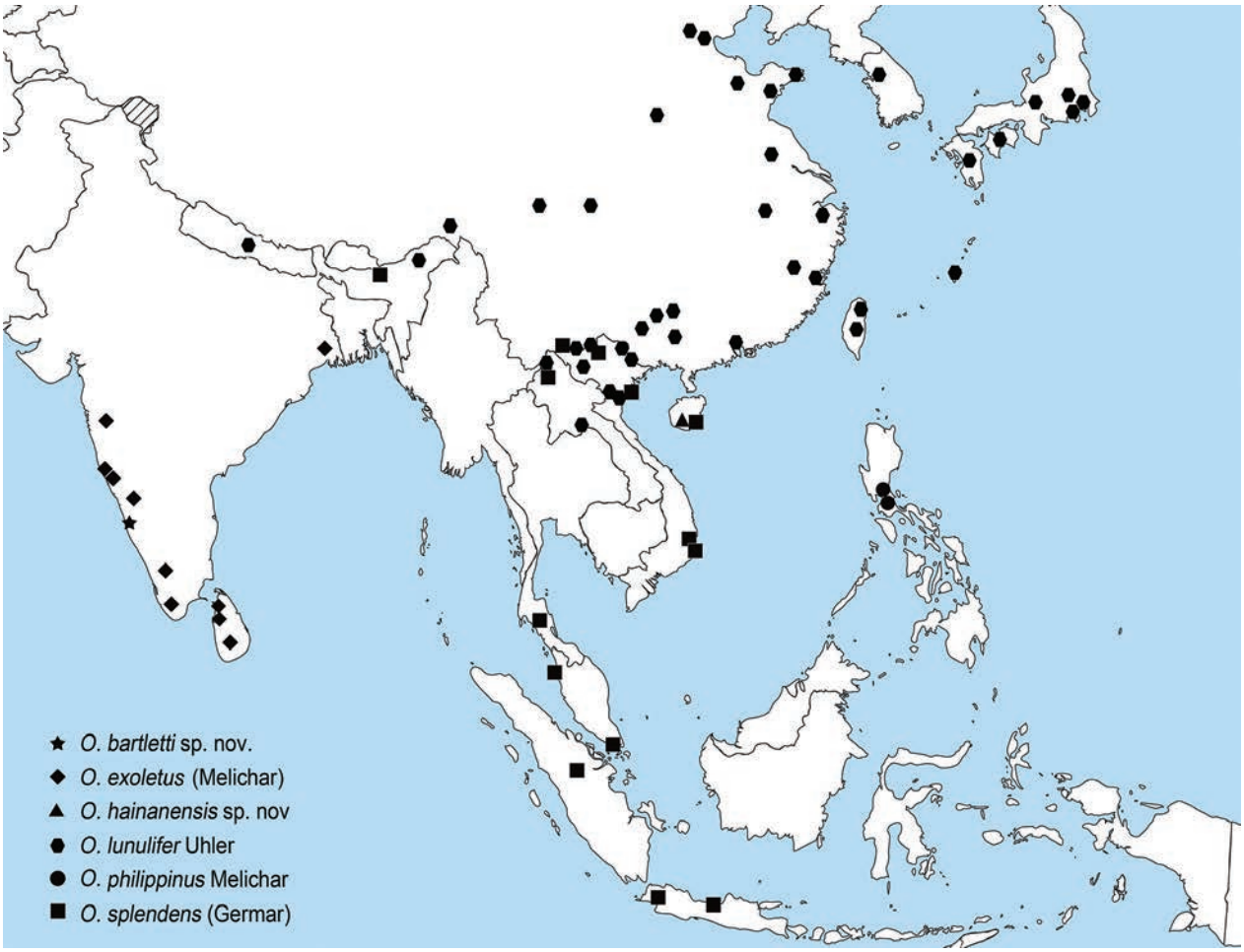


**Figure 11.** Geographical distribution of the *Orthopagus* species.

According to the phylogenetic hypothesis by Song et al. (2016d, 2018), *Orthopagus* is most closely related to the monotypic genus *Dictyomeria* Song, Webb & Liang, 2016, represented by *D. simulata* (Distant, 1906) from India which has been known only from the female holotype so far (Song et al. 2016d). Both genera share a similar forewing shape, venation and dark brown pattern on the forewing membrane. *Orthopagus* can be distinguished from *Dictyomeria* by the following characters: short and nearly straight head (in contrast, the cephalic process in *Dictyomeria* is strongly upturned in front of eyes); frons with median carina ridged and intermediate carinae approaching frontoclypeal suture (with median carina robust and strongly convex and intermediate carinae extending to anterior margin of eyes in *Dictyomeria*); pronotum with intermediate carinae distinct in basal half (indistinct in *Dictyomeria*); fore femora flattened and dilated, with a large and blunt spine near apex (fore femora not flattened and dilated, with a small spine in *Dictyomeria*).

*Orthopagus* is also similar, e.g. in the head morphology, to another monotypic genus *Truncatomeria* Song & Liang, 2011, established for *T. viridistigma* (Kirby, 1891) (= *Udugama fletcheri* Kirkaldy, 1908) from Sri Lanka. *Orthopagus* can be distinguished from *Truncatomeria* by the following characters: frons with median carina moderately ridged (very strongly produced in *Truncatomeria*); fore femora flattened and dilated, with a large blunt spine near apex (slender with a short small spine near apex in *Truncatomeria*); the hind tibiae with seven black-tipped apical spines (eight spines in *Truncatomeria*); the forewing relatively shorter, broader and with membrane bearing well-developed dark brown markings (clear in *Truncatomeria*); and the long, apically pointed endosomal processes of the aedeagus (short and apically obtuse in *Truncatomeria*; see Song and Liang 2011). The similarity in the head shape between the two genera might be a symplesiomorphy or a convergence; according to Song et al. (2016d, 2018), *Truncatomeria* is more closely related to *Centromeria* Stål, 1870 and a few other genera.

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# References

Bierman CJH (1907) Homopteren aus Semarang (Java) gesammelt von Herrn Edw. Jacobson. Entomologische Berichten 2: 161–163.

Bierman CJH (1908) Homopteren aus Semarang (Java) gesammelt von Herrn E. Jacobson. Notes from the Leyden Museum Leyden 29: 151–169.

Bourgoin T (1993) Female genitalia in Hemiptera Fulgoromorpha, morphological and phylogenetic data. Annales de la Société Entomologique de France (Nouvelle Série) 29: 225–244.

Bourgoin T (2018) FLOW (Fulgoromorpha Lists On the Web): a world knowledge base dedicated to Fulgoromorpha. Version 8. http://hemiptera-databases.org/flow/ [Accessed on 18 January 2018]

Bourgoin T, Wang RR, Asche M, Hoch H, Soulier-Perkins A, Stroiński A, Yap S, Szwedo J (2015) From micropterism to hyperpterism: recognition strategy and standardized homology-driven terminology of the forewing venation patterns in planthoppers (Hemiptera: Fulgoromorpha). Zoomorphology 134: 63–77. https://doi.org/10.1007/s00435-014-0243-6

Distant WL (1906) The Fauna of British India, including Ceylon and Burma. Rhynchota Vol. III (Heteroptera-Homoptera). Taylor & Francis, London, 503 pp.

Emeljanov AF (1983) Dictyopharidae from the Cretaceous deposits on the Taymyr Peninsula (Insecta, Homoptera). Paleontologicheskii Zhurnal 3: 79–85.

Emeljanov AF (2011) Improved tribal delimitation of the subfamily Dictyopharinae and description of new genera and new species (Homoptera, Fulgoroidea, Dictyopharidae). Entomological Review 91(9): 1122–1145. https://doi.org/10.1134/s0013873811090053

Germar EF (1830) Species Cicadarium enumeratae et sub genera distributae. Thon‘s Entomologisches Archiv 2(2): 1–57.

ICZN [International Commission on Zoological Nomenclature] (1999) International Code of Zoological Nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature c/o the Natural History Museum, London, 271 pp. https://doi.org/10.5962/bhl.title.50608

Kirkaldy GW (1904) Bibliographical and nomenclatorial notes on the Hemiptera No. 3. The Entomologist. An illustrated Journal of Entomology London 37: 279–283.

Kirkaldy GW (1908) Notes on some Sinhalese Rhynchota. Annales de la Société entomologique de Belgique Bruxelles 52: 9–14.

Kirkaldy GW, Muir F (1913) On some new species of leaf-hoppers. Report of the work of the Experiment Station of the Hawaiian Sugar Planters' Association, Entomological series, Bulletin Honolulu 12: 1–90.

Krstić O, Cvrković T, Mitrović M, Toševski I, Jović J (2016). *Dictyophara europaea* (Hemiptera: Fulgoromorpha: Dictyopharidae): description of immatures, biology and host plant associations. Bulletin of Entomological Research 106: 395–405. https://doi.org/10.1017/S0007485316000067

Liang AP (1996) Lectotype designations and taxonomic notes on P. R. Uhler's Japanese Fulgoroidea (Homoptera) in the U. S. National Museum of Natural History. Japanese Journal of Systematic Entomology 2(1): 45–50.

Liang AP, Song ZS (2006) Revision of the Oriental and eastern Palaearctic planthopper genus *Saigona* Matsumura, 1910 (Hemiptera: Fulgoroidea: Dictyopharidae), with descriptions of five new species. Zootaxa 1333: 25–54.

Matsumura S (1905a) Nippon Senchu Zukai. Descriptions and illustrations of the species. 1000 Insects of Japan 2: 42–70. [In Japanese]

Matsumura S (1905b) Die Hemipterenfauna von Riukiu (Okinawa). Transactions of the Sapporo Natural History Society 1: 15–38.

Matsumura S (1910) Die schädlichen und nützlichen Insekten vom Zuckerrohr Formosas. Keiseisha, Tokyo, 52 pp + 30 pls. https://doi.org/10.5962/bhl.title.35666

Melichar L (1903) Homopteren Fauna von Ceylon. Berlin: Verlag von Felix L. Dames, 248 pp.

Melichar L (1912) Monographie der Dictyophorinen (Homoptera). Abhandlungen der K. K. Zoologisch-Botanischen Gesellschaft in Wien 7(1): 1–221.

Melichar L (1914) Neue Homopteren von den Philippinen. Philippine Journal of Science 9: 173–181.

Metcalf ZP (1946) General catalogue of the Hemiptera, Fasci. IV. Fulgoroidea, Part 8 *Dictyopharidae*. Smith College, Northampton, USA, 246 pp. https://doi.org/10.1093/aesa/40.3.544

Muir F (1923) On the Classification of the Fulgoroidea (Homoptera). Proceedings of Hawaiian Entomological Society 5: 205–247.

Oshanin VT (1908) Verzeichnis der palaearktischen Hemipteren mit besonderer Berücksichtung ihrer Verteilung im Russischen Reiche. II. Band. Homoptera. III. Lieferung. Annuaire du Musée Zoologique de l'Académie Impériale des Sciences de St.-Pétersbourg 13: 385–492.

Pu GQ, Mao JP (2012) A list of mulberry pests in Jiangsu Province (continued). Jiangsu Sericulture 2: 7–12.

Schumacher F (1915) Homoptera in H. Sauter's Formosa-Ausbeute. Supplementa Entomologica 4: 108–142.

Song ZS, Bartlett CR, O'Brien LB, Liang AP, Bourgoin T (2018) Morphological phylogeny of Dictyopharidae (Hemiptera: Fulgoromorpha). Systematic Entomology. https://doi.org/10.1111/syen.12293

Song ZS, Deckert J, Liang AP (2012) Revision of the Oriental genus *Leprota* Melichar (Hemiptera, Fulgoromorpha, Dictyopharidae), with description of a new species from northern Borneo, Malaysia. Deutsche Entomologische Zeitschrift 59: 219–226. https://doi.org/10.1002/mmnd.201200017

Song ZS, Deckert J, Liang AP (2016a) Review of the oriental genus *Protolepta* Melichar, with description of the second species from Sulawesi, Indonesia (Hemiptera: Auchenorrhyncha: Dictyopharidae: Orthopagini). Entomologica Americana 122(3): 467–475. https://doi.org/10.1664/1947-5144-122.3.467

Song ZS, Liang AP (2006a) First record of the genus *Dictyopharina* Melichar (Hemiptera: Fulgoroidea: Dictyopharidae) from China, with descriptions of two new species. Zootaxa 1166: 21–33.

Song ZS, Liang AP (2006b) Two new species of the genus *Dictyopharina* Melichar (Hemiptera: Fulgoroidea: Dictyopharidae) from Southeast Asia. Acta Zootaxonomica Sinica 31: 595–600. https://doi.org/10.3969/j.issn.1000-0739.2006.03.031

Song ZS, Liang AP (2007) A new species of the Oriental planthopper genus *Tenguna* Matsumura, 1910 (Hemiptera: Fulgoroidea: Dictyopharidae) from Xizang, China. Zootaxa 1439: 57–64. https://doi.org/10.11646/zootaxa.1439.1.3

Song ZS, Liang AP (2011) Two new genera and two new species of Oriental dictyopharid planthoppers (Hemiptera: Fulgoromorpha: Dictyopharidae) from Sri Lanka and southern India. Zootaxa 2740: 24–34.

Song ZS, Liang AP (2012a) Taxonomic revision of the Oriental genus *Metaurus* Stål (Hemiptera: Fulgoromorpha: Dictyopharidae), with description of a new species. Journal of Natural History 46: 2563–2575. https://doi.org/10.1080/00222933.2012.708456

Song ZS, Liang AP (2012b) *Dictyotenguna choui*, a new genus and species of Dictyopharinae (Hemiptera: Fulgoromorpha: Dictyopharidae) from China. Entomotaxonomia 34(2): 207–214.

Song ZS, Malenovský I, Liang AP (2016b) Revision of the Afrotropical genus *Fernandea* Melichar, 1912 (Hemiptera: Fulgoromorpha: Dictyopharidae), with description of a new species from Equatorial Guinea. Zootaxa 4139: 106–116.

Song ZS, Malenovský I, Liang AP (2017) Revision of the Afrotropical planthopper genus *Centromeriana* Melichar, 1912 (Hemiptera, Dictyopharidae). European Journal of Taxonomy 278: 1–21. https://doi.org/10.58552/ejt.2017.278

Song ZS, Szwedo J, Wang RR, Liang AP (2016c) Systematic revision of Aluntiini Emeljanov (Hemiptera: Fulgoromorpha: Dictyopharidae: Dictyopharinae): reclassification, phylogenetic analysis, and biogeography. Zoological Journal of the Linnean Society 176: 349–398. https://doi.org/10.1111/zoj.12319

Song ZS, Webb MD, Liang AP (2014) Systematic revision of the Oriental planthopper genus *Miasa* Distant (Hemiptera: Fulgoromorpha: Dictyopharidae), with description of a new genus from southern India. Arthropod Systematics & Phylogeny 72: 137–164.

Song ZS, Webb MD, Liang AP (2016d) Phylogenetic analysis of the Oriental genera of Orthopagini, 1983 (Hemiptera: Fulgoromorpha: Dictyopharidae: Dictyopharinae), with a systematic revision of the genus *Centromeria* Stål, 1870. Zoological Journal of the Linnean Society 178: 33–87.

Stål C (1861) Miscellanea hemopterologica. Entomologische Zeitung. Herausgegeben von dem entomologischen Vereine zu Stettin 22: 129–153. https://doi.org/10.1111/zoj.12401

Tsaur SC (2005) Some Fulgoroids (Insecta: Hemiptera) collected on Turtle Island, Taiwan. Zoological Studies 44: 1–4.

Uhler PR (1897) Summary of the Hemiptera of Japan presented to the United States National Museum by Professor Mitzukuri. Proceedings of the United States National Museum. Washington 19: 255–297. https://doi.org/10.5479/si.00963801.1108.255

Walker F (1851) List of the specimens of Homopterous insects in the collection of the British Museum. Part I.II. Printed by order of the Trustees, London, 636 pp.

Walker F (1854) List of the specimens of lepidopterous insects in the collection of the British Museum 2: 279–581.

Westwood JO (1839) On the family Fulgoridae, with a monograph of the genus *Fulgora* of Linnaeus. Transactions of the Linnean Society of London, Second series, Zoology 18: 133–153.

Wilson MR, Malenovský I (2007) Tropiduchidae described by Leopold Melichar (Hemiptera, Fulgoromorpha). Acta Musei Moraviae, Scientiae biologicae. Brno 92: 137–153.

Wilson SW, O'Brien LB (1987) A survey of planthopper pests of economically important plants (Homoptera, Fulgoroidea). Proceedings of the 2nd International Workshop on Leafhoppers and Planthoppers of Economic Importance. CAB International Institute of Entomology, London, 343–360.

Wilson SW, Mitter C, Denno RF, Wilson MR (1994) Evolutionary patterns of host plant use by delphacid planthoppers and their relatives. Planthoppers, Their Ecology and Management (ed. by R.F. Denno and T.J. Perfect), 7–45. Chapman and Hall, New York.

Yang CT, Yeh WB (1994) Nymphs of Fulgoroidea (Homoptera: Auchenorrhyncha) with descriptions of two new species and notes on adults of Dictyopharidae. Chinese Journal of Entomology Special Publication 8: 1–189.

Young DA, Soós A (1964) Types of Cicadellinae (Homoptera, Cicadellidae) in the Hungarian Natural History Museum. Annales historico-naturales Musei nationalis hungarici. Budapest 56: 465–467.

Zhao DY, Liao FY, Qin CS (2013) Studies on occurrence regularity of diseases and pests of Camellia oleifera in Guangdong. Guangdong Agricultural Sciences 40(12): 86–89, 98. https://doi.org/10.16768/j.issn.1004-874x.2013.12.037

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# Taxonomic status of *Aphyocharax avary* Fowler, 1913, *Aphyocharax pusillus* Günther, 1868 and *Chirodon alburnus* Günther, 1869 (Characiformes, Characidae)

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## Abstract

The obscure taxonomic histories of three species of *Aphyocharax* (*A. alburnus*, *A. avary* and *A. pusillus*) are revised, based on both morphological and literature data. *Aphyocharax avary* is resurrected as a valid species and removed from synonymy with *A. alburnus*. Based on examinations of type specimens, *A. alburnus* is considered a junior synonym of *A. pusillus*.

## Key Words

Aphyocharacinae
Characoids
junior synonym
taxonomy

## Introduction

*Aphyocharax* Günther, 1868, is a genus of small-sized characids, no larger than 80 mm standard length (Souza–Lima 2003), occurring in most river basins in the Neotropical region: the Orinoco, Amazon, and La Plata River systems (Taphorn and Thomerson 1991, Tagliacollo et al. 2012). According to Tagliacollo et al. (2012), Froese and Pauly (2018) and Eschmeyer et al. (2018), the genus comprises 11 valid species: *Aphyocharax agassizii* (Steindachner, 1882), *A. alburnus* (Günther, 1869), *A. anisitsi* Eigenmann & Kennedy, 1903, *A. colifax* Taphorn & Thomerson, 1991, *A. dentatus* Eigenmann & Kennedy, 1903, *A. erythrurus* Eigenmann, 1912, *A. gracilis* Fowler, 1940, *A. nattereri* (Steindachner, 1882), *A. pusillus* Günther, 1868 (the type species of the genus), *A. rathbuni*

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Eigenmann, 1907, and *A. yekwanae* Willink, Chernoff & Machado-Allison, 2003. However, some authors (e.g., Souza-Lima 2007, Ferreira et al. 2011, Lima et al. 2013) consider *A. avary* Fowler, 1913, as a 12th valid species. *Aphyocharax avary* was described by Fowler (1913) based on a single specimen from the "Madeira River, about 200 miles east of Long. 62°20'W., Brazil". Fowler (1940) later considered it a synonym of *A. alburnus*. That taxonomic decision was based on a supposed morphological colour pattern variation and the number of teeth of those species – and influenced by their confusing taxonomic histories, principally the work published by Eigenmann (1915). Géry (1977) considered *A. avary* a valid species and included it in his species identification key of the genus. More recently, Souza-Lima (2003), following the taxonomic decision of Fowler (1940), also considered it a synonym of *A. alburnus*. Shortly afterwards, however, Souza-Lima (2007) considered *A. avary* to be a valid species. Tagliacollo et al. (2012) did not consider *A. avary* as one of the 11 valid species of the genus, although those authors did not specify which species they would consider it a synonym of; we presume that they again considered it a junior synonym of *A. alburnus*.

*Aphyocharax pusillus* was described by Günther (1868) based on three specimens from "Huallaga and Xeberos" [Amazon River Basin, Peru] (Souza-Lima 2003, 2007, Eschmeyer et al. 2018). Günther described the genus *Aphyocharax* in that same work, comprising only *A. pusillus*, which could be distinguished from *Chirodon* mainly by the presence of maxillary teeth (vs. their absence in *Chirodon*). Just one year later, Günther (1869) described *Chirodon alburnus* (currently a species of *Aphyocharax*) based on four specimens from the Peruvian Amazon, without providing a more precise and specific locality (Souza-Lima 2003, 2007, Eschmeyer et al. 2018), and did not detect maxillary teeth. Eigenmann (1915) re-described *Chirodon alburnus* without examining the type material, repeating the briefly informative description of the species provided by Günther (1869). Eigenmann (1915) examined five new lots from several distant localities, and characterized the species as possessing a humeral blotch and "maxillary with ten to sixteen teeth on over half the length of the maxillary" – character traits never before proposed for that species. Those specimens examined by Eigenmann probably corresponded to another species. The confusion caused by Eigenmann was followed by subsequent studies of the genus (e.g. Taphorn and Thomerson 1991, Willink et al. 2003).

Some authors who have recently described new species of *Aphyocharax*, or proposed phylogenetic relationships for the group, have overlooked the type locality of *A. alburnus* and examined and included material solely from Venezuelan river drainages (e.g., Taphorn and Thomerson 1991, Willink et al. 2003, Tagliacollo et al. 2012).

The aim of the present paper was to untangle the confusing taxonomic histories of those three species names, clarify their taxonomic statuses, and present, for the first time, the morphological features of their type specimens. We also provide a diagnosis for the species considered herein as valid, based on the examinations of type materials, information from the original descriptions, and the literature. The resolution of this confusing taxonomic history, the description of some morphological features of the type materials, and determinations of which of the three species are valid, will be important to solving taxonomical incongruities in the literature related to the genus and to enabling descriptions of new species of *Aphyocharax*. According to Souza-Lima (2007), there are at least four undescribed species, and the taxonomic identifications of several populations are still inaccurate (e.g. Lima et al. 2013, Ohara et al. 2017).

## Materials and methods

Measurements and counts were made according to Fink and Weitzman (1974), except for the perforated lateral line scales (which are interrupted) and the last scale on caudal-fin base (Y) which is counted separately from the other perforated scales (X), following the formula (X + Y). Internal counts, as well as some fins counts, were made only on radiograph images. The four modified vertebrae that constitute the Weberian apparatus were not included in the vertebrae counts, and the fused PU1 + U1 was considered a single element. Osteological nomenclature follows Weitzman (1962). C&S means cleared and stained, prepared according to Taylor and Van Dyke (1985). The materials herein examined are deposited in the following institutions: Academy of Natural Sciences of Philadelphia (ANSP), California Academy of Sciences, San Francisco, California, U.S.A. (CAS), Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais da Universidade Federal do Maranhão (CICCAA), Coleção Ictiológica do Instituto de Biologia da Universidade Federal do Rio de Janeiro (UFRJ), British Museum of Natural History (BMNH) and Field Museum of Natural History (FNMH), and the Information about *Aphyocharax* was based on both examined material and the literature (e.g., Günther 1868, 1869, Fowler 1940, Eigenmann 1915, Géry 1977, Taphorn and Thomerson 1991, Souza-Lima 2003, 2007, Willink et al. 2003, Tagliacollo et al. 2012, Eschmeyer et al. 2018, Froese and Pauly 2018).

## Taxonomy

### *Aphyocharax pusillus* Günther, 1868

*Aphyocharax pusillus* Günther, 1868: 480. Type locality: Huallaga and Xeberos [Amazon River Basin, Peru]. Syntypes: (1) BMNH 1867.6.13.46, (2) BMNH 1867.6.13.58-59.

*Chirodon alburnus* Günther, 1869:424: Type locality: Peruvian Amazons [Amazon River Basin, Peru]. Lectotype: BMNH 1869.5.21.10; Paralectotypes: (3) BMNH 1869.5.21.11-13. **New synonym.**

**Material examined.** BMNH 1867.6.13.46, 1 (Syntype), Huallaga and Xeberos [Amazon River Basin, Peru], Mr. Bartlett. BMNH 1867.6.13.58-59, 2 (Syntypes), Huallaga

**Figure 1.** Syntypes of *Aphyocharax pusillus*: BMNH 1867.6.13.58-59. Photographed by Kevin Webb.

**Figure 2.** Lectotype of *Aphyocharax alburnus*: BMNH 1869.5.21.10. Photographed by Harry Taylor.

**Table 1.** Meristic data of *Chirodon alburnus* and *Aphyocharax pusillus.*

| Measurements | BMNH 1867.6.13.46 *A. pusillus* | BMNH 1867.6.13.58-59 *A. pusillus* | BMNH 1869.5.21.10 *C. alburnus* | BMNH 1869.5.21.11-13 *C. alburnus* |
|---|---|---|---|---|
| Pored lateral-line scales | 11+1 | 11–12+1 | 11+1 | 11+1 |
| Longitudinal scales | 36 | 36–38 | 37 | 37 |
| Transverse scales | 11 | 10–11 | 11 | 11 |
| Dorsal-fin rays | 10 | 10–11 | 11 | 10–11 |
| Pectoral fin-rays | 10 | 10–11 | 11 | 11 |
| Pelvic-fin rays | 7 | 7 | 7 | 7 |
| Anal-fin rays | 19 | 18–19 | 18 | 17–18 |
| Premaxillary teeth | 6 | 7 | 7 | 7 |
| Maxillary teeth | 8 | 8 | 8 | 7–8 |
| Dentary teeth | 15 | 16–18 | 16 | 15–18 |
| Vertebrae | 34 | 31–33 | 33 | 32–33 |

and Xeberos [Amazon River Basin, Peru], Mr. Bartlett. BMNH 1869.5.21.10, 1 (Lectotype of *Chirodon alburnus*), [Amazon River, Peru]. BMNH 1869.5.21.11-13, 3 (Paralectotypes of *Chirodon alburnus*), [Amazon River, Peru].

**Diagnosis.** *Aphyocharax pusillus* differs from all of its congeners, except *A. avary*, by having black or dark brown middle caudal-fin rays (Figs 1–3; Günther 1869, fig. 2). *Aphyocharax pusillus* is distinguished from *A. avary* by having fewer maxillary teeth, spread along the proximal half of the bone (Fig. 4B–G) [vs. more maxillary teeth spread along 2/3 of the maxillary extension (Fig. 4A].

**Morphological notes.** Meristic data of the type specimens are presented in Table 1. Body shape generally fusiform, slightly elongate, greatest body depth slightly anterior to dorsal-fin base. Dorsal body profile straight or slightly convex from snout to vertical through anterior nostrils; straight or slightly convex from posterior nostrils to tip of supraoccipital bone; straight or slightly convex from this point to dorsal-fin origin; slightly convex along dorsal-fin base; postdorsal profile straight from base of last dorsal-fin ray to adipose-fin origin; slightly concave from adipose-fin to end of caudal peduncle. Ventral profile convex from snout to pelvic-fin insertion; straight or

**Figure 3.** Recently preserved specimens of *Aphyocharax pusillus*: ANSP 178013, 4, 33.1–54.8 mm SL, Peru, Loreto, Rio Napo (Amazon river basin), right bank just upstream from mouth of Mazan river, near town of Mazan (3°29'10"S, 73°6'24"W). Photographed by Mark Sabaj Perez.

slightly convex from this point to anal-fin origin; straight and posterodorsally-aligned along anal-fin base; postventral profile slightly concave from base of last anal-fin ray to end of caudal peduncle. Snout rounded. Mouth terminal, Lower jaw protrudes slightly beyond upper jaw when mouth closed; aligned approximately to middle of eye. Long and truncated snout, with its length larger than orbital diameter. Mouth terminal; upper jaw slightly larger than lower one. Maxillary bone surpassing a vertical line through middle of the eye; maxillary teeth small and conical, spread along proximal half of the bone. Lateral line interrupted; last scale on caudal-fin base.

There no mention of the presence of a humeral spot in the original description of either *A. pusillus* or *C. alburnus*. Additionally, the type materials of both species do not show any humeral spot. The illustration of *C. alburnus* by Günther (1869, fig. 2), however, gives the impression of the possible presence of a very inconspicuous humeral spot. However, the examination of recently preserved specimens of *A. pusillus* evidences that a very inconspicuous humeral spot could be present on larger recently preserved specimens (Fig. 3). Thus, we conclude that: it does not exhibit a humeral spot on smaller specimens or on specimens preserved several years ago; or it could possess a very inconspicuous humeral spot on larger and recently preserved specimens (Figs 1–3; Günther 1869, fig. 2). Nominal taxa have black or dark brown middle caudal-fin rays (Figs 1–3; Günther 1869, fig. 2).

**Remarks.** *Aphyocharax pusillus* is the type species of the genus, designated by monotypy. The specimens were collected by Mr. Bartlett in Xeberos and Huallaga (Amazon River Basin, Peru), and succinctly described by Günther (1868); A. Günther did not, however, state the number of specimens used for the description. Eschmeyer et al. (2018) cite two lots as syntypes (BMNH 1867.6.13.46, 1 syntype, and BMNH 1867.6.13.58-59, 2 syntypes), and this material was examined in the present work.

*Chirodon alburnus* was succinctly described by Günther (1869) as belonging to the genus *Chirodon* Günther, 1864 because he overlooked the presence of maxillary teeth on the type specimens of *C. alburnus*. According to Maclaine (per. com.), Rosana Souza-Lima designated the specimens BMNH 1869.5.21.10 and BMNH 1869.5.21.11-13 as the lectotype and paralectotypes, respectively, of *Chirodon alburnus* in 2003, but that designation was never published. We concur, and so formally designate BMNH 1869.5.21.10 and BMNH

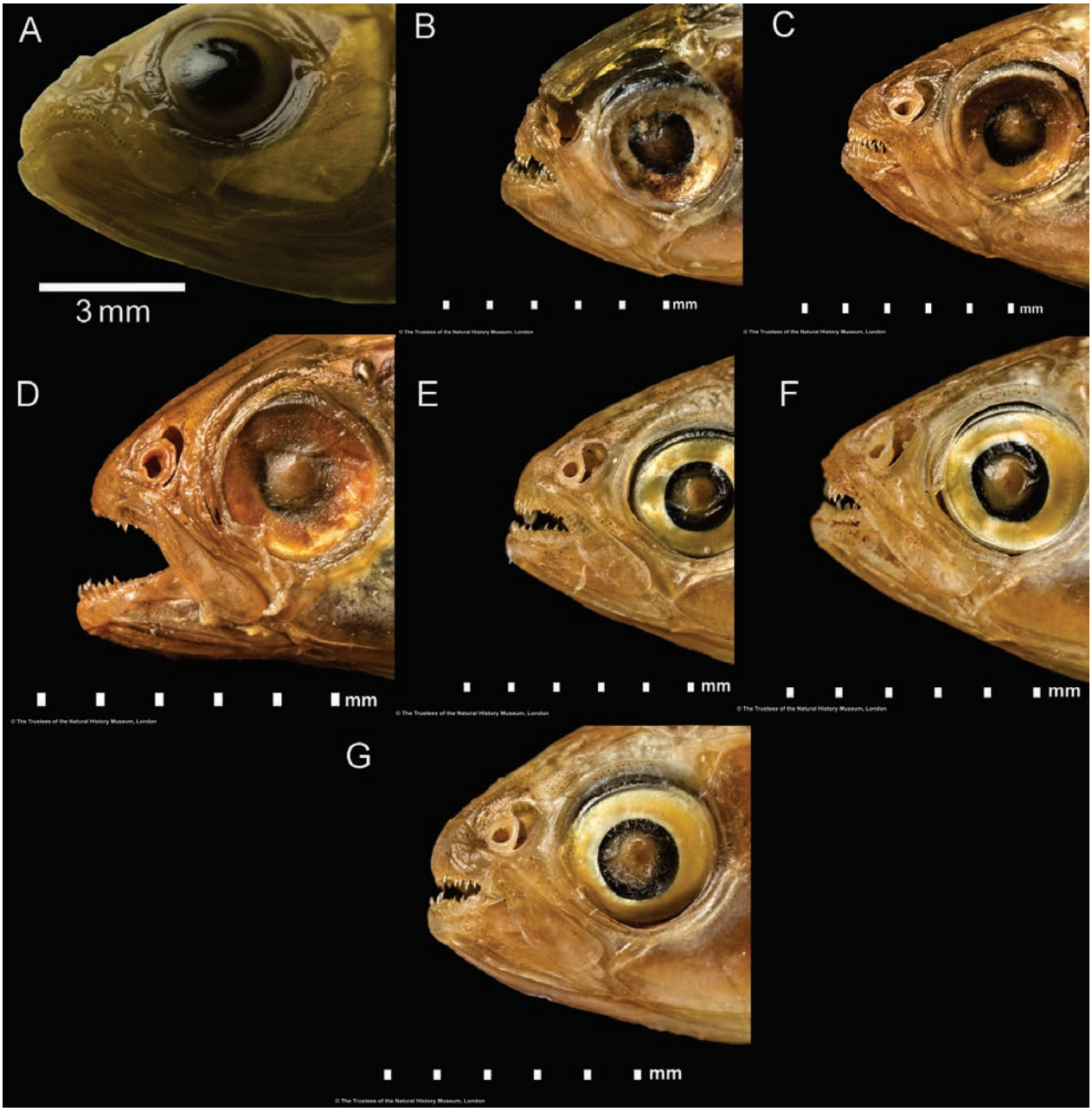


**Figure 4.** Maxillary teeth of: **A,** Holotype of *Aphyocharax avary*: ANSP 39217; **B,** Syntype of *Aphyocharax pusillus*: BMNH1867.6.12.46; **C, D;** Syntypes of *Aphyocharax pusillus*: BMNH 1867.6.13.58-59; **E,** Lectotype of *Chirodon alburnus*: BMNH 1869.5.21.10; **F, G,** two of the three paralectotypes of *Chirodon alburnus* (head damaged in a third): BMNH 1869.5.21.11-13. **A,** Photographed by Axel Katz; and **B-G,** Photographed by Kevin Webb.

1869.5.21.11-13 as the lectotype and paralectotypes, respectively, of *Chirodon alburnus*.

Based on the information presented here (type material examination, original descriptions, and the literature) we conclude that *Chirodon alburnus* is a junior synonym of *Aphyocharax pusillus* – as there are no clear diagnostic character states that distinguish those two species. Additionally, the type locality of *Chirodon alburnus* is imprecise ("Peruvian Amazon"), encompassing the type locality of *Aphyocharax pusillus*.

### *Aphyocharax avary* Fowler, 1913

*Aphyocharax avary* Fowler, 1913:532. Type locality: Madeira River, about 200 miles east of Long. 62°20'W., Brazil. Holotype: ANSP 39217.

**Material examined.** ANSP 39217, 41.7, 1 (Holotype), Madeira River, about 200 miles east of 62°20'W, Brazil, Sept 1912, Edgar A. Smith.

**Diagnosis.** *Aphyocharax avary* differs from all of its congeners, except *A. pusillus*, by having black or dark brown

**Figure 5.** Holotype of *Aphyocharax avary*: ANSP 39217, 41.7 mm SL. Photographed by Axel Katz.

**Table 2.** Meristic data of *Aphyocharax avary.*

| Measurements | ANSP 39217 |
|---|---|
| Pored lateral-line sclaes | 13+1 |
| Longitudinal scales | 38 |
| Transverse scales | 11 |
| Dorsal-fin rays | 11 or 12 |
| Pectoral fin-rays | 13 |
| Pelvic-fin rays | 7 |
| Anal-fin rays | 19 |
| Premaxillary teeth | 6 or 7 |
| Maxillary teeth | 14 |
| Dentary teeth | 13 or 14 |
| Vertebrae | 34 |

middle caudal-fin rays (Fig. 5; Fowler, 1913, fig. 8). It can be distinguished from *A. pusillus* by having more teeth on the maxillary, spread along 2/3 of the bone's extension (Fig. 4A) [vs. fewer teeth on the maxillary, spread along only the proximal half of the bone (Fig. 4B–G)].

**Morphological notes.** Meristic data of the holotype are presented in Table 2.

Body slightly compressed. Dorsal profile slightly convex in the snout region, somewhat flat in the anterior limit of the nasal bone to the extremity of the supraoccipital spine; convex from that point to the dorsal-fin origin; straight through dorsal-fin base; slightly convex after dorsal fin; slightly concave through caudal peduncle. Ventral profile slightly convex from snout to pelvic-fin insertion; straight from this point to the anal-fin origin and through that fin base; slightly concave through caudal peduncle. Snout rounded; eye large compared to the head and snout length; mouth terminal; lower maxilla slightly shorter than upper one. Lateral line interrupted; last scale on caudal-fin base.

Humeral spot conspicuous and middle caudal-fin rays black or dark brown (Fig. 5; Fowler 1913, fig. 8).

**Remarks.** *Aphyocharax avary* was described by Fowler (1913) based on a single specimen collected by Edgar A. Smith in September 1912 in the Madeira River, approximately 200 miles East of 62°20'W. This collection site is located between the municipalities of Novo Aripuanã-AM and Borba-AM, in the lower Madeira River Basin. The number of maxillary teeth was cited as 4 in the original inscription, although our examination of the holotype counted 14.

Based on the information presented here, we conclude that *A. avary* is a valid species, distinguishable from *A. pusillus* mainly by the number of teeth on the maxillary and their different distributions along that bone (14 maxillary teeth spread along 2/3 of the bone extension vs. 7–8 maxillary teeth spread along the proximal half of the bone) (Fig. 4).

### Comparative material

***Aphyocharax anisitsi***: CICCAA 00867, 14, 25.1–27.9 mm SL, Brazil, Mato Grosso do Sul state, Rio Verde municipality. CICCAA 01267, 6 C&S, 22-26.9 mm SL, Brazil, Mato Grosso do Sul state, Rio Verde municipality. CAS 59697, 1, 41.0 mm SL, Paraguay, Asuncion municipality (Radiograph and photograph of Holotype. ***Aphyocharax dentatus***: ANSP 128718, 21, 25.4–34.9 mm SL, Colombia, Lake Mozambique; UFRJ 5571, 2, 23.3–26.0 mm SL, Brazil, Mato Grosso state, Poconé municipality. CAS 59722, 1, 71.0 mm SL, Paraguay, Asuncion municipality, Laguna del Río Paraguay (Radiograph and photograph of Holotype). ***Aphyocharax erythrurus* Eigenmann, 1912**: FMNH 53406, 1, Guyana: Rockstone sandbank (Photograph of paratype). ***Aphyocharax nattereri***: UFRJ 5783, 2, Brazil, Mato Grosso State, Poconé municipality. ***Aphyocharax pusillus***: ANSP 178013, 4, 33.1–54.8 mm SL (photographs of recently preserved specimens), Peru, Loreto, Rio Napo (Amazon river basin), right bank just upstream from mouth of Mazan river, near town of Mazan (3°29'10"S, 73°6'24''W). ***Aphyocharax rathbuni***: CAS 76467, 1, 26.0 mm SL, Paraguay, Arroyo Chagalalina, Paraguay basin. Paraguay (Radiograph and photograph of a Holotype). ***Aphyocharax yekwanae***: FMNH 109278, 1, Venezuela, Bolivarian Republic of (Radiograph of paratype). ***Aphyocharax* sp.**: CICCAA 00865, 11, 29.9–36.2 mm SL, Brazil, Mato Grosso State, Pontes e Lacerda municipality. CICCAA 2330, 4 C&S, 27.3–32.4, Brazil, Mato Grosso State, Pontes e Lacerda municipality.

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## References

Eigenmann CH (1915) The Cheirodontinae, a subfamily of minute characid fishes of South America. Memoirs of the Carnegie Museum 7(1): 1–99.

Eschmeyer WN, Fricke R, van der Laan R [Eds] (2018) Catalog of fishes: genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [accessed on 10/06/2018]

Fink W, Weitzman S (1974) The so called Cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). Smithsonian Contributions to Zoology 172: 1–46. https://doi.org/10.5479/si.00810282.172

Fowler HW (1913) Fishes from the Madeira River, Brazil. Proceedings of the Academy of Natural Sciences of Philadelphia 65: 517–579.

Froese R, Pauly D [Eds] (2018) FishBase. http://www.fishbase.org [accessed on 10/06/2018]

Géry J (1977) Characoids of the world – TFH-publications, Neptune City, 672 pp.

Günther A (1868) Diagnoses of some new freshwater fishes from Surinam and Brazil, in the collection of the British Museum. Annals and Magazine of Natural History 1(6): 475–481. https://doi.org/10.1080/00222936808695733

Günther A (1869) Descriptions of some species of fishes from the Peruvian Amazons. Proceedings of the Zoological Society of London 2: 423–429. https://doi.org/10.1111/j.1469-7998.1869.tb07347.x

Lima FCT, Pires THS, Ohara WM, Jerep FC, Carvalho FR, Marinho MMF, Zuanon J (2013) Characidae. In: Queiroz LJ, Torrente-Vilara G, Ohara WM, Pires THS, Zuanon J, Dória CRC (Eds) Peixes do rio Madeira (1 edn). Dialeto Latin American Documentary, São Paulo, 213–395.

Ohara WM, Lima FCT, Salvador GN, Andrade MC (2017) Peixes do rio teles pires: diversidade e guia de Identificação (1 edn). Gráfica Amazonas e Editora Ltda, Goiás, 408.

Souza-Lima R (2003) The subfamily Aphyocharacinae. In: Reis RE, Kullander SE, CJ Ferraris (Eds) Check List of the Freshwater Fishes of South and Central America. Porto Alegre, EDIPUCRS, 197–199.

Souza-Lima R (2007) Família Characidae: Aphyocharacinae. In: Buckup PA, Menezes NA, Ghazzi MS (Eds) Catálogo das espécies de peixes de água doce do Brasil. Rio de Janeiro, Museu Nacional, 32–33.

Tagliacollo VA, Souza-Lima R, Benine RC, Oliveira C (2012) Molecular phylogeny of Aphyocharacinae (Characiformes, Characidae) with morphological diagnoses for the subfamily and recognized genera. Molecular Phylogenetics and Evolution 64: 297–307. https://doi.org/10.1016/j.ympev.2012.04.007

Taphorn DC, Thomerson JE (1991) Un characido nuevo, *Aphyocharax colifax*, de las cuencas de los rios Caroni y Caura en Venezuela. Revista Unellez de Ciencia y Tecnologia 4(1–2): 113–115.

Taylor W, Van Dyke G (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–119.

Weitzman SH (1962) The osteology of *Brycon meeki*, a generalized characid fish, with an osteological definition of the family. Stanford Ichthyological Bulletin 8: 3–77.

Willink PW, Chernoff B, Machado-Allison A, Provenzano F, Petry P (2003) *Aphyocharax yekwanae*, a new species of bloodfin tetra (Teleostei: Characiformes: Characidae) from the Guyana Shield of Venezuela. Ichthyological Exploration of Freshwaters 14(1): 1–8.

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# Deciphering conserved identical sequences of mature miRNAs among six members of great apes

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## Abstract

MicroRNAs (miRNAs) are a group of small RNA molecules which act as negative regulators of gene expression by controlling post-transcriptional regulation through binding to their corresponding mRNAs. Due to their small size, their nucleotide compositions are expected to be similar, but until now, the extent of similarity has not been reported in humans and their six phylogenetically closely related members of hominids. The present study allows direct comparison among six members of hominid species (*Homo sapiens*, *Gorilla gorilla*, *Pan paniscus*, *Pongo pygmaeus*, *Pan troglodytes* and *Symphalangus syndactylus*) in terms of their miRNA repertoire, their evolutionary distance to human, as well as, the categorization of identical species-specific miRNAs. For this purpose, a total of 2694, 370, 157, 673, 590 and 10 mature miRNA sequences of *Homo sapiens*, *Gorilla gorilla*, *Pan paniscus*, *Pongo pygmaeus*, *Pan troglodytes* and *Symphalangus syndactylus* respectively were retrieved from miRbase 22. A total of 12, 4, 4 and 3 conserved clusters with identical miRNA sequences that belong to the same gene families were found in *Homo sapiens*, *Gorilla gorilla*, *Pongo pygmaeus*, *Pan troglodytes* respectively by neighbor-joining method using MEGA7 software. Interestingly, cross-species comparison has also shown a set of conserved identical miRNA sequences. Homologs of human mature miRNAs with 100% sequence identity are expected to have similar functions in the studied primates. Further *in-vitro* study is required to investigate common targets for identical miRNAs in the studied primates.

## Introduction

MiRNAs are small (19–23nt) RNA molecules that regulate messenger RNA through binding to their 3'-UTR, mediated by the RNA induced silencing (RISC) complex in all living organisms (Zhang et al. 2018). Binding of miRNA to their corresponding target mRNA leads to translational repression and/ or mRNA degradation (Unterbruner et al. 2018). To date, considerable number of mature miRNAs have been identified in *Homo sapiens*, *Gorilla gorilla*, *Pan paniscus*, *Pongo pygmaeus* and *Pan troglodytes*, *Symphalangus syndactylus* (no=2694, 370, 157, 673, 590, 10) respectively, as shown in miRbase database (http://www.mirbase.org/) (Griffiths-Jones et al. 2006; Kozomara and Griffiths-Jones 2013).

It is already known that multiple miRNAs are produced from the same primary transcript and majority of miRNA clusters are transcribed as a single unit (Marco et al. 2013). The evolutionary importance of miRNA clusters has been the subject of much speculation (Wang et al. 2016). Many clusters contain members of the same family, suggesting an important role of gene duplication in their evolution (Berezikov 2011). On the other hand, some miRNA clusters also contain members of different miRNA families, particularly in animal kingdom (McCreight et al. 2017). Like other gene families, miRNAs are also prone to forming paralogs, with the result that many miRNAs appear as members of families as homologs (Hertel et al. 2006). However, the origin and evolution of these miRNA clusters has not been investigated in detail (Altuvia et al. 2005; Tanzer and Stadler 2004). Phylogenetic studies have shown that miRNAs are present throughout the evolution of metazoans. Comparison of pre-miRNA sequences demonstrate that they are less conserved and

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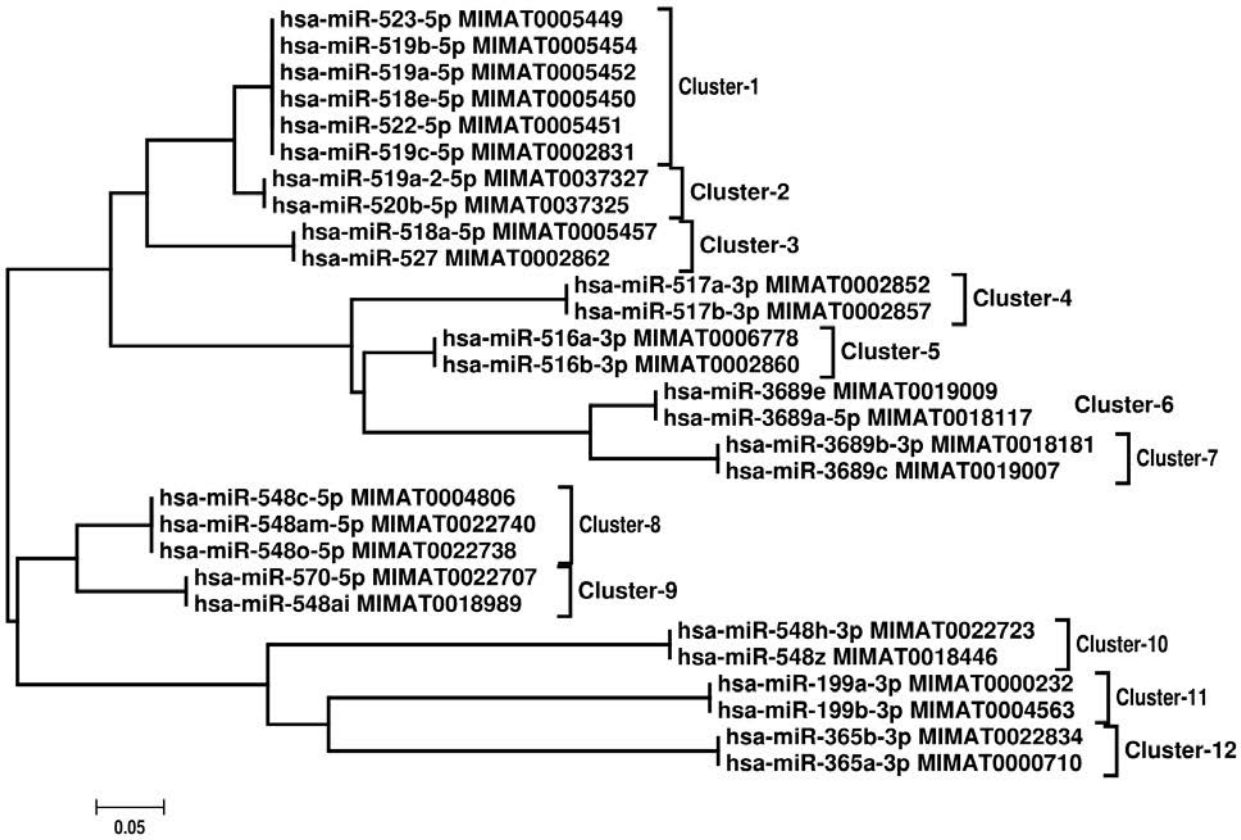


**Figure 1.** Shows evolutionary relationships of taxa for all mature miRNAs in *Homo sapiens*. The analysis involved 29 nucleotide sequences.

therefore are more prone to phylogenetically preserved than the mature sequences alone. High degree of identity across different species was observed for mature miRNAs (Li et al. 2010). It is also noted that many matured miRNAs are prevailing in several species and are highly conserved and are confined to specific lineages. There are several polycistronic transcripts that suggest a potential mode of evolution for polycistronic miRNAs (Truscott et al. 2016). It is already known that the miRNA repertoire has continuously increased during evolution of metazoan. However, the advent ratio of these molecules is diverse over evolutionary time (Bartel 2018). The expansions of miRNA have been linked with evolutionary innovations that lead to the diversification of bilaterians. Till now, identification of orthologous miRNAs in different species has been investigated in primates.

Among the six members of great apes, *Homo sapiens* are the deepest explored group with 2694 mature miRNAs miRNAs described. In the present study, we took advantage of a recently available set of mature miRNA from six members of the great ape population to systematically detect identical miRNA by comparing patterns of intra- and inter-species sequence similarity and their evolutionary distance. Interestingly, it was found that intra- and inter-species sequence set of identical mature miRNA exists in great apes including humans. Further *in-vitro* study is required to investigate common targets for identical miRNAs in the studied primates.

# Materials and methods

### Alignment of sequences and phylogenetic analysis

For miRNA, a very limited open and free data is available. The miRBase is one of the highly referred databases, easily accessible and in its latest release 10883 pre-miRNAs are available. Dataset of mature miRNAs sequences of *Homo sapiens* (no=2694), *Gorilla gorilla* (no=370), *Pan paniscus* (no=157), *Pongo pygmaeus* (no=673 mature), *Pan troglodytes* (no=590 mature) and *Symphalangus syndactylus* (no=10 mature) were retrieved from miRBase sequence database (a data repository of published miRNA sequences and its annotation) (release 22.0) at http://microrna.sanger.ac.uk. ClustalW was used to generate multiple alignments of nucleic acid sequences (Chenna et al. 2003) and MEGA7 was used to generate phylogenetic analyses using Neighbor-Joining method (Kumar et al. 2016).

# Results

### Identification of Homologous mature miRNA sequences in intra-species in hominides

Homologous sequences in *Homo sapiens* were clustered based on their phylogenetic relationship and sequence identity using ClustalW. Multiple alignment of mature miRNAs revealed a conserved consensus. Neighbor-Join-

**Table 1.** List of miRNAs grouped into clusters, their genomic coordinates, gene family names and their matured miRNA sequences in *Homo sapiens*.

| S. No | Members | Gene Family Name | Genomic coordinate | Mature miRNA sequence |
|---|---|---|---|---|
| **Cluster 1** | hsa-miR-523-5p | MIPF0000020; mir-515 | chr19: 53698385-53698471 [+] | CUCUAGAGGGAAGCGCUUUCUG |
| | hsa-miR-519b-5p | | chr19: 53695213-53695293 [+] | |
| | hsa-miR-519a-5p | | chr19: 53752397-53752481 [+] | |
| | hsa-miR-518e-5p | | chr19: 53729838-53729925 [+] | |
| | hsa-miR-522-5p | | chr19: 53751211-53751297 [+] | |
| | hsa-miR-519c-5p | | chr19: 53686469-53686555 [+] | |
| **Cluster 2** | hsa-miR-519a-2-5p | | chr19: 53762344-53762430 [+] | CUGCAAAGGGAAGCCCUUUC |
| | hsa-miR-519b-2-5p | | chr19: 53754018-53754102 [+] | |
| **Cluster 3** | hsa-miR-518a-5p | | chr19: 53731006-53731090 [+] | |
| | hsa-miR-527 | | chr19: 53754018-53754102 [+] | |
| **Cluster 4** | hsa-miR-517a-3p | | chr19: 53712268-53712354 [+] | AUCGUGCAUCCCUUUAGAGUGU |
| | hsa-miR-517b-3p | | chr19: 53721076-53721142 [+] | |
| **Cluster 5** | hsa-miR-516a-3p | | chr19: 53756741-53756830 [+] | UGCUUCCUUUCAGAGGGU |
| | hsa-miR-516b-3p | | chr19: 53736845-53736934 [+] | |
| **Cluster 6** | hsa-miR-3689e | MIPF0001144; mir-3689 | chr9: 134850570-134850641 [-] | UGUGAUAUCAUGGUUCCUGGGA |
| | hsa-miR-3689a-5p | | chr9: 134849487-134849564 [-] | |
| **Cluster 7** | hsa-miR-3689b-3p | | chr9: 134850125-134850272 [-] | CUGGGAGGUGUGAUAUUGUGGU |
| | hsa-miR-3689c | | chr9: 134849298-134849369 [-] | |
| **Cluster 8** | hsa-miR-548c-5p | MIPF0000317; mir-548 | chr12: 64622509-64622605 [+] | AAAAGUAAUUGCGGUUUUUGCC |
| | hsa-miR-548am-5p | | chrX: 16627012-16627085 [-] | |
| | hsa-miR-570-5p | | chr3: 195699401-195699497 [+] | AAAGGUAAUUGCAGUUUUUCCC |
| **Cluster 9** | hsa-miR-548ai | | chr6: 99124609-99124696 [+] | |
| **Cluster 10** | hsa-miR-548h-3p | | chr8: 27048853-27048963 [-] | CAAAAACCGCAAUUACUUUUGCA |
| | hsa-miR-548z | | chr12: 64622509-64622605 [-] | |
| **Cluster 11** | hsa-miR-199a-3p | MIPF0000040; mir-199 | chr19: 10817426-10817496 [-] | ACAGUAGUCUGCACAUUGGUUA |
| | hsa-miR-199b-3p | | chr9: 128244721-128244830 [-] | |
| **Cluster 12** | hsa-miR-365b-3p | MIPF0000061; mir-365 | chr17: 31575411-31575521 [+] | UAAUGCCCCUAAAAAUCCUUAU |
| | hsa-miR-365a-3p | | chr16: 14309285-14309371 [+] | |

ing method was used for inferring the evolutionary history. The optimal tree with the sum of branch length = 2.32453654 is shown in Figure 1. The p-distance method was used for computing the evolutionary distances. The analysis involved 29 nucleotide sequences. All uncertain positions were deleted for each sequence pair. In the final dataset, there was a total of 36 positions. Evolutionary analyses were conducted in MEGA7. The conserved miRNA sequences were grouped into 12 clusters. Number of miRNA members in each cluster, showing 100% identity in their sequences and their corresponding genomic coordinates, are shown in Table 1. It is interesting to note that the genomic location of all the identical miRNAs are in clustered form. This indicates that the genes for these identical miRNAs are originated through gene duplication during evolution and speciation. Identical miRNA sequences were also noted in *Gorilla gorilla* using the same phylogenetic model. The optimal tree with the sum of branch length = 0.40606061 is shown in Figure 2. The analysis involved 12 nucleotide sequences. There were a total of 35 positions in the final dataset. However, only 12 miRNAs were found to be 100% identical. These 12 miRNAs are grouped into four clusters. Number of miRNA members in each cluster showing 100% identity in their sequences and their corresponding genomic coordinates are shown in Table 2. Interestingly, all the identified conserved miRNAs belong to a single gene family (MIPF0000020; mir-515). Similarly, identical miRNA sequences were also noted in *Pongo pygmaeus* using the same phylogenetic model as shown in Figure 3. The total number of identical miRNAs were 8 that were grouped into 4 clusters as shown in Table 3. The optimal tree with the sum of branch length = 1.37798461 is shown in Figure 3. The analysis involved 8 nucleotide sequences. There were a total of 32 positions in the final dataset. Likewise, identical miRNA sequences were also noted in *Pan troglodytes* using the same phylogenetic model. Seven conserved miRNAs were also found in *Pan troglodytes* that were grouped into three clusters as shown in Table 4. The optimal tree with the sum of branch length = 0.71717172 is shown in Figure 4. The analysis involved 7 nucleotide sequences. There were a total of 26 positions in the final dataset.

### Identification of Homologous mature miRNA Sequences in inter-species in Hominides

To assess whether any cross-species conserved miRNA in hominids exists, all known matured miRNAs were aligned to generate multiple alignments of nucleic acid sequences using ClustalW, and MEGA7 was used to generate phylogenetic analyses. The optimal tree with the sum of branch length = 3.16412289 is shown in Figure 4. There were a total of 31 positions in the final dataset.

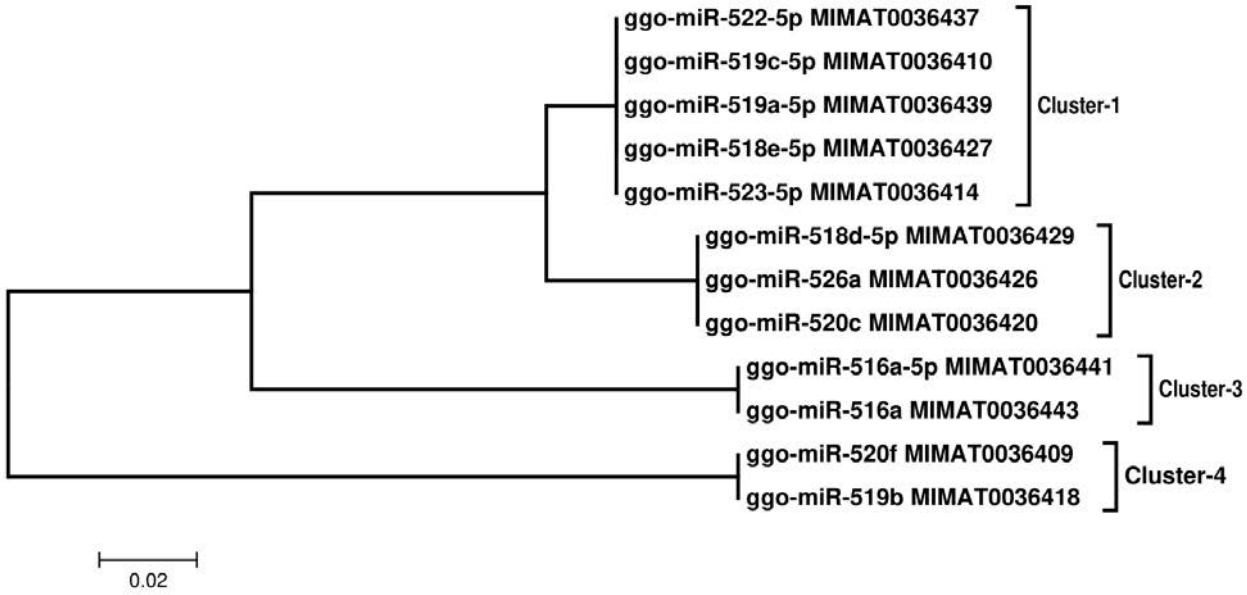


**Figure 2.** Represents evolutionary relationships of taxa for *Gorilla gorilla*. The optimal tree with the sum of branch length = 0.40606061 is shown. The analysis involved 12 nucleotide sequences.

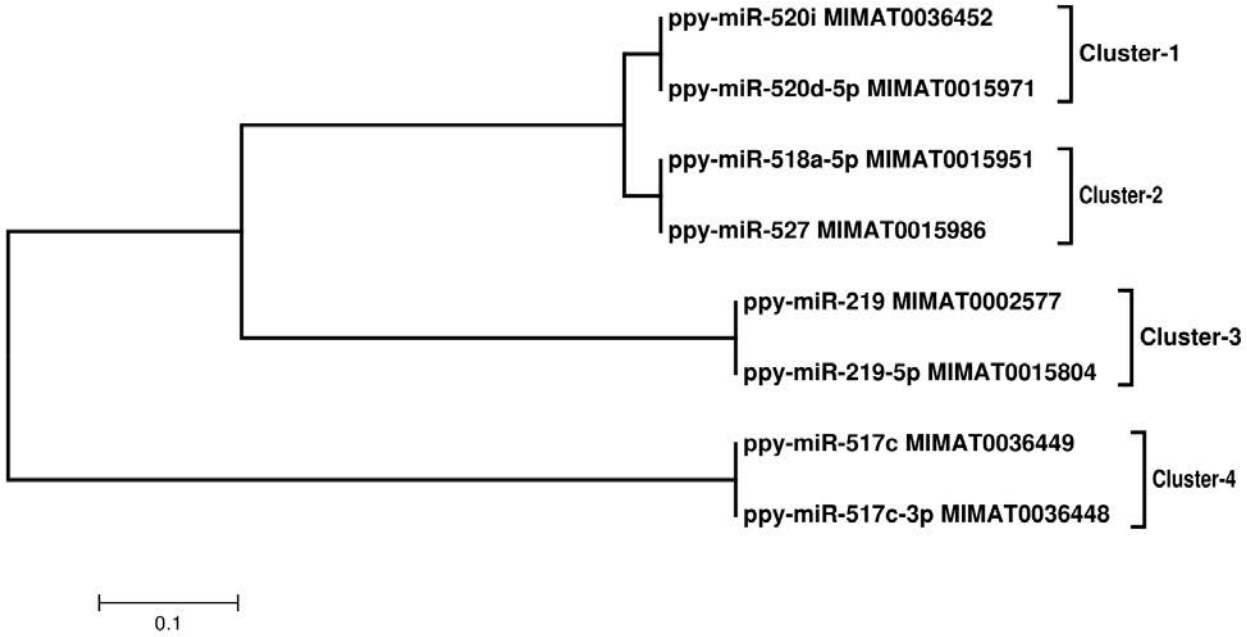


**Figure 3.** Illustrate evolutionary relationships of taxa for *Pongo pygmaeus*. The analysis involved 8 nucleotide sequences.

Using online tool jvenn (Bardou et al. 2014), it was interesting to note that one miRNA (CUCUAGAGGGAAGCGCUUUCUG) was conserved and overlapping in *Homo sapiens* and *Gorilla gorilla*. Similarly, another conserved sequence (CUGCAAAGGGAAGCCCUUUC) was overlapping in all the three members of hominde species (*Homo sapiens*, *Pan troglodytes* and *Pongo pygmaeus*) as shown in Figures 5, 6. It was also noted that one miRNA (ACAGUAGUCUGCACAUUGGUUA) was overlapping in *Homo sapiens* and *Pan troglodytes*.

# Discussion

MiRNA-mediated gene regulation is novel mechanism among all lineages in animal kingdom (Zhang et al. 2004). Due to their smaller size, many known miRNA genes in animal genomes are found as clusters. MiRNA clusters are a group of related miRNAs closely localized in the genome with an evolution that remains poorly understood (Chen et al. 2015). Therefore, most of the clusters are transcribed as a single polycistronic transcripts (Lagos-Quintana et

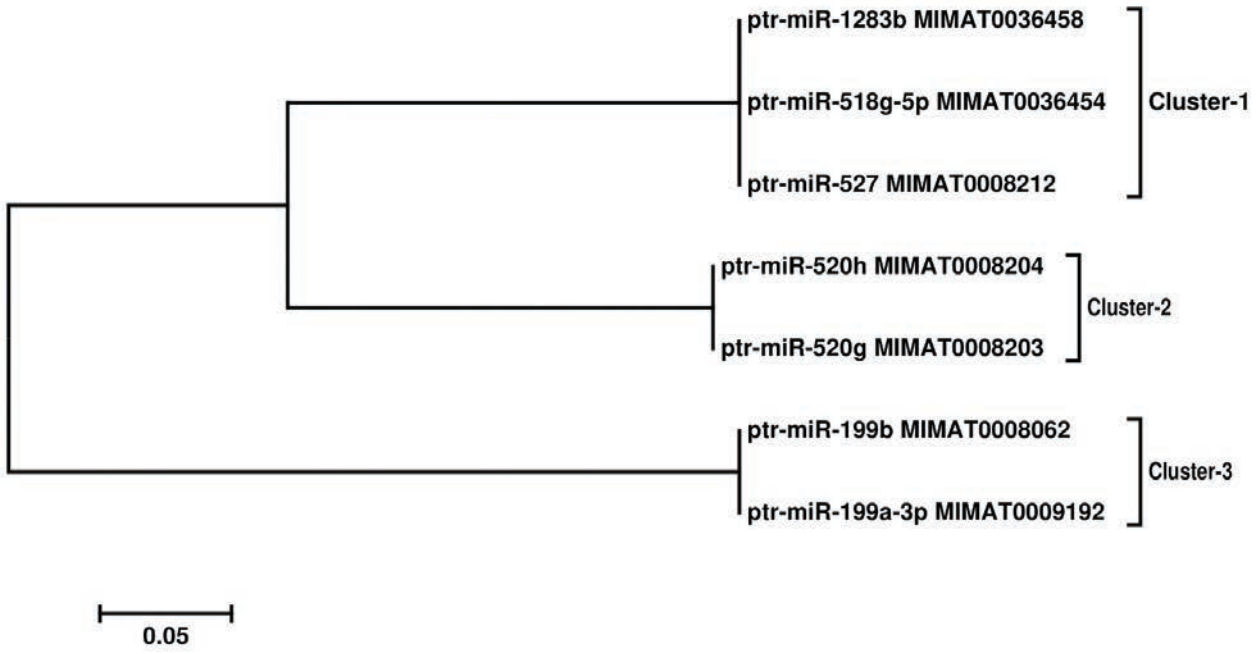


**Figure 4.** Represents evolutionary relationships of taxa for *Pan troglodytes*. The optimal tree with the sum of branch length = 0.71717172 is shown. The analysis involved 7 nucleotide sequences.

**Table 2.** List of miRNAs grouped into clusters, their genomic coordinates, gene family names and their matured miRNA sequences in *Gorilla gorilla*.

| S. No | No. of members | Gene family | Genomic coordinate | Mature miRNA sequence |
|---|---|---|---|---|
| Cluster 1 | ggo-miR-522-5p | MIPF0000020; mir-515 | chr19: 53095952-53096058 [+] | CUCUAGAGGGAAGCGCUUUCUG |
| | ggo-miR-519c-5p | | chr19: 53038851-53038969 [+] | |
| | ggo-miR-519a-5p | | chr19: 53097153-53097260 [+] | |
| | ggo-miR-518e-5p | | chr19: 53073319-53073416 [+] | |
| | ggo-miR-523-5p | | chr19: 53042042-53042160 [+] | |
| Cluster 2 | ggo-miR-518d-5p | | chr19: 53078317-53078435 [+] | |
| | ggo-miR-526a | | chr19: 53069990-53070073 [+] | |
| | ggo-miR-520c | | chr19: 53049906-53050015 [+] | |
| Cluster 3 | ggo-miR-516a-5p | | chr19: 53101171-53101280 [+] | UUCUCGAGGAAAGAAGCACUUUC |
| | ggo-miR-516a | | chr19: 53105582-53105691 [+] | |
| Cluster 4 | ggo-miR-520f | | chr19: 53025391-53025489 [+] | AAGUGCUUCCUUUUAGAGGGUU |
| | ggo-miR-519b | | chr19: 53044871-53044969 [+] | |

**Table 3.** List of miRNAs grouped into clusters, their genomic coordinates, gene family names and their matured miRNA sequences in *Pongo pygmaeus*.

| S. No | No. of members | Gene Family | Genomic coordinate | Mature miRNA sequence |
|---|---|---|---|---|
| Cluster 1 | ppy-miR-520i | MIPF0000020; mir-515 | chr19: 55513927-55514025 [+] | CUACAAAGGGAAGCCCUUUC |
| | ppy-miR-520d-5p | | chr19: 55491990-55492076 [+] | |
| Cluster 2 | ppy-miR-518a-5p | | chr19: 55505640-55505726 [+] | CUGCAAAGGGAAGCCCUUUC |
| | ppy-miR-527 | | chr19: 55533164-55533250 [+] | |
| Cluster 3 | ppy-miR-219 | MIPF0000044; mir-219 | NW_002874576.1: 1044940-1045049 [+] | UGAUUGUCCAAACGCAAUUCU |
| | ppy-miR-219-5p | | chr9: 125361031-125361127 [-] | |
| Cluster 4 | ppy-miR-517c | MIPF0000020; mir-515 | chr19: 55485875-55485961 [+] | AUCGUGCAUCCCUUUAGAGUGU |
| | ppy-miR-517c-3p | | | |

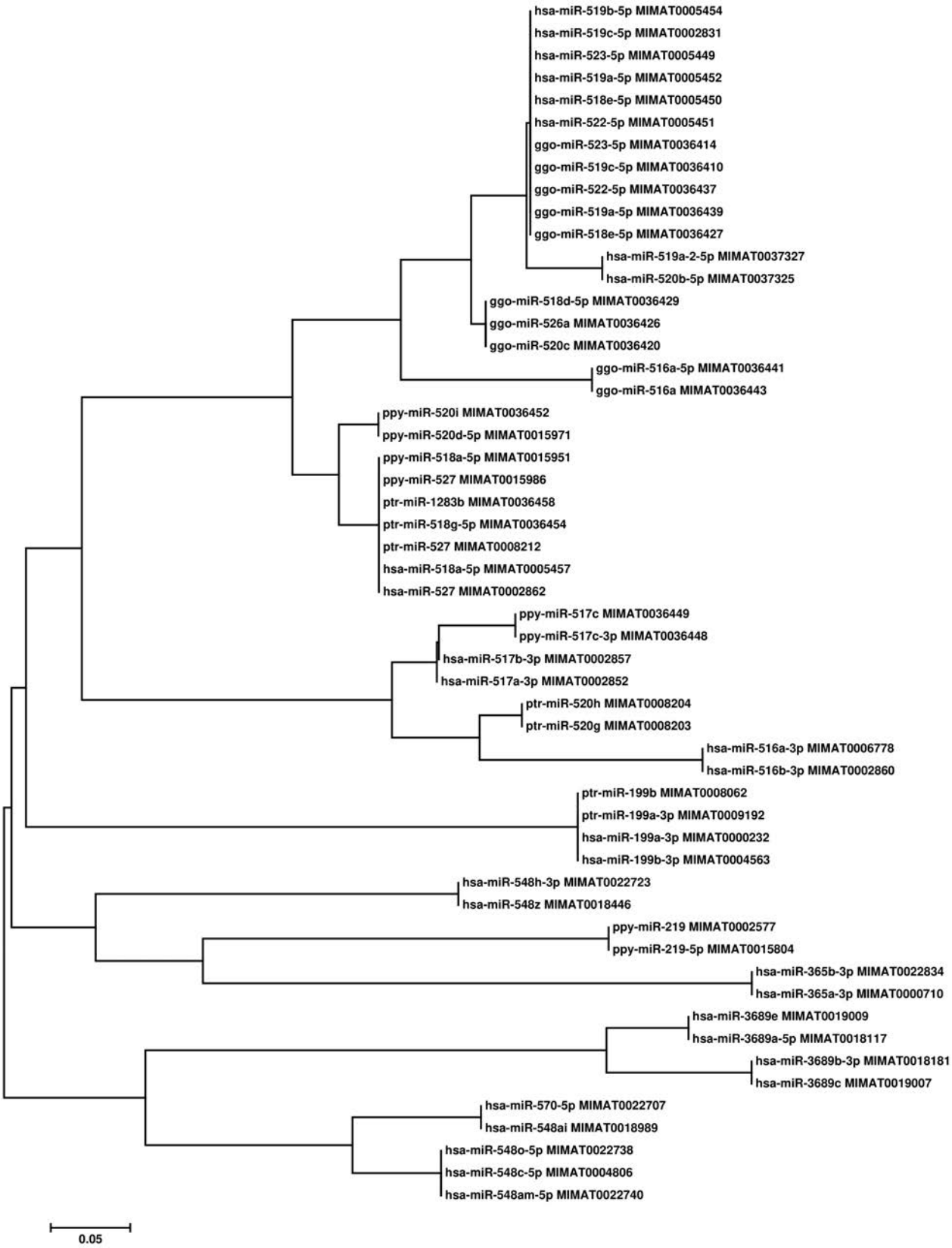


**Figure 5.** A comparative evolutionary relationships of taxa for *Homo sapiens*, *Gorilla gorilla*, *Pongo pygmaeus* and *Pan troglodytes*. The analysis involved 54 nucleotide sequences.

**Table 4.** List of miRNAs grouped into clusters, their genomic coordinates, gene family names and their matured miRNA sequences in *Pan troglodytes*.

| S. No | No. of members | Gene family | Genomic coordinate | Mature miRNA sequence |
|---|---|---|---|---|
| **Cluster 1** | ptr-miR-1283b | MIPF0000020; mir-515 | chr19: 56105638-56105756 [+] | CUGCAAAGGGAAGCCCUUUC |
| | ptr-miR-518g-5p | | chr19: 56078436-56078532 [+] | |
| | ptr-miR-527 | | chr19: 56101441-56101526 [+] | |
| **Cluster 2** | ptr-miR-520h | | chr19: 56089945-56090033 [+] | ACAAAGUGCUUCCCUUUAGAGUGU |
| | ptr-miR-520g | | chr19: 56069121-56069209 [+] | |
| **Cluster 3** | ptr-miR-199b | MIPF0000040; mir-199 | chr9: 106385739-106385847 [-] | ACAGUAGUCUGCACAUUGGUUA |
| | ptr-miR-199a-3p | | chr19: 11396978-11397047 [-] | |

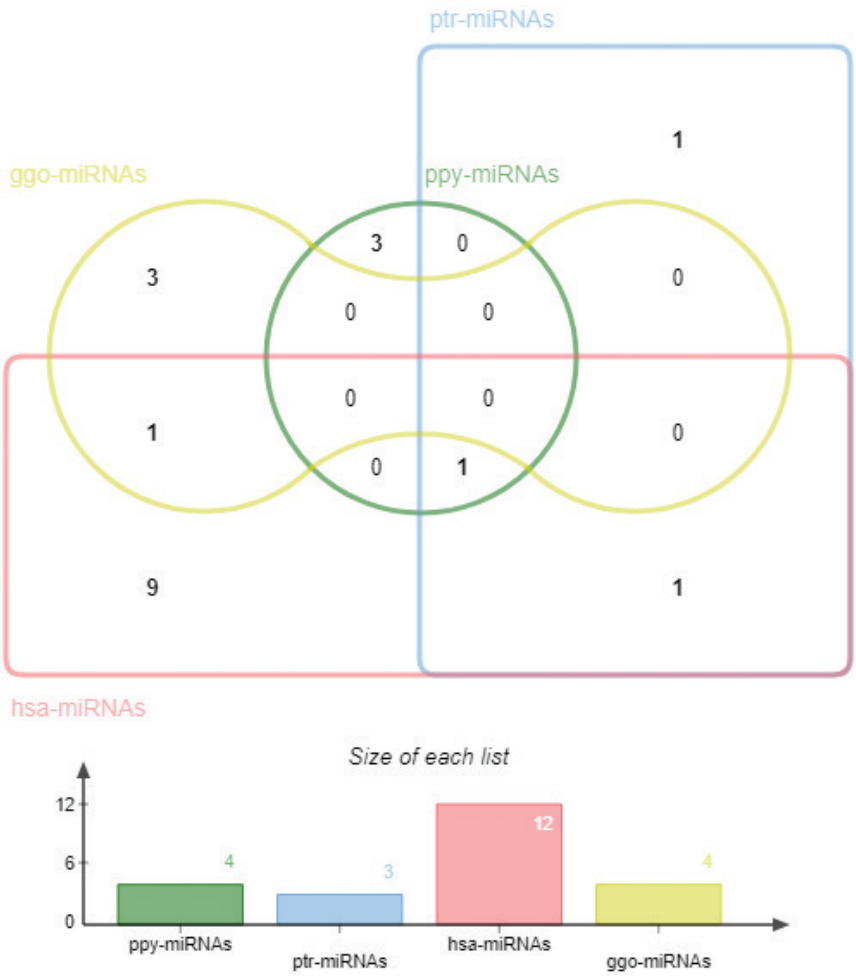


**Figure 6.** Venn diagram representing number of overlapping miRNA in *Homo sapiens*, *Gorilla gorilla*, *Pongo pygmaeus* and *Pan troglodytes*.

al. 2003; Mourelatos et al. 2002). It was found that these clusters are highly conserved in most mammals. Insertions of new miRNAs, deletions of individual miRNAs, and a cluster duplication observed in different species suggest an actively evolving cluster. In the present study, intra-species and inter-species conserved identical miRNAs were identified in the six species of hominoids. Interestingly, there were few miRNAs that were conserved across all studied species, indicating their evolutionary distance to humans, as well as, the categorization of identical species-specific miRNAs were identified in six members of great apes. It was investigated that most conserved miRNA clusters in all the studied members of hominoids belong to the two families i.e., mir-515 and mir-199, suggesting that the ancestral clusters may be originated by tandem duplication. It has been demonstrated that some miRNA genes exhibited the phenomena of clustering (Kurkewich et al. 2018). Interestingly, several studies have shown that miRNA clusters comprise of two or more miRNA genes that display high level of identity in sequences. Moreover, they are situated contiguously with each other in the genome (Gonzalez-Vallinas et al. 2018). Through experimental

and computational identification, the miRBase database is one of the primary repository resources for collecting miRNA genes (Griffiths-Jones et al. 2007; Kozomara and Griffiths-Jones 2013). No futuristic data related to miRNA clusters is available in miRBase. Similarly, no further information about miRNA gene clusters was rendered to explore the evolutionary conservation between miRNA clusters across several species. The present data highlighted intra and inter phylogenetic relationship of matured miRNA in six species of great apes.

## Conclusion

In this comparative study, conserved identical miRNA sequences were found among four hominid species. The applied prediction algorithm (mentioned in the materials and method section) proves several criteria based on similarity to identified conserved sequences of miRNAs to detect both more distantly-related and closely-related homologs. Further study is required to identify potential targets for identical miRNAs in the studied primates.

## Compliance with ethical standards

**Conflict of interest:** No conflict of interest exists.

**Ethical approval:** This article does not contain any study with human participants or animals performed by any of the authors.

## References

Altuvia Y et al. (2005) Clustering and conservation patterns of human microRNAs Nucleic acids research 33: 2697–2706. https://doi.org/10.1093/nar/gki567

Bardou P, Mariette J, Escudie F, Djemiel C, Klopp C (2014) jvenn: an interactive Venn diagram viewer BMC Bioinformatics 15: 293. https://doi.org/10.1186/1471-2105-15-293

Bartel DP (2018) Metazoan MicroRNAs Cell 173:20–51. https://doi.org/10.1016/j.cell.2018.03.006

Berezikov E (2011) Evolution of microRNA diversity and regulation in animals Nature Reviews Genetics 12: 846. https://doi.org/10.1038/nrg3079

Chen L, Heikkinen L, Emily Knott K, Liang Y, Wong G (2015) Evolutionary conservation and function of the human embryonic stem cell specific miR-302/367 cluster Comp Biochem Physiol Part D Genomics Proteomics 16: 83–98. https://doi.org/10.1016/j.cbd.2015.08.002

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs Nucleic acids research 31: 3497–3500. https://doi.org/10.1093/nar/gkg500

Gonzalez-Vallinas M, et al. (2018) Epigenetically Regulated Chromosome 14q32 miRNA Cluster Induces Metastasis and Predicts Poor Prognosis in Lung Adenocarcinoma Patients Mol Cancer Res 16: 390–402. https://doi.org/10.1158/1541-7786.MCR-17-0334

Griffiths-Jones S, Grocock RJ, Van Dongen S, Bateman A, Enright AJ (2006) miRBase: microRNA sequences, targets and gene nomenclature Nucleic acids research 34: D140–D144. https://doi.org/10.1093/nar/gkj112

Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2007) miRBase: tools for microRNA genomics Nucleic acids research 36: D154–D158. https://doi.org/10.1093/nar/gkm952

Hertel J et al. (2006) The expansion of the metazoan microRNA repertoire BMC genomics 7: 25. https://doi.org/10.1186/1471-2164-7-25

Kozomara A, Griffiths-Jones S (2013) miRBase: annotating high confidence microRNAs using deep sequencing data Nucleic acids research 42: D68–D73. https://doi.org/10.1093/nar/gkt1181

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets Molecular biology and evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Kurkewich JL, Boucher A, Klopfenstein N, Baskar R, Kapur R, Dahl R (2018) The mirn23a and mirn23b microrna clusters are necessary for proper hematopoietic progenitor cell production and differentiation Exp Hematol 59: 14–29. https://doi.org/10.1016/j.exphem.2017.12.007

Lagos-Quintana M, Rauhut R, Meyer J, Borkhardt A, Tuschl T (2003) New microRNAs from mouse and human RNA 9: 175–179. https://doi.org/10.1261/rna.2146903

Li SC, Chan WC, Hu LY, Lai CH, Hsu CN, Lin WC (2010) Identification of homologous microRNAs in 56 animal genomes Genomics 96: 1–9. https://doi.org/10.1016/j.ygeno.2010.03.009

Marco A, Ninova M, Ronshaugen M, Griffiths-Jones S (2013) Clusters of microRNAs emerge by new hairpins in existing transcripts Nucleic acids research 41: 7745–7752. https://doi.org/10.1093/nar/gkt534

McCreight JC, Schneider SE, Wilburn DB, Swanson WJ (2017) Evolution of microRNA in primates. PloS ONE 12: e0176596. https://doi.org/10.1371/journal.pone.0176596

Mourelatos Z et al. (2002) miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs Genes Dev 16: 720–728. https://doi.org/10.1101/gad.974702

Tanzer A, Stadler PF (2004) Molecular evolution of a microRNA cluster Journal of molecular biology 339: 327–335. https://doi.org/10.1016/j.jmb.2004.03.065

Truscott M, Islam AB, Frolov MV (2016) Novel regulation and functional interaction of polycistronic miRNAs RNA 22: 129–138. https://doi.org/10.1261/rna.053264.115

Unterbruner K, Matthes F, Schilling J, Nalavade R, Weber S, Winter J, Krauss S (2018) MicroRNAs miR-19, miR-340, miR-374 and miR-542 regulate MID1 protein expression PLoS One 13:e0190437. https://doi.org/10.1371/journal.pone.0190437

Wang Y, Luo J, Zhang H, Lu J (2016) microRNAs in the same clusters evolve to coordinately regulate functionally related genes Molecular biology and evolution 33: 2232–2247. https://doi.org/10.1093/molbev/msw089

Wheeler BM, Heimberg AM, Moy VN, Sperling EA, Holstein TW, Heber S, Peterson KJ (2009) The deep evolution of metazoan microRNAs Evolutionary Development 11, 50–68. https://doi.org/10.1111/j.1525-142X.2008.00302.x

Zhang J, Yang J, Zhang X, Xu J, Sun Y, Zhang P (2018) MicroRNA-10b expression in breast cancer and its clinical association PLoS One 13: e0192509. https://doi.org/10.1371/journal.pone.0192509

Zhang P, Min W, Li WH (2004) Different age distribution patterns of human, nematode, and Arabidopsis duplicate genes Gene 342: 263–268. https://doi.org/10.1016/j.gene.2004.08.001

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# The smallest 'true chameleon' from Madagascar: a new, distinctly colored species of the *Calumma boettgeri* complex (Squamata, Chamaeleonidae)

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## Key Words

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Integrative taxonomy
Micro-computed tomography
Osteology
*Calumma nasutum* group

## Abstract

On a recent expedition to eastern Madagascar, we discovered a distinct new species of the genus *Calumma* that we describe here using an integrative approach combining morphology, coloration, osteology and molecular genetics. *Calumma roaloko* **sp. n.** has a dermal rostral appendage and occipital lobes, and belongs to the *C. boettgeri* complex, within the Madagascar-endemic phenetic *C. nasutum* species group. It is readily distinguished from other species of the *C. boettgeri* complex by a characteristic two-toned body coloration and small body size with a snout-vent length of 45.6 mm in an adult male. The osteology of the skull, with a prominent maxilla and broad parietal, is similar to the closest related species, *C. uetzi*. Analysis of uncorrected genetic distances within the *C. nasutum* group using the mitochondrial gene ND2 shows a minimum pairwise distance of 11.98% to *C. uetzi* from the Sorata massif and Marojejy National Park >500 km north of the type locality of *C. roaloko* **sp. n.**. Given an apparently small range (potentially <300 km$^2$), located entirely outside of any nationally-protected areas, we recommend this new species be classified as Endangered under criterion B1ab(iii) of the IUCN Red List. The discovery of clearly distinct species like *C. roaloko* **sp. n.** in an area of Madagascar that is comparatively thoroughly surveyed highlights the critical role of continued field surveys for understanding the true extent of Madagascar's spectacular biodiversity.

## Introduction

The biota of Madagascar is recognized as exceptional, both in terms of endemism and density of species (Myers et al. 2000). In recent years, revised estimates of species richness for the island have revealed a significant underestimation of animal species richness by current taxonomy, e.g., in primates (Yoder et al. 2000), anurans (Vieites et al. 2009) and squamates (Nagy et al. 2012). In addition to the recognition of many morphologically 'cryptic' species (Bickford et al. 2006), often identified by the application of integrative taxonomy (Dayrat 2005, Padial et al. 2010), biodiversity field surveys in Madagascar continue to reveal morphologically distinct and often deeply divergent species, frequently characterized by restricted ranges and/or highly secretive habits (e.g., among herpetofauna, Nussbaum and Raxworthy 1994, Glaw et al. 1998, 2006, Vieites et al. 2010, Gehring et al. 2011, Rosa et al. 2014, Scherz et al. 2015, 2017, Lambert et al. 2017).

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With currently 90 endemic species (Glaw 2015, Prötzel et al. 2017, 2018) chameleons are among the most diverse squamate families on Madagascar. The application of widespread genetic sampling and species delimitation methods (Gehring et al. 2012) confirmed long-standing suspicions that *Calumma nasutum* and other species are actually complexes of species (e.g., Hillenius 1959, Brygoo 1971, Glaw and Vences 2007). As many as 33 potential species (OTUs) were identified by Gehring et al. (2012) in the *C. nasutum* species group, but a higher taxonomic resolution awaits the completion of ongoing detailed morphological and genetic analyses (Prötzel et al. 2015, 2016, 2017, 2018).

The small-bodied chameleons of the *Calumma nasutum* group, usually characterized by their dermal rostral appendages, are distributed across the forests of eastern and northern Madagascar. Within this group, the species *C. boettgeri* (Boulenger, 1888), *C. guibei* (Hillenius, 1959) and *C. linotum* (Müller, 1924) differ from the others by the possession of well-defined occipital lobes and are referred to as the *C. boettgeri* complex (Gehring et al. 2012). Recently the number of species in the *C. boettgeri* complex has more than doubled with the description of *C. gehringi* Prötzel et al., 2017, *C. juliae* Prötzel et al., 2018, *C. lefona* Prötzel et al., 2018, and *C. uetzi* Prötzel et al., 2018 due to discoveries on recent expeditions. So far, *C. juliae* has been the only member of the *C. boettgeri* complex that occurs in eastern Madagascar; the other species are from northern Madagascar.

During fieldwork in a forest fragment within the Réserve de Ressources Naturelles du Corridor Ankeniheny-Zahamena just south of Andasibe-Mantadia National Park in 2015/2016, we discovered a small-bodied chameleon with distinct coloration belonging to the *Calumma nasutum* group. Integrating morphological, molecular, and osteological data, we describe this new species of the *C. boettgeri* complex.

## Materials and methods

### Specimen collection

We located specimens at night using targeted searches of arboreal habitats during the rainy season, using flashlights to locate sleeping individuals. Following euthanasia, we removed a portion of liver tissue and transferred it immediately into 95% ethanol for use in DNA extractions for genetic analyses. Specimens were fixed in 10% formalin (buffered to pH 7.0 with sodium phosphate), and transferred to 75% ethanol for long-term storage after approximately two weeks. We deposited the holotype and four paratypes at the University of Kansas Biodiversity Institute, Lawrence, KS (KU). Two of the paratypes will be repatriated to the Université d'Antananarivo, Mention de Zoologie et Biologie Animale (UADBA) probably during 2018 and one paratype was exchanged with the Zoologische Staatssammlung München (ZSM). All type specimens will maintain their original KU museum number so that they can more easily be referenced in the future. SML refers to field numbers of S. M. Lambert.

### Morphological investigation

Terms of morphological measurements taken on these specimens were adapted from previous studies (Prötzel et al. 2015, 2017). The following characters (Table 1) were measured with a digital caliper to the nearest 0.1 mm, counted using a binocular dissecting microscope, evaluated by eye or calculated: snout-vent length (SVL) from the snout tip (not including the rostral appendage) to the cloaca; tail length (TaL) from the cloaca to the tail tip; total length (TL) as a sum of SVL + TaL; ratio of TaL and SVL (RTaSV); length of the rostral appendage (LRA) from the upper snout tip; ratio of LRA and SVL (RRASV); diameter of rostral appendage (DRA), measured dorsoventrally at the widest point; ratio of DRA and SVL (RDRSV); number of scales across DRA (NDRA); number of tubercle scales (diameter >0.3 mm) on rostral appendage, counted on the right side (NSRA); ratio of NSRA and LRA (RNLRA); distinct rostral crest (RC) presence (+) or absence (–); lateral crest (LC), running from the posterior of the eye horizontally, presence (+) or absence (–); temporal crest, running dorsally to the LC, curving toward the midline, absence (–) or number of tubercles on left side (TCL) and right side (TCR); parietal crest (PC) presence (+) or absence (–); occipital lobes (OL) completely separated (s) or still, at least slightly, connected (c); depth of the dorsal notch in the occipital lobes (OLND); ratio of OLND and SVL (ROLSV); lateral diameter of OL (OLD); ratio of OLD and SVL (RODSV); width of OL measured at the broadest point (OLW); ratio of OLW and SVL (ROWSV); diameter of largest scale on OL (DSOL); diameter of largest scale on temporal region (DSCT), measured on the right side; dorsal crest (DC) absence (–) or number of dorsal cones visible to the naked eye without the use of a binocular microscope according to Eckhardt et al. (2012); diameter of broadest scales on the lower arm (DSA), defined as the area from the elbow to the manus in lateral view on the right side; number of scales on lower arm in a line from elbow to manus on the right side (NSA); number of supralabial scales (NSL), counted from the first scale next to the rostral to the last scale that borders directly and entirely (with one complete side) to the mouth slit of the upper jaw on the right side; and number of infralabial scales (NIL), analogous to the definition of NSL above, on the right side. Terminology of hemipenial structures follows Klaver and Böhme (1986) and Prötzel et al. (2017). For the "diagnosis", only adult specimens were considered. "Adult" is defined for specimens with 90–100% SVL of the largest specimen and additionally for males with completely developed hemipenes; "subadult" (subad.) refers to specimens with 70–90% of SVL and already developed hemipenes; "juvenile" is defined as <70% of SVL without any distinct sexual characteristics.

**Table 1.** Morphological measurements of *Calumma roaloko* sp. n. All measurements in mm. For abbreviations, see Materials and methods.

| Final museum no. | KU 343178 | KU 343168 | ZSM 244/2018 | UADBA-R (uncatalogued) | UADBA-R (uncatalogued) |
|---|---|---|---|---|---|
| original museum no. | KU 343178 | KU 343168 | KU 343177 | KU 343176 | KU 343167 |
| field no. | SML 213 | SML 177 | SML 210 | SML 178 | SML 166 |
| sex | adult male | adult female | subad. male | subad. male | subad. female |
| type status | holotype | paratype | paratype | paratype | paratype |
| altitude [m] | 1100 | 1100 | 1100 | 1100 | 1100 |
| SVL | 45.6 | 44.5 | 37.6 | 38.6 | 40.0 |
| TaL | 48.1 | 41.0 | 44.3 | 42.3 | 34.8 |
| TL | 93.7 | 85.5 | 81.9 | 80.9 | 74.8 |
| RTaSV | 105% | 92% | 118% | 110% | 87% |
| LRA | 5.2 | 2.3 | 4.6 | 3.9 | 2.7 |
| RRASV | 11.4% | 5.2% | 12.2% | 10.1% | 6.8% |
| DRA | 2.6 | 1.9 | 2.3 | 2.7 | 1.6 |
| RDRSV | 5.7% | 4.3% | 6.1% | 7.0% | 4.0% |
| NDRA | 5 | 6 | 4 | 5 | 7 |
| NSRA | 16 | 29 | 28 | 33 | 31 |
| RNLRA | 3.1 | 12.6 | 6.1 | 8.5 | 11.5 |
| RC | + | + | + | + | + |
| LC | + | + | + | + | + |
| TCL | – | – | – | – | – |
| TCR | – | – | – | – | – |
| PC | + | – | – | – | – |
| OL | c | c | c | c | c |
| OLND | 0.4 | 0.2 | 0.3 | 0.2 | 0.3 |
| RODSV | 8.8% | 10.1% | 10.4% | 10.6% | 10.3% |
| OLD | 4.0 | 4.5 | 3.9 | 4.1 | 4.1 |
| ROLSV | 4.6% | 4.3% | 5.1% | 5.2% | 4.5% |
| OLW | 2.1 | 1.9 | 1.9 | 2.0 | 1.8 |
| ROWSV | 0.9% | 0.4% | 0.8% | 0.5% | 0.8% |
| DSOL | 0.7 | 0.5 | 0.7 | 0.6 | 0.7 |
| DSCT | 0.7 | 0.6 | 0.7 | 0.6 | 0.6 |
| DC | 2 | 0 | 1 | 0 | 0 |
| DSA | 0.7 | 0.5 | 0.5 | 0.4 | 0.4 |
| NSA | 11 | 14 | 13 | 15 | 15 |
| NSL | 13 | 13 | 13 | 13 | 13 |
| NIL | 13 | 14 | 13 | 13 | 12 |

## Micro-CT

For internal morphology, micro-Computed Tomography (micro-CT) scans of the head were prepared for the male holotype KU 343178 and the female paratype KU 343168. For micro-CT scanning, specimens were placed in a closed plastic vessel slightly larger than the specimen with the head oriented upwards and stabilized with ethanol-soaked paper. To avoid artifacts, it was ensured that the paper did not cover the head region. Micro-CT scanning was performed with a phoenix|x nanotom m (GE Measurement & Control, Wunstorf, Germany) using a tungsten or diamond target at a voltage of 130 kV and a current of 80 µA for 29 minutes (1800 projections). 3D data sets were processed with VG Studio Max 2.2 (Visual Graphics GmbH, Heidelberg, Germany); the data were visualized using the Phong volume renderer to show the surface of the skull and reflect a variety of different levels of x-ray absorption following recommendations of Scherz et al. (2017). Osteological terminology follows Rieppel and Crumly (1997). Measurements were taken in VG Studio Max 2.2 using the caliper tool, given the following abbreviations (Table 2, Fig. 1): nasal length (NaL); frontal width measured at prefrontal border (FWPf); frontal width measured at anterior border to postorbitofrontal (FWPo); frontal width measured at frontal-parietal-border (FWPa); parietal width measured at posterior border to postorbitofrontal (PWPo); parietal width at midpoint (PWm); parietal length (PL); frontal length (FL); snout-casque length, measured from tip of upper jaw to posterior end of parietal (SCL); skull length, measured from tip of upper jaw to skull capsule (SkL); the respective ratios, divided by SkL, are indicated with an 'R' in front of the character-abbreviations.

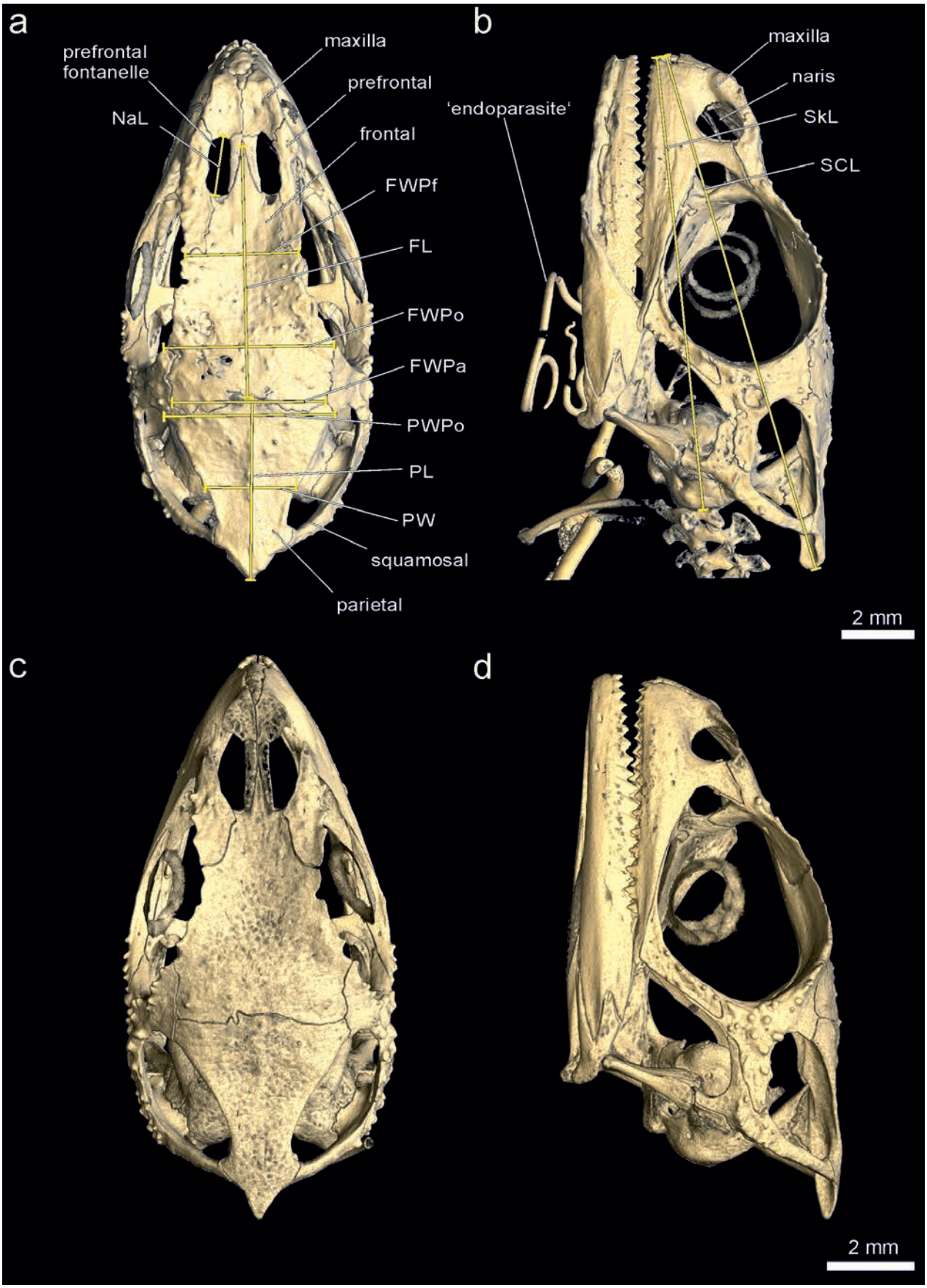


**Figure 1.** Micro-CT scans of skulls of *Calumma roaloko* sp. n. Male holotype KU 343178 in dorsal view (**a**) and lateral view (**b**), note the worm-like structure (presumably an endoparasite) in the throat of the holotype; female KU 343168 in dorsal view (**c**) and lateral view (**d**). See Materials and methods for abbreviations. See also Suppl. material 3 and 4 for a 360° movie of the skull.

**Table 2.** Osteological measurements based on micro–CT scans of the skulls of the male holotype and an adult female of *Calumma roaloko* sp. n. All measurements in mm. For abbreviations, see Materials and methods.

| Collection no. | KU 343178 | KU 343168 |
|---|---|---|
| **field no.** | **SML 213** | **SML 177** |
| **sex** | male | female |
| **type status** | holotype | paratype |
| **NaL** | 1.4 | 1.8 |
| **RNaL** | 11.6% | 15.7% |
| **FWPf** | 3.2 | 2.8 |
| **RFWPf** | 26.4% | 24.3% |
| **FWPo** | 4.5 | 4.1 |
| **RFWPo** | 37.2% | 35.7% |
| **FWPa** | 4.1 | 3.9 |
| **RFWPa** | 33.9% | 33.9% |
| **PWPo** | 4.7 | 4.0 |
| **RPWPo** | 38.8% | 34.8% |
| **PWm** | 2.7 | 2.2 |
| **RPWm** | 22.3% | 19.1% |
| **PL** | 5.1 | 4.9 |
| **RPL** | 42.1% | 42.6% |
| **FL** | 7.0 | 6.0 |
| **RFL** | 57.9% | 52.2% |
| **SCL** | 14.5 | 13.5 |
| **RSCL** | 119.8% | 117.4% |
| **SkL** | 12.1 | 11.5 |

### DNA sequencing and phylogenetic analysis

We extracted genomic DNA from tissue samples at the KU Biodiversity Institute using a phenol-chloroform protocol. We amplified two mitochondrial gene fragments, COI and ND2, using standard protocols. Primers and protocols used for ND2 are described in Gehring et al. (2011) for ND2 and in Nagy et al. (2012) for COI. For ND2 alignments, we used previously published sequences from Gehring et al. (2012) and Prötzel et al. (2018), supplemented by sequences of the new species described herein (Fig. 2). For COI alignments, we downloaded all available sequences for the *C. nasutum* group taxa from GenBank. All newly generated sequences were submitted to GenBank (accession numbers MH668289–MH668297). We aligned sequences using MUSCLE (Edgar 2004) in Geneious version 6 (Kearse et al. 2012), under default settings. We manually inspected alignments for accuracy and open reading frames, but no changes were necessary. We calculated uncorrected pairwise genetic distances from our alignments in R v3.3.2 (R Development Core Team 2017), using the dist.dna function of the ape package (Paradis et al. 2004), with deletion of non-shared sites for each pairwise comparison. We used *C. oshaughnessyi* (FGZC 4577) as an outgroup.

Prior to phylogenetic analysis of the ND2 gene, conducted using maximum-likelihood in RAxML 8.2.6 (Stamatakis 2014), we used PartitionFinder2 (Lanfear et al. 2012) to select an optimal partitioning scheme. We provided the first, second, and third positions as initial partitions. As RAxML can only use a single model across all partitions, we evaluated only the 'GTR+G' model of sequence evolution. We then used the '-f a' option in RAxML to run 1000 rapid bootstraps and searched for the best-scoring maximum-likelihood tree, providing the optimal partitioning scheme identified by PartitionFinder2 using the '-q' option.

### Registration of nomenclature

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:2433A9DD-8AC1-4139-A639-E24053D5C33F. The online version of this work will be archived and made available from the following digital repositories: CLOCKSS and Zenodo.

## Results

### Genetic differentiation in the *Calumma boettgeri* complex

The ND2 alignment contained 513 sites and a total of 235 variable sites, of which 177 were parsimony informative. The genetic analysis of the ND2 gene fragment (Fig. 2) revealed strong differences of the newly discovered form to all other species of the *C. boettgeri* complex including the three recently described species. Comparisons of genetic distance using mitochondrial genes show minimum distances of 11.98% in ND2 to *C. uetzi*, a species from Sorata and Marojejy (>500 km north from the type locality of our novel species) that was described only recently (Prötzel et al. 2018), and maximum distances to *C. boettgeri* and *C. gehringi* (>17%, Suppl. material 1). The intraspecific variation is small at 0.00–0.19%. In the COI sequences there is at least 12.20% distance between the collected specimens and all sequences available for the *C. nasutum* group, however *C. uetzi* is not included in this dataset (Suppl. material 2). According to the ND2 phylogeny (Fig. 2) *C. uetzi* is the sister taxon to the new species and together they form a clade which is sister to a clade including *C. boettgeri*, *C. linotum* and *C. juliae*. However, bootstrap values are relatively low, possibly due to the limited number of informative base-pairs. Supported by the genetic data provided we describe the following new species:

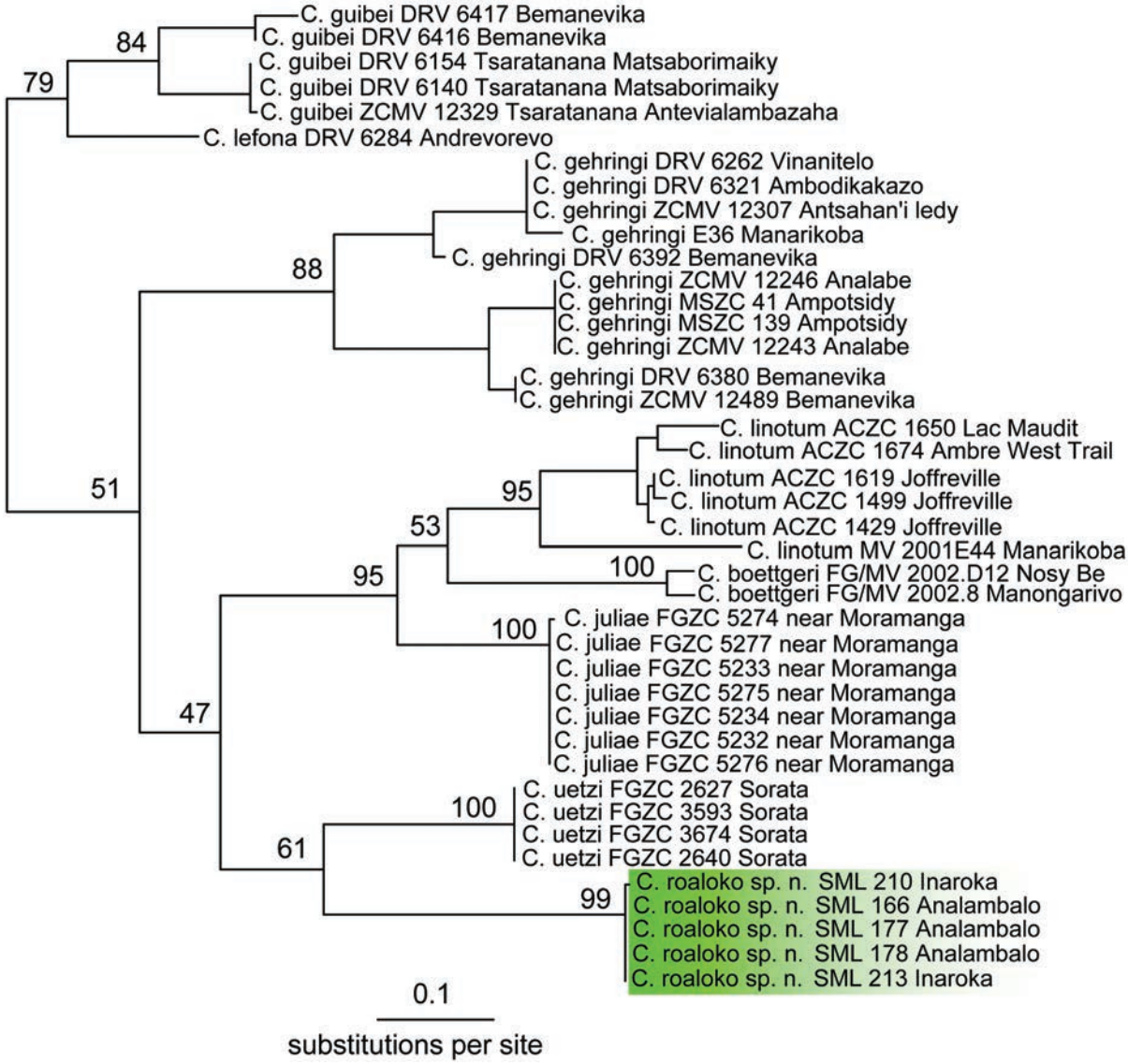


**Figure 2.** Maximum-likelihood tree of the *Calumma boettgeri* complex, based on 513 base-pairs of the mitochondrial ND2 gene. Nodal support values indicate the proportional support from 1000 rapid bootstrap replicates. Support values for intra-specific relationships are not shown. Outgroup (*C. oshaughnessyi* FGZC 4577) not shown for graphical reasons.

***Calumma roaloko* sp. n.**

http://zoobank.org/B2018AA8-8C9A-4F1C-8B18-FA49ADA627EA

**Suggested common English name:** The two-toned soft-nosed chameleon
**Suggested common Malagasy name:** Tanalahy roa loko

**Holotype.** KU 343178 (field number SML 213), adult male in a good state of preservation with incompletely everted hemipenes (Fig. 3), collected on January 12th, 2016 by Shea M. Lambert, Carl R. Hutter, Kerry A. Cobb and Ginah Tsiorisoa Andrianasolo in mid-altitude rainforest, locally known as Inaroka (ca. 19.0050°S, 48.4613°E, ca. 1100 m a.s.l., Fig. 4) near Vohidrazana, Alaotra-Mangoro Region, in central-eastern Madagascar.

**Paratypes.** ZSM 244/2018 (KU 343177, field number SML 210), subadult male, same locality and collectors as holotype; KU 343168 (field number SML 177), adult female, UADBA-R uncatalogued (KU 343176, field number SML 178), subadult male, and UADBA-R uncatalogued (KU 343167, field number SML 166), subadult female, all three collected on December 28th, 2015 (SML 166) and December 29th, 2015 (SML 177, 178) by Shea M. Lambert, Carl R. Hutter, Kerry A. Cobb and Ginah Tsiorisoa Andrianasolo in mid-elevation rainforest, locally known as Analambalo (ca. 18.9659°S, 48.4888°E, ca. 1100 m a.s.l., Figs 4–6) near Vohidrazana, Alaotra-Mangoro Region, in central-eastern Madagascar.

**Diagnosis.** *Calumma roaloko* sp. n. is a member of the phenetic *C. nasutum* species group (Prötzel et al. 2016), on the basis of the presence of a soft, dermal unpaired rostral appendage, absence of gular and ventral crests, and heterogeneous scalation on the lower

**Figure 3.** Preserved holotype (KU 343178) of *Calumma roaloko* sp. n. Scale bar = 10 mm.

arm, consisting mostly of tubercles of 0.4–0.7 mm diameter. With 44.5–45.6 mm SVL and 85.5–93.7 mm total length in adult specimens it is currently the smallest known species in the genus *Calumma*. The body of the chameleon is uniquely two-colored with beige/white on the ventral and bright green on the dorsal half. Furthermore, it is characterized by a prominent and distally rounded rostral appendage, occipital lobes that are slightly notched, a distinctly elevated rostral crest, absence of a dorsal crest (or presence of at most two cones) in both sexes, absence of axillary pits, and a unique skull morphology.

*Calumma roaloko* sp. n. differs from *C. fallax*, *C. gallus*, *C. nasutum*, *C. peyrierasi*, *C. vatosoa* and *C. vohibola* of the *C. nasutum* group by the presence of occipital lobes; from *C. boettgeri*, *C. gehringi*, *C. guibei*, *C. lefona*, *C. linotum* and *C. juliae* in the generally smaller body size with a maximum SVL of 45.6 mm and a maximum TL of 93.7 mm (vs. a range of SVL maxima in the former species of 49.1–59.6 mm and TL maxima of 98.7–126.1 mm), and a straight-lined dorsal margin of the supralabial scales vs. serrated (character 'en dents de scie' in Angel 1942); additionally from *C. gehringi*, *C. guibei*, and *C. lefona* in the slightly notched occipital lobes of 0.2–0.4 mm (vs. clearly notched with 0.5–1.8 mm) and in the absence of frontoparietal fenestra; from *C. boettgeri* by the large juxtaposed tubercle scales on the extremities (vs. isolated from each other).

From the most similar taxon *Calumma uetzi*, *C. roaloko* sp. n. differs in the absence of a dorsal crest or presence of at most two cones (vs. presence of 5–14 cones), absence of a temporal crest (vs. presence of 1–2 temporal tubercles), greater number of supralabial scales (13 vs. 10–12) and infralabial scales (12–14 vs. 11–12), a longer rostral appendage in adult males of 5.2 mm with large tubercle scales (vs. 3.8 mm, small and smooth tubercle scales; note: n = 1 each), and less heterogeneous scalation on the head with diameter of largest scale in temporal region of 0.6–0.7 mm (vs. 1.0–1.3 mm). The osteology of the skull is similar in both species; *C. roaloko* sp. n. differs from *C. uetzi* only in the absence of elevated protuberances at the anterior end of the maxilla that characterize the skull of male *C. uetzi*. *Calumma roaloko* sp. n. furthermore differs from all other species by distinct differences in the mitochondrial genes ND2 and COI and a unique two-colored life-coloration.

**Description of the holotype.** Adult male (Figs 3, 5b) in a good state of preservation; mouth slightly open; both hemipenes incompletely everted; SVL 45.6 mm, tail length 48.1 mm, for further measurements see Table 1; distinct and elevated rostral ridges that form a concave cup on the snout and fuse on the anterior snout at the base of a tapering, laterally compressed dermal rostral appendage that projects straight forward over a length of 5.2 mm with a diameter of 2.6 mm, rounded distally; 13 infralabi-

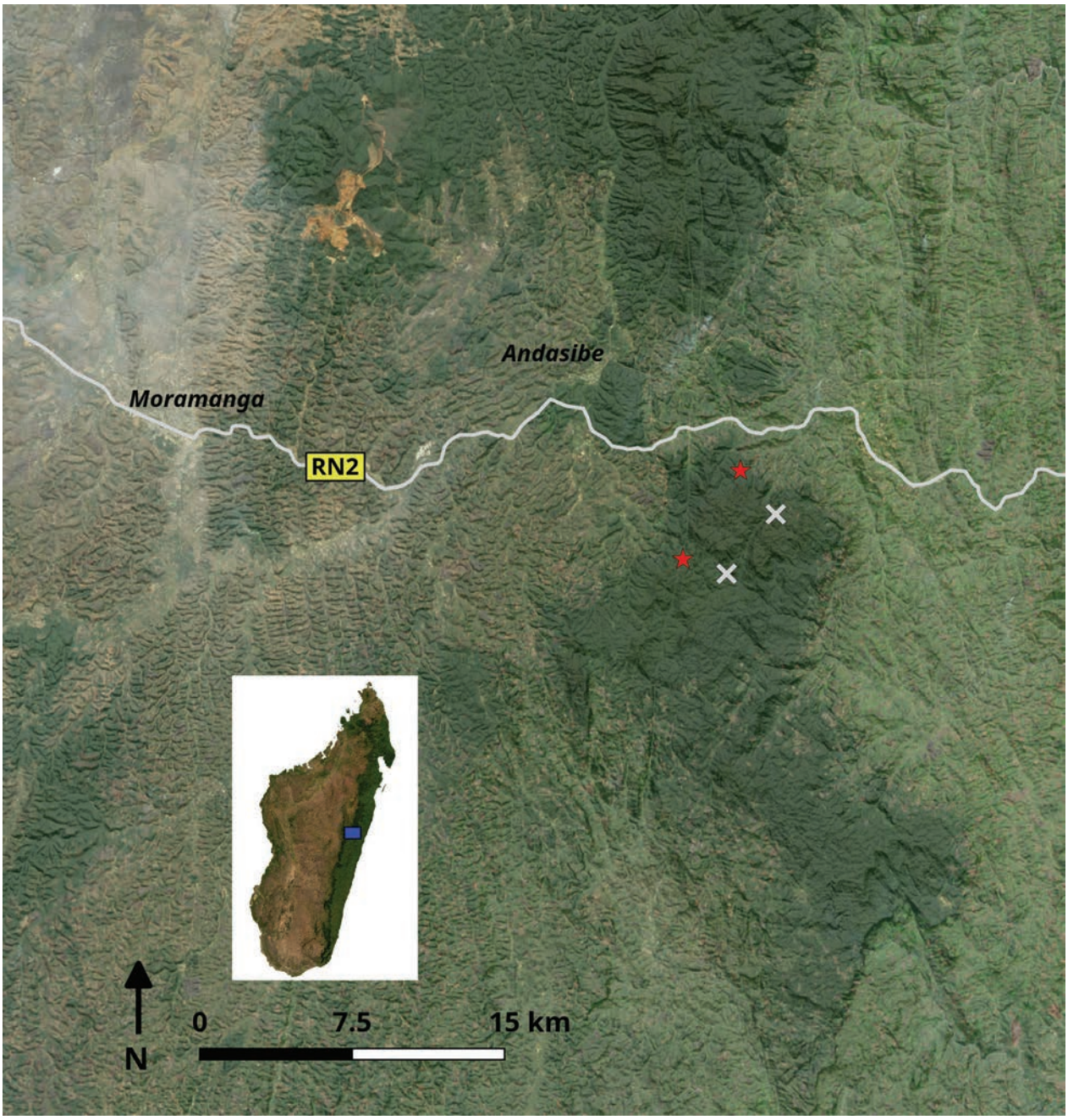


**Figure 4.** Map showing the location of the known range of *Calumma roaloko* sp. n. in central-eastern Madagascar. Red stars indicate localities where *C. roaloko* sp. n. was found, gray "X" indicate localities surveyed but with no detection of the species. The map is a composite of Landsat 7 and SRTM (Shuttle Radar Topographic Mission; Farr and Kobrick 2000) digital elevation data (U.S. Geological Survey (USGS) Earth Resources Observation and Science (EROS) Center) created in QGIS v2.18.

al and 13 supralabial scales; supralabials with a straight dorsal margin; no supra-orbital crest; distinct lateral crest running horizontally; no temporal crest; indistinct parietal crest; occipital lobes clearly developed and slightly notched (0.4 mm); casque raised; dorsal crest absent, only two single cones 0.7 and 1.1 mm from the base of the notch between the occipital lobes; no caudal crest; no traces of gular or ventral crest. Body laterally compressed with fine homogeneous scalation, slightly more heterogeneous on the extremities and head region; limbs with rounded tubercle scales with maximum of 0.7 mm diameter; heterogeneous scalation on the head with largest scale on temporal region with diameter of 0.7 mm; 16 large, oval tubercle scales (diameter >0.3 mm) on the right side of the rostral appendage; no axillary or inguinal pits.

**Skull osteology of the holotype.** Description based on a micro-CT scan (Fig. 1a, b). Skull length 12.1 mm; snout-casque length 14.5 mm; maxillae dorsolaterally forming ridges—externally seen as rostral crest; narrow paired nasals tightly bordering anteriorly and separating frontal from maxillae; anterior tip of frontal exceeding more than

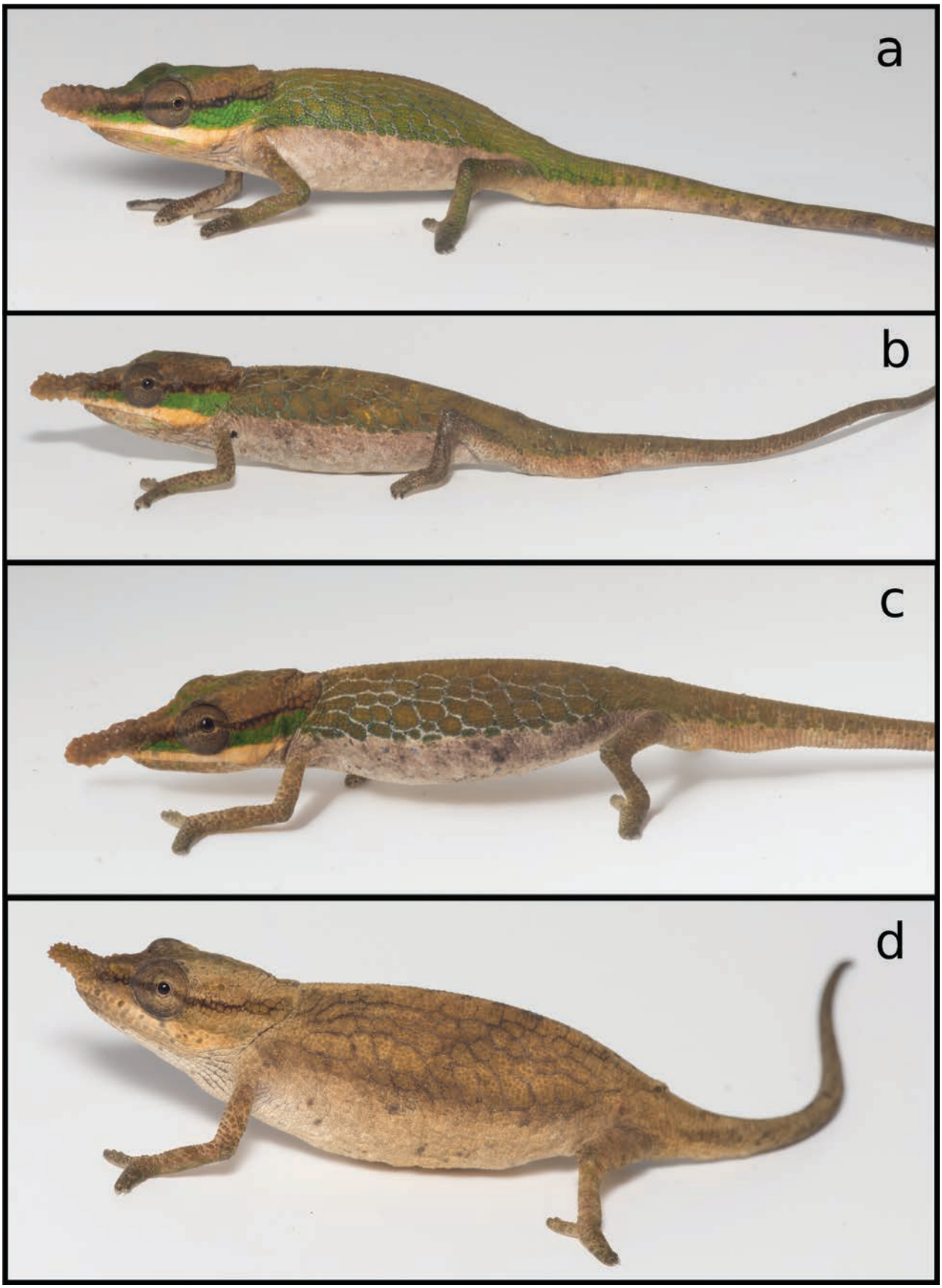


**Figure 5.** In-life photos of four specimens of *Calumma roaloko* sp. n.; (**a**) subadult male (ZSM 244/2018, KU 343177); (**b**) the holotype, adult male (KU 343178); (**c**) subadult male (UADBA-R, KU 343167); (**d**) adult female (KU 343168).

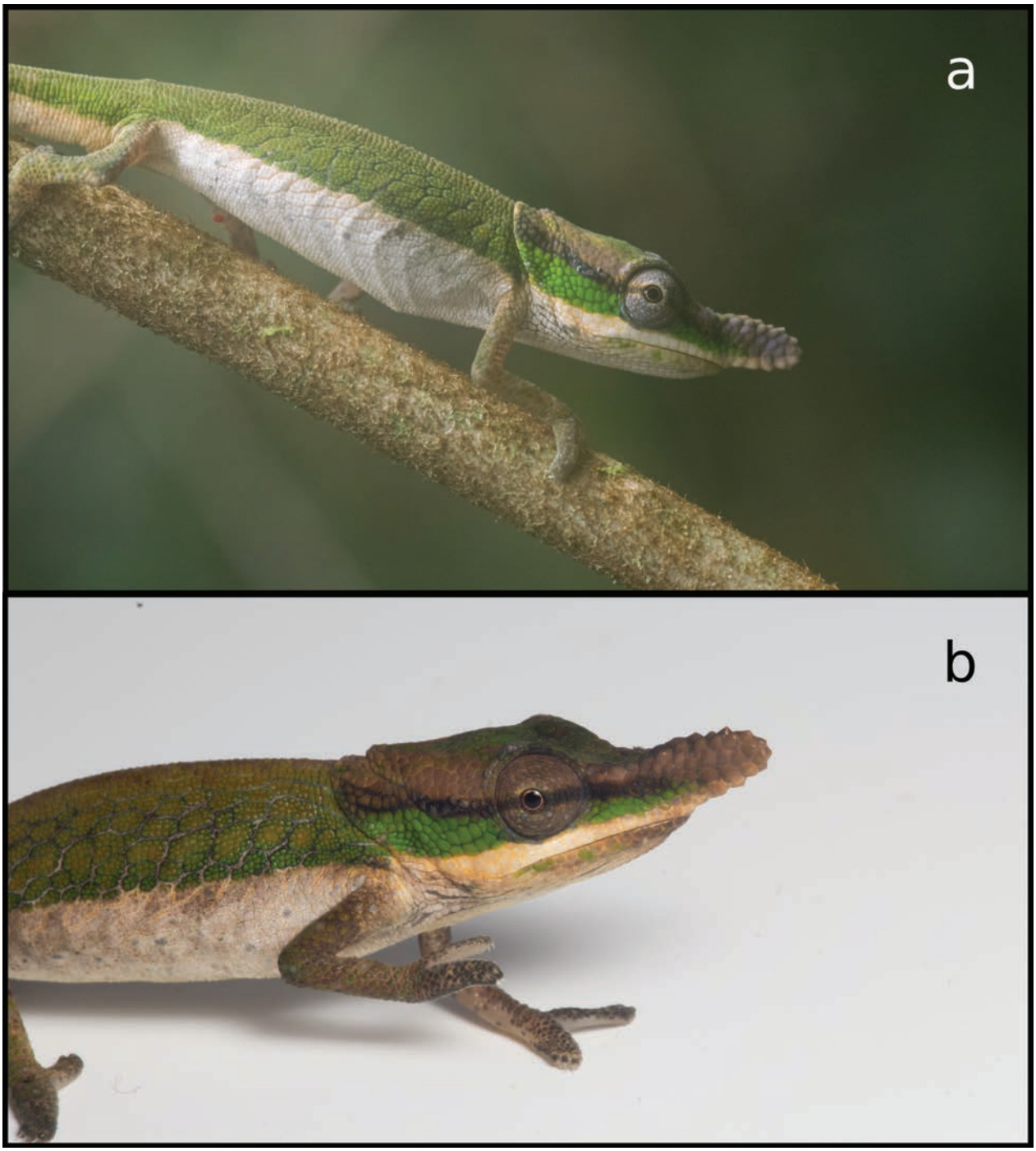


**Figure 6.** Posed photos of a subadult male specimen of *Calumma roaloko* sp. n. (ZSM 244/2018, KU 343177); (**a**) Indigo coloration on the rostral appendage and head scalation is apparent; (**b**) portrait of the same specimen.

half of the naris; prefrontal fontanelle and naris separated by contact of prefrontal with maxilla; frontal and parietal smooth with few tubercles; frontal with a width of 3.2 mm (26.4% of skull length) at border to prefrontal, extending to 4.5 mm (37.2%) at border to postorbitofrontal; broad parietal tapering more or less constantly from a width of 4.7 mm (38.8%) at the border to frontal and still broad at midpoint at 2.7 mm (22.3%) until it meets the squamosals, then narrowing to a tip; posterodorsally directed parietal in broad lateral contact with the squamosal; squamosal thick with a few tubercles. For further measurements, see Table 2 and also Suppl. material 3 for a 360° video of the skull. The skull of *Calumma roaloko* sp. n. shows notable similarity to *C. uetzi* except for the shape of the maxilla.

The micro-CT scan uncovered a worm-like structure that lies curled and fractured in the throat and proceeds posteriorly into the chameleon's body presumably via the esophagus. We suppose that this shows an endoparasite trying to leave the dying chameleon after the processing, but note that it is remarkably strongly mineralized.

**Coloration of the holotype in preservative.** The body of the holotype in preservative (Fig. 3) is of gray and blue/violet color; the rostral appendage and the head are dark brown with a beige stripe from the snout tip via the supralabial scales to the ventral margin of the occipital lobes and a dark blue temporal region; broad lateral stripe on the body violet with a diffuse net-like pattern, tubercles on extremities also violet; ventral half of the body and inner side of extremities beige-white, dorsal margin of the body and tail of same dark brown color as the head.

**Variation.** The four paratypes agree well with the holotype in most characters of morphology and osteology. However, all four paratypes have more tubercle scales on rostral appendage on right side (28–33 vs. 16) and all paratypes lack a parietal crest (vs. indistinctly present); dorsal crest absent in UADBA-R (KU 343167), KU 343168, and UADBA-R (KU 343176). In osteology of the skull the only other micro-CT scanned specimen (the female KU 343168; Fig. 1c, d, Suppl. material 4) differs by the fused prefrontal fontanelle and naris, and the slightly narrower parietal with 34.8% of skull length at postorbitofrontal border (vs. 38.8%) and 19.1% of skull length at midpoint (vs. 22.3%). Both osteological characters can be attributed to sexual dimorphism or intraspecific variation.

**Coloration in life.** Based on observations and photographs of the type specimens (Figs 5–7) the species is sexually dichromatic, with males showing a body coloration with an olive green to bright green dorsal half of the body and beige to white ventral half that is continuing on the tail. Females are generally brown and tan or cream ventrally. Both sexes can display a netlike pattern caused by skin between scales in dark brown or beige. Extremities indistinct brown or beige; throat and upper labial scales beige in both sexes; rostral appendage of same brown color as the upper head region, can turn violet in males (Fig. 6a), as well as the eyes, with a beige line on ventral side; in females the appendage can turn yellowish (Fig. 7); dark lateral stripe from the base of the appendage crossing the eyes and ending at the occipital lobes; cheek region highlighted in bright green in the males, continued anteriorly to the base of the appendage.

**Hemipenial morphology.** The hemipenes of the three male specimens (the holotype KU 343178, UADBA-R (KU 343176), and ZSM 244/2018) are not completely everted and consequently we can only provide a preliminary and possibly incomplete description. On the asulcal side of the truncus the hemipenis shows large calyces with smooth ridges. The apex is ornamented with two pairs of rotulae, which are larger on the sulcal side (with 12–14 tips) and with 8–10 tips on asulcal side. In the holotype KU 343178 and UADBA-R (KU 343176) there is a small peak between the lobes on the posterior side that might be the tip of a cornuculum (Prötzel et al. 2017), but this interpretation is in need of confirmation due to the incomplete eversion of the hemipenes. The top of the apex has a papillary field of several fleshy papillae.

**Available names.** There are no available names that could be attributed to a species of the *C. nasutum* group with occipital lobes.

**Etymology.** The specific epithet "roaloko" is a combination of the Malagasy words "roa" meaning "two" and "loko" meaning "color", in reference to the characteristic two-toned body colorations of males (green and white) and females (brown and tan) of this species. The epithet is to be treated as an invariable noun in apposition.

**Natural history.** The specific natural history of *C. roaloko* sp. n. is little-known, but assumed to be similar to other small-bodied *Calumma*. As with other *C. nasutum* group species, individuals of *C. roaloko* sp. n. were encountered sleeping at night on leaves (Fig. 7) or small branches, and most often spotted ~2–5 m above the ground. *Calumma roaloko* sp. n. may be restricted to higher-elevation habitats, as it has only been found at ca. 1100 m a.s.l., although this is difficult to determine with certainty as most forests below ~1000 m a.s.l. in the area have been cleared. Interestingly, it is known from only two sites, both on the periphery of the forest fragment, and characterized by qualitatively more degraded habitat and/or secondary forest growth as compared to two sites located with more intact primary forest, where it was not encountered (Fig. 4). In summary, either *C. roaloko* sp. n. may have a higher detection probability in disturbed habitats, and/or may be out-competed in primary forest by close relatives (e.g., *C. nasutum* complex species that we found in all four sites). Several specimens were observed to have small red acarid ectoparasites (visible on the hindlimb in Fig. 6a).

**Distribution.** Given current evidence, the distribution of *C. roaloko* sp. n. is potentially restricted to a small fragment (~300 km$^2$) of mid-elevation rainforest that lies outside of nearby Analamazaotra Special Reserve and Andasibe-Mantadia National Park in central-eastern Madagascar (Fig. 4), but within the Réserve de Ressources Naturelles du Corridor Ankeniheny-Zahamena newly protected area. However, we believe that *C. roaloko* sp. n. may still be discovered in nearby areas, including Andasibe-Mantadia National Park, although it has never been found over dozens of surveys in nearby protected areas over the last century, including our own surveys (Hutter, Lambert, Scherz, Prötzel, Glaw, etc. unpubl. data). It is also possible that *C. roaloko* sp. n. could be found in other smaller and more fragmented forests located to the west of the type locality of *C. roaloko* sp. n., south of the city of Moramanga, but recent work in one remnant forest fragment in that area discovered *C. juliae* there, and no specimens of *C. roaloko* sp. n. were found (Prötzel et al. 2018).

**Figure 7.** In-situ photograph of an uncollected (in sleeping position) female of *Calumma roaloko* **sp. n.**, from the same locality as KU 343168.

**Suggested Conservation Status.** The ~300 km$^2$ fragment of mid-elevation rainforest from which *C. roaloko* sp. n. is known is managed by several local government councils, and has recently been established as a new protected area (Réserve de Ressources Naturelles du Corridor Ankeniheny-Zahamena) within the scope of the expansion of Madagascar's national parks (Gardner et al. 2018). Forest in this area is dramatically fragmented and its area is decreasing. We suggest to evaluate the species as Endangered under the IUCN Red List criterion B1 (Extent of occurrence <5000 km$^2$) subcriteria a (severely fragmented or known from fewer than five threat-defined locations) and b(iii) (continuing decline in the area, extent, and/or quality of habitat). However, potentially suitable habitat for *C. roaloko* sp. n. also exists in other nearby protected areas (Andasibe-Mantadia, Analamazaotra) and private reserves (Vohimana). Although field surveys to these areas have not yet uncovered *C. roaloko* sp. n., they have revealed the presence of several other undescribed species of amphibians and reptiles, found originally in the same forest fragment as *C. roaloko* sp. n. (Hutter, unpubl. data). Furthermore, current evidence suggests that *C. roaloko* sp. n. is amenable to disturbed habitat (see Natural History). As such, the conservation status of *C. roaloko* sp. n. as suggested herein may need revision pending future survey work, particularly in nearby protected areas.

## Discussion

The discovery of *C. roaloko* adds to a growing understanding of the diversity of small-bodied *Calumma* in Madagascar (Gehring et al. 2011, 2012, Prötzel et al. 2015, 2016, 2017, 2018). The *C. nasutum* group has grown significantly over the past few years, and is likely to continue to grow as taxonomic revision on it continues, and given the number of OTUs identified for the group by Gehring et al. (2012). Yet with this contribution and those of Prötzel et al. (2017, 2018), the *C. boettgeri* complex has expanded from three known species (Prötzel et al. 2015) to eight.

Biogeographically the pattern of diversity in the *C. nasutum* group currently suggests a complex history, possibly involving several major dispersal events, especially within the *C. boettgeri* complex, with *C. roaloko* being

sister to *C. uetzi*, a species found >500 km to the north in the Sorata massif and Marojejy NP (Prötzel et al. 2018), and a similar situation in the recently described *C. juliae*, whose sister species are *C. boettgeri* and *C. linotum*, separated also by over 500 km. These distributions highlight the north central east of Madagascar as an important biogeographic gap in the *C. boettgeri* complex that may yield intermediate members connecting these species over their long sister-pair distances.

With a total length of 93.7 mm and a body size of 45.6 mm in the largest specimen (the male holotype), *C. roaloko* represents the smallest member of the "true" chameleons, subfamily Chamaeleoninae, (excluding the small, mostly ground dwelling species of the subfamily Brookesiinae) on Madagascar, and one of the smallest members of the Chamaeleoninae in the world. Within the *C. nasutum* group *C. uetzi* (with maximum TL of 101.2 mm and maximum SVL of 45.7 mm) and *C. vohibola* (with maximum TL of 90.5 mm – resulting from a measuring error due to a cut-off tail for DNA analysis – and maximum SVL of 49.8 mm, Gehring et al. 2011) are only slightly larger. Other small species are *C. guillaumeti* and *C. peyrierasi* with below 110 mm (TL), but all of these data are based on relatively small sample sizes (Prötzel et al. 2016).

Interestingly, *Calumma roaloko* and its sister taxon *C. uetzi* are among the only species within the *C. nasutum* group with strong sexual dichromatism. Males of both species differ clearly from the females by a conspicuous display coloration that contrasts well from the green and brown overall background of their habitat as shown for some chameleon species of the genus *Bradypodion* (Stuart-Fox et al. 2007). Displaying male *C. roaloko* are still well-camouflaged however when seen from above due to the green color on the dorsal part of their bodies. Laterally, from a conspecific's eye perspective, they may signal with the white ventral body part and the violet rostral appendage—a strategy employed still more strongly in several other lizards, e.g. *Algyroides*, *Sceloporus*, *Uta* (Ossip-Drahos et al. 2016).

The increase in species richness in chameleons may come not only from the splitting of currently recognized and often widespread species (e.g., in the African chameleon genus *Kinyongia*, Hughes et al. 2017), but from the continued discovery of clearly distinct, previously unknown species, often with geographically or elevationally restricted ranges, and/or low detection probabilities, such as *C. roaloko* (see also Gehring et al. 2011 and Prötzel et al. 2018). Such discoveries highlight the unabated importance of field research for phylogenetic systematics. Indeed, far more examples of "unexpected" species discoveries, also revealed by recent field surveys, are found in other squamate lineages and tropical regions (Welton et al. 2010, Mahler et al. 2016), but also occur in other Malagasy herpetofauna (e.g. Glaw et al. 2006), as well as in other tropical vertebrate clades (e.g., in mammals, Helgen et al. 2013, Hrbek et al. 2014). Clearly, if we are to understand the evolutionary extent of Madagascar's many endemic radiations, and of biodiversity in the tropics generally, a great deal of basic field survey work yet remains.

## Acknowledgements

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## References

Angel F (1942) Les lézards de Madagascar. Mémoires de l'Académie Malgache 36: 1–193.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I (2006) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22(3): 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Brygoo ER (1971) Reptiles Sauriens Chamaeleonidae – Genre *Chamaeleo*. Faune de Madagascar 33: 1–318.

Dayrat B (2005) Towards integrative taxonomy. Biological Journal of the Linnean Society 85(3): 407–415. https://doi.org/10.1111/j.1095-8312.2005.00503.x

Eckhardt FS, Gehring PS, Bartel L, Bellmann J, Beuker J, Hahne D, Korte J, Knittel V, Mensch M, Nagel D, Pohl M, Rostosky C, Vierath V, Wilms V, Zenk J, Vences M (2012) Assessing sexual dimorphism in a species of Malagasy chameleon (*Calumma boettgeri*) with a newly defined set of morphometric and meristic measurements. Herpetology Notes 5: 335–344.

Edgar RC (2004) *MUSCLE*: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Farr TG, Kobrick M (2000) Shuttle Radar Topography Mission produces a wealth of data. Eos, Transactions American Geophysical Union 81: 583–585. https://doi.org/10.1029/EO081i048p00583

Gardner CJ, Nicoll ME, Birkinshaw C, Harris A, Lewis RE, Rakotomalala D, Ratsifandrihamanana AN (2018) The rapid expansion of Madagascar's protected area system. Biological Conservation 220: 29–36. https://doi.org/10.1016/j.biocon.2018.02.011

Gehring P-S, Ratsoavina FM, Vences M, Glaw F (2011) *Calumma vohibola*, a new chameleon species (Squamata: Chamaeleonidae) from the littoral forests of eastern Madagascar. African Journal of Herpetology 60(2): 130–154. https://doi.org/10.1080/21564574.2011.628412

Gehring P-S, Tolley KA, Eckhardt FS, Townsend TM, Ziegler T, Ratsoavina F, Glaw F, Vences M (2012) Hiding deep in the trees: discovery of divergent mitochondrial lineages in Malagasy chameleons of the *Calumma nasutum* group. Ecology and Evolution 2(7): 1468–1479. https://doi.org/10.1002/ece3.269

Glaw F (2015) Taxonomic checklist of chameleons (Squamata: Chamaeleonidae). Vertebrate Zoology 65: 167–246.

Glaw F, Hoegg S, Vences M (2006) Discovery of a new basal relict lineage of Madagascan frogs and its implications for mantellid evolution. Zootaxa 1334: 27–43.

Glaw F, Vences M (2007) A Field Guide to the Amphibians and Reptiles of Madagascar. Third Edition. Vences & Glaw Verlag, Köln, 496 pp.

Glaw F, Vences M, Böhme W (1998) Systematic revision of the genus *Aglyptodactylus* Boulenger, 1919 (Anura: Ranidae) and analysis of its phylogenetic relationships with other ranid genera from Madagascar (*Tomopterna*, *Boophis*, *Mantidactylus* and *Mantella*). Journal of Zoological Systematics and Evolutionary Research 36: 17–37. https://doi.org/10.1111/j.1439-0469.1998.tb00775.x

Helgen KM, Pinto, CM, Kays R, Helgen LE, Tsuchiya MTN, Quinn A, Wilson DE, Maldonado JE (2013) Taxonomic revision of the olingos (*Bassaricyon*), with description of a new species, the Olinguito. ZooKeys 324: 1–83. https://doi.org/10.3897/zookeys.324.5827

Hillenius D (1959) The differentiation within the genus *Chamaeleo* Laurenti, 1768. Beaufortia 8: 1–92.

Hrbek T, da Silva VMF, Dutra N, Gravena W, Martin AR, Farias IP (2014) A new species of river dolphin from Brazil or: How little do we know our biodiversity. PLoS ONE 9 (1): e83623. https://doi.org/10.1371/journal.pone.0083623

Hughes DF, Kusamba C, Behangana M, Greenbaum E (2017) Integrative taxonomy of the Central African forest chameleon, *Kinyongia adolfifriderici* (Sauria: Chamaeleonidae), reveals underestimated species diversity in the Albertine Rift. Zoological Journal of the Linnean Society 181: 1–39. https://doi.org/10.1093/zoolinnean/zlx005

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Klaver C, Böhme W (1986) Phylogeny and classification of the Chamaeleonidae (Sauria) with special reference to hemipenis morphology. Bonner Zoologische Monographien 22: 1–64.

Lambert SM, Hutter CR, Scherz MD (2017) Diamond in the rough: a new species of fossorial diamond frog (*Rhombophryne*) from Ranomafana National Park, southeastern Madagascar. Zoosystematics and Evolution 93(1): 143–155. https://doi.org/10.3897/zse.93.10188

Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29(6): 1695–1701. https://doi.org/10.1093/molbev/mss020

Mahler DL, Lambert SM, Geneva AJ, Ng J, Hedges SB, Losos JB, Glor RE (2016) Discovery of a giant chameleon-like lizard (*Anolis*) on Hispaniola and its significance to understanding replicated adaptive radiations. The American Naturalist 188(3): 357–364. https://doi.org/10.1086/687566

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501

Nagy ZT, Sonet G, Glaw F, Vences M (2012) First large-scale DNA barcoding assessment of reptiles in the biodiversity hotspot of Madagascar, based on newly designed COI primers. PLoS ONE 7(3): e34506. https://doi.org/10.1371/journal.pone.0034506

Nussbaum RA, Raxworthy CJ (1994) A new rainforest gecko of the genus *Paroedura* Günther from Madagascar. Herpetological Natural History 2(1): 43–49.

Ossip-Drahos AG, Morales JRO, Vital-García C, Zúñiga-Vega JJ, Hews DK, Martins EP (2016) Shaping communicative colour signals over evolutionary time. Royal Society Open Science, 3(11): 160728. https://doi.org/10.1098/rsos.160728

Padial JM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. Frontiers in Zoology 7: 16. https://doi.org/10.1186/1742-9994-7-16

Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20(2): 289–290. https://doi.org/10.1093/bioinformatics/btg412

Prötzel D, Ruthensteiner B, Glaw F (2016) No longer single! Description of female *Calumma vatosoa* (Squamata, Chamaeleonidae) including a review of the species and its systematic position. Zoosystematics and Evolution 92(1): 13–21. https://doi.org/10.3897/zse.92.6464

Prötzel D, Ruthensteiner B, Scherz MD, Glaw F (2015) Systematic revision of the Malagasy chameleons *Calumma boettgeri* and *C. linotum* (Squamata: Chamaeleonidae). Zootaxa 4048(2): 211–231. https://doi.org/10.11646/zootaxa.4048.2.4

Prötzel D, Vences M, Hawlitschek O, Scherz MD, Ratsoavina FM, Glaw F (2018) Endangered beauties: micro-CT cranial osteology, molecular genetics and external morphology reveal three new species of chameleons in the *Calumma boettgeri* complex (Squamata: Chamaeleonidae). Zoological Journal of the Linnean Society zlx112. https://doi.org/10.1093/zoolinnean/zlx112

Prötzel D, Vences M, Scherz MD, Vieites DR, Glaw F (2017) Splitting and lumping: an integrative taxonomic assessment of Malagasy chameleons in the *Calumma guibei* complex results in the new species *C. gehringi* sp. nov. Vertebrate Zoology 67(2): 231–249.

R Development Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R–project.org.

Rieppel O, Crumly C (1997) Paedomorphosis and skull structure in Malagasy chamaeleons (Reptilia: Chamaeleoninae). Journal of Zoology 243(2): 351–380. https://doi.org/10.1111/j.1469-7998.1997.tb02788.x

Rosa GM, Crottini A, Noël J, Rabibisoa N, Raxworthy CJ, Andreone F (2014) A new phytotelmic species of *Platypelis* (Microhylidae: Cophylinae) from the Betampona Reserve, eastern Madagascar. Salamandra 50(4): 201–214.

Scherz MD, Hawlitschek O, Andreone F, Rakotoarison A, Vences M, Glaw F (2017) A review of the taxonomy and osteology of the *Rhombophryne serratopalpebrosa* species group (Anura: Microhylidae) from Madagascar, with comments on the value of volume rendering of micro-CT data to taxonomists. Zootaxa 4273(3): 301–340. https://doi.org/10.11646/zootaxa.4273.3.1

Scherz MD, Rakotoarison A, Hawlitschek O, Vences M, Glaw F (2015) Leaping towards a saltatorial lifestyle? An unusually long-legged new species of *Rhombophryne* (Anura, Microhylidae) from the Sorata massif in northern Madagascar. Zoosystematics and Evolution 91(2): 105–114. https://doi.org/10.3897/zse.91.4979

Stamatakis A (2014) RaxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformaics/btu033

Stuart-Fox D, Moussalli A, Whiting MJ (2007) Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. The American Naturalist 170(6): 916–930. https://doi.org/10.1086/522835

Vieites DR, Ratsoavina FM, Randrianiaina R-D, Nagy ZT, Glaw F, Vences M (2010) A rhapsody of colours from Madagascar: discovery of a remarkable new snake of the genus *Liophidium* and its phylogenetic relationships. Salamandra 46(1): 1–10.

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences of the USA 106(20): 8267–8272. https://doi.org/10.1073/pnas.0810821106

Welton LJ, Siler CD, Bennett D, Diesmos A, Duya MR, Dugay R, Rico ELB, Van Weerd M, Brown RM (2010) A spectacular new Philippine monitor lizard reveals a hidden biogeographic boundary and a novel flagship species for conservation. Biology Letters 6(5): 654–658. https://doi.org/10.1098/rsbl.2010.0119

Yoder AD, Rasoloarison RM, Goodman SM, Irwin JA, Atsalis S, Ravosa MJ, Ganzhorn JU (2000) Remarkable species diversity in Malagasy mouse lemurs (primates, *Microcebus*). Proceedings of the National Academy of Sciences of the USA 97(21): 11325–11330. https://doi.org/10.1073/pnas.200121897

# Supplementary material 1

## Genetic distances of ND2

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .ods spreadsheet

Explanation note: Uncorrected genetic distances for all pairwise comparisons of ND2.

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Link: https://doi.org/10.3897/zse.94.27305.suppl1

# Supplementary material 2

## Genetic distances of COI

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .ods spreadsheet

Explanation note: Uncorrected genetic distances for all pairwise comparisons of COI.

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Link: https://doi.org/10.3897/zse.94.27305.suppl2

# Supplementary material 3

## Movie of 3D model of the skull

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .avi video file

Explanation note: Movie of micro-CT scan of the skull of the male holotype of *Calumma roaloko* (KU 343178).

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Link: https://doi.org/10.3897/zse.94.27305.suppl3

# Supplementary material 4

## Movie of 3D model of the skull

Authors: David Prötzel, Shea M. Lambert, Ginah Tsiorisoa Andrianasolo, Carl R. Hutter, Kerry A. Cobb, Mark D. Scherz, Frank Glaw

Data type: .avi video file

Explanation note: Movie of micro-CT scan of the skull of the female *Calumma roaloko* (KU 343168).

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# Exploring the evolutionary potential of parasites: Larval stages of pathogen digenic trematodes in their thiarid snail host *Tarebia granifera* in Thailand

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cercariae
intermediate hosts

## Abstract

Minute intestinal flukes from several distinct families of endoparasitic platyhelminths are a medically important group of foodborne trematodes prevalent throughout Southeast Asia and Australasia. Their lifecycle is complex, with freshwater snails as primary intermediate hosts, with infecting multiple species of arthropods and fish as second intermediate hosts, and with birds and mammals including humans as definitive hosts. In Southeast Asian countries, the diversity of snail species of the Thiaridae which are frequently parasitized by trematode species is extremely high. Here, the thiarid *Tarebia granifera* in Thailand was studied for variation of trematode infections, by collecting the snails every two months for one year from each locality during the years 2004–2009, and during 2014–2016 when snails from the same localities were collected and new localities found. From ninety locations a total of 15,076 *T. granifera* were collected and examined for trematode infections. With 1,577 infected snails the infection rate was found to be 10.46 %. The cercariae were categorized into fifteen species from eight morphologically distinguishable types representing several distinct families, viz. (i) virgulate xiphidiocercariae (*Loxogenoides bicolor*, *Loxogenes liberum* and *Acanthatrium histaense*), (ii) armatae xiphidiocercariae cercariae (*Maritreminoides caridinae* and *M. obstipus*); (iii) parapleurophocercous cercariae (*Haplorchis pumilio*, *H. taichui* and *Stictodora tridactyla*); (iv) pleurophocercous cercariae (*Centrocestus formosanus*); (v) megarulous cercariae (*Philophthalmus gralli*); (vi) furcocercous cercariae (*Cardicola alseae*, *Alaria mustelae* and *Transversotrema laruei*); as well as (vii) echinostome-type cercariae, and (viii) gymnocephalous-type cercariae. In addition, a phylogenetic marker (internal transcribed spacers 2, ITS2) was employed in generic and infrageneric level classifications of these trematodes, using sequences obtained from shed cercariae isolated from *T. granifera* specimens of the second study period collected in various regions in Thailand. We obtained ITS2 sequences of cercariae from nine species (of seven types): *Loxogenoides bicolor*, *Loxogenes liberum*, *Maritreminoides obstipus*, *Haplorchis taichui*, *Stictodora tridactyla*, *Centrocestus formosanus*, *Philophthalmus gralli*, as well as from one species each of echinostome cercariae and gymnocephalous cercariae. Thus, this analysis combines the parasites' data on morphology and geographical occurrence with molecular phylogeny, aiming to provide the groundwork for future studies looking into more details of the parasite-snail evolutionary relationships.

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## Introduction

Trematodes (or flatworms) are endoparasitic platyhelminths that not only infect fishes, birds and other wildlife worldwide but also mammals as well as humans. As foodborne parasites they are of medical importance resulting in significant morbidities and mortalities worldwide. For example, the disability adjusted life years (also known as DALYs) for the foodborne trematodiases including *Fasciola* spp., *Clonorchis* spp., *Opisthorchis* spp., *Paragonimus* spp. and the minute intestinal trematodes such as *Fasciolopsis buski*, *Heterophyes* spp. and *Metagonimus* spp., are estimated to be 2.02 million worldwide (Torgerson et al. 2015).

Especially as liver flukes and intestinal flukes human infecting parasites are highly prevalent in Southeast Asian countries (Wongratanacheewin et al. 2001, Chai et al. 2005, 2013, Krailas et al. 2014). Infections caused by these flukes have a major public health impact and are also of economic importance in veterinary medicine. Humans or domestic animals become infected when they eat raw, salted, pickled or smoked fish containing the infective metacercariae (e.g. *Opisthorchis* spp.,) or contaminated to raw or uncooked vegetables (e.g. *Fasciola* spp.) (see e.g. Krailas et al. 2011, Krailas et al. 2014). Examples include the liver fluke *Opisthorchis viverrini*, which can cause cholangiocarcinoma, a kind of cancer in the bile ducts. The intestinal fluke *Haplorchis taichui* is a possible agent of irritable bowel syndrome-like symptoms, and *Centrocestus formosanus* may cause epigastric pain and indigestion accompanied by occasional diarrhea (Watthanakulpanich et al. 2010, Sripa et al. 2010, Chai et al. 2013). The prevalence of human trematode infections of the mentioned species was found to be the highest in the northern and northeastern regions of Thailand (Srisawangwong et al. 1997, Pungpak et al. 1998, Radomyos et al. 1998, Sukontason et al. 1999). In Northeast Thailand alone, for example, about six million people are infected with the liver fluke, *O. viverrini* (Shin et al. 2010). As Thailand has the highest incidence of cholangiocarcinoma associated with *O. viverrini* (Sripa et al. 2007), opisthorchiasis received greater attention for research than infection with the minute intestinal flukes, such as *Haplorchis taichui*, for which no such associations have been documented. Nevertheless, Thai people have considerably underestimated these trematodes in the past by continually eating traditional Thai food prepared from raw freshwater fish (Chuboon et al. 2005). Hence, the prevalence of trematodes in Thailand remains a continuous problem (Krailas et al. 2014).

Trematodes often have very complex life cycles involving at least one, sometimes two or four, but usually three different hosts, of which the first is almost always a mollusc (Galaktionov and Dobrovolskij 2003). Eggs are released by the definitive host and either the first larval stage, i.e. the miracidium, hatches from the egg in a suitable medium (usually water) being adapted for actively recognizing and penetrating the first intermediate host; or the miracidium remains embryonated within the egg and infects the first intermediate host through passive uptake and subsequent hatching and penetration within the host. The miracidium develops directly into a (mother) sporocyst that may produce daughter sporocysts or rediae (sometimes rediae also produce a second generation of rediae). Another larval form, i.e. the cercariae, then develops either within the sporocyst or within the redia in the first intermediate host and is typically released into the environment where it either actively searches and penetrates the host or is passively taken up. Within the second host cercariae encyst and develop into metacercariae. Through predation metacercariae are taken up by the definitive host and then develop into the adult trematode completing the life cycle. Deviations from this typical life cycle occur either in the number of different life cycle stages that actually develop or in the number of hosts involved in the development (for a detailed overview, see Galaktionov and Dobrovolskij 2003).

The occurrence of trematodes depends on the presence of first and second intermediate host species, as well as the eating habit of local people (Radomyos et al. 1998). In Thailand, medically-important freshwater snails have been investigated since 1980 for trematode infections (Upatham et al. 1980, Nithiuthai et al. 2002, Krailas et al. 2003, 2008, 2014, Sri-aroon et al. 2005, Dechruksa et al. 2007, 2013, 2017, Ukong et al. 2007). For example, the liver fluke *Opisthorchis viverrini* (Family: Opisthorchiidae) is found in Thailand in freshwater snails *Bithynia funiculata*, *B. siamensis goniomphalos* and *B. siamensis siamensis* (Bithyniidae). However, despite the importance of the snail intermediate host(s) to the lifecycle of trematodes, the faunistic and biosystematic knowledge of these limnic molluscs is scarce in general. In particular, among the Cerithioidea which is ecologically and phylogenetically a highly important caenogastropod group of molluscs (Glaubrecht 1996, 2009, 2011; Strong et al. 2011), several freshwater gastropods are known especially in the Thiaridae Gill, 1871 to be important first intermediate hosts of trematodes. For example, species of the intestinal lung fluke *Paragonimus* have been identified in paludomids and/or thiarids, such as e.g. species of *Paludomus* as well as in *Melanoides tuberculata* and *Tarebia granifera*. Pinto and de Melo (2011) list 37 species of cercariae and another 81 trematode larval forms for *Melanoides tuberculata* Müller, 1774. For Thailand Brandt (1974) lists five snail species, viz. *Melanoides tuberculata*, *M. jugicostis* Hanley & Theobald, 1876, *Sermyla riqueti* Grateloup, 1840, *Neoradina prasongi* Brandt, 1974 and *Tarebia granifera* Lamarck, 1816 (see Lamarck 1816), that are currently assigned to the Thiaridae (Glaubrecht 1996, 1999, 2011, Lydeard et al. 2002, Glaubrecht et al. 2009, Strong et al. 2011). Most recently, Krailas et al. (2014) and Dechruksa et al. (2017) investigated the cercarial fauna of *M. tuberculata* and *M. jugicostis* populations from Thailand in detail, reporting 18 different cercariae from the former and four from the latter; among them *C.*

*formosanus*, *H. taichui*, *Haplorchis pumilio* Looss, 1896 and *Stictodora tridactyla* Martin & Kuntz, 1955 that are known to be human pathogen (Watson 1960, Malek and Cheng 1974, Upatham et al. 1995, Pointier and Jourdane 2000, Dechruksa et al. 2007, Ukong et al. 2007).

In the present study, the cercarial fauna of *Tarebia granifera* populations from Thailand is investigated. This thiarid species is widespread in the Oriental region, with an autochthonous range including South and Southeast Asia, South China and numerous islands of the Western Pacific (Brandt 1974, Glaubrecht 1996). The species has been introduced to Africa, the Near East, North and Central America as well as to the Caribbean region and is considered to be invasive there (Abbott 1952, Chaniotis et al. 1980, Prentice 1983, Vargas et al. 1991, Férnandez et al. 1992, Gutierrez et al. 1997, Pointier et al. 1998, Appleton 2002, Mukaratirwa et al. 2005, Facon and David 2006, Appleton et al. 2009, Miranda et al. 2010, 2011, Miranda and Perissinotto 2012). A parallel study on *Tarebia granifera* (also published in this journal; see Veeravechsukij et al. 2018) shows this species to be widely distributed throughout Thailand, with several named and described congeneric constituent populations, as is revealed by respective collections carried out in the North, Northeast, South, East, and Central region, and morphological documentation conducted detailing the biometrical parameters of the adult shells. In addition, molecular phylogenies using fragments of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and 16 S rRNA genes have been constructed, as well as the reproductive strategy documented (i.e. the various stages of embryos and juveniles in the brood pouch) and analysed as to the effect of cercariae infection in female snails.

Here we apply, aside from traditional morphological methods, molecular genetic techniques in order to delimit species of cercariae; i.e. sequencing parts of the nuclear ribosomal RNA gene cluster that have been shown to be efficient for the identification of species of trematodes from their distinct life stages (Skov et al. 2009, Prasad et al. 2011, Davies et al. 2015, Anucherngchai et al. 2016, 2017). With this combination of molecular phylogeny and the parasites' data on morphology and geographical occurrence, we attempt to provide the groundwork for future studies determining the parasite's evolutionary potential within the complex snail-host relationship.

## Materials and methods

### Sampling

Specimens of *Tarebia granifera* were collected in streams, ponds, rivers, brooks, trenches and mountain creeks in all major regions of Thailand (North, South, East, Central and Northeast). Geographic coordinates (WGS84 datum) of sampling sites were determined with the global positioning system (GPS) (Garmin PLUS III, Taiwan). Where GPS data for sampling sites were unavailable, coordinates were determined as accurately as possible from a map. Sampling sites were mapped on a dot-by-dot basis on a public domain map (ArcGIS, Esri, Redlands, California, USA) and then compiled using Photoshop CS6 (Adobe Systems, San Jose, California, USA).

### Collection methods and determination of snails

Snail collections were done during two periods. In the first period, from 2004 to 2009, the snails were collected every two months for one year from each of all the locations. During the second period, from 2014 to 2016, the same localities were visited again, but additional samples were also taken at several new localities, this time collected once only from each location. The snails were collected using the counts per unit of time sampling method (Olivier and Schneiderman 1956). Five researchers collected samples by handpicking and scooping every 10 minutes at each sampling site. The snails were transferred and studied in the laboratory of the Parasitology and Medical Malacology Research Unit, Silpakorn University, Nakhon Pathom, Thailand (PaMaSU: code SUT). The snails were identified according to their shell morphology , following essentially Brandt (1974), and subsequently examined for trematode infections.

### Cercarial study

Collected snails were investigated for trematode infections by using shedding and crushing methods. Descriptions of their morphology were based on living cercariae that had escaped from the snails. The emerged cercariae were studied unstained or vitally stained with 0.5% neutral red. Details of the cercariae were drawn using a camera lucida and identified according to Schell (1970), Yamaguti (1975), Ito (1980) and Krailas et al. (2014). Sample measurements (average size) in micrometers were taken, using an ocular micrometer, from 10 specimens fixed in 10% formalin. Some cercariae (c. 20 specimens from each location) belonging to identified trematode species were then preserved in 95% ethanol for further DNA analysis.

### Molecular study of cercariae

The preserved cercariae were processed for molecular identification at the Department of Animal Diversity, Zoological Museum of the Center for Natural History (CeNak), Universität Hamburg, Germany. Genomic DNA from the cercariae was extracted using the DNeasy blood and animal tissue kit (QIAGEN, Venlo, The Netherlands). Amplification by polymerase chain reaction (PCR) of the nuclear internal transcribed spacer 2 (ITS2) region were performed with the following primers ITS2-F (5'-CTT GAACGC ACA TTG CGG CCA TGG G-3') and ITS2-R: (5'-GCG GGT AAT CACGTC TGA GCC GAG G-3') (Sato et al. 2009). Reactions were set up in 20 µl volumes containing 1.0 µl dNTPs (2 mM each), 2.0 µl 10× mM DreamTaq

Green buffer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.3 µl GreenTaq DNA polymerase (5 U/µl, Thermos Fisher Scientific), 1.0 µl of each primer (10 µM) and 14.7 µl $ddH_2O$. The DNA samples were initially denatured at 94 °C for 4 min followed by 35 cycles (denaturation at 94 °C for 1 min, annealing at 60 °C for 30 s, and elongation at 72 °C for 2 min; see Sato et al. 2009) and a final elongation step at 72 °C for 7 min. The PCR products were purified according to the protocol for enzymatic PCR product clean-up with exonuclease I (20 U/µl, Thermo Fisher Scientific) and FastAP thermosensitive alkaline phosphatase (1 U/µL, Thermo Fisher Scientific). Purified PCR products were sequenced at Macrogen Europe Lab. (Amsterdam, The Netherlands). Alignments of forward and reverse strands were conducted using Geneious 10.1.3 (Biomatters Ltd., Auckland, New Zealand). The ITS2 consensus sequences were aligned in MEGA 7 (Kumar et al. 2016) using MUSCLE (Edgar 2004) under default settings. A Neighbor joining (NJ) analysis was performed based on p-distances with 1,000 bootstrap replicates. For details on sequences used for this study, see Table 1.

# Results

### Geographical origin of collected snails

Specimens of *Tarebia granifera* were found at 90 sampling sites in five regions of Thailand (Fig. 1). During the first sampling period (2004–2009), infected snails were reported from 18 sampling sites. In the second period (2014–2016), infected snails were reported from 51 sampling sites. At a total of 58 localities in four regions of Thailand snails with cercarial infections were found. For information on sampling sites including geographic coordinates and the number of infected snails, see Table 2.

### Occurrence of trematodes obtained from *Tarebia granifera* in Thailand

The various trematode cercariae (distinguished and described in more detail below) exhibit a certain geographical pattern within the various water bodies in Thailand. Only two among the fifteen trematode species found in the thiarid *T. granifera*, viz. *Loxogenoides bicolor* and *Stictodora tridactyla*, were recorded in the present study from almost all major river systems in Thailand (Fig. 2).

In contrast, several species exhibit a more restricted distribution. For example, *Haplorchis taichui* was only detected in *T. granifera* samples from the Nan River (Chao Phraya river system) and the Loei River (Mekong river system), whereas *Philophthalmus gralli* and gymnocephalous cercaria were only detected in the Phachi River (Mae Klong river system). Echinostome cercaria were only present in the *T. granifera* population from the Khek River (Chao Phraya river system).

Cercariae of *Loxogenes liberum*, *Centrocestus formosanus* and *Maritreminoides obstipus* had again a somewhat wider distribution in Thai *T. granifera* populations, being present in several rivers of the Chao Phraya, Mae Klong and Gulf of Thailand drainages (Fig. 2).

### Cercarial diversity and infection rates

A total of 15,076 snails of *T. granifera* were collected and examined for trematode infections. With 1,577 parasitized snails the overall infection rate was found to be 10.46 %. The obtained cercariae were classified into a total of fifteen species from eight morphologically distinguishable types representing at least seven distinct trematode families, viz. (i) virgulate xiphidiocercariae (*Loxogenoides bicolor*, *Loxogenes liberum* and *Acanthatrium histaense*), (ii) armatae xiphidiocercariae (*Maritreminoides caridinae* and *Maritreminoides obstipus*), (iii) parapleurophocercous cercariae (*Haplorchis pumilio*, *Haplorchis taichui* and *Stictodora tridactyla*), (iv) pleurophocercous cercariae (*Centrocestus formosanus*), (v) megarulous cercariae (*Philophthalmus gralli*), (vi) furcocercous cercariae (*Cardicola alseae*, *Alaria mustelae* and *Transversotrema laruei*), as well as (vii) echinostome cercariae, and (viii) gymnocephalous cercariae. The virgulate xiphidiocercariae were the dominant cercarial type infecting snails (5.10%), while infections with other cercarial types were found at rates of (ii) 0.15%, (iii) 3.73%, (iv) 1.14%, (v) 0.02%, (vi) 0.25%, (vii) 0.07%, (viii) 0.01%, respectively; see Table 3 for details.

In this study, neither double trematode infections nor triple trematode infections of collected *Tarebia granifera* were found.

### Morphology of infecting cercariae

The cercariae were categorized by their morphology and organ characters, using as reference previous morphological descriptions (e.g. Schell 1970, Yamaguti 1975, Frandsen and Christensen 1984, Krailas et al. 2014). They are described as follows for the eight distinct morphological cercarial types known and found to date, attributable to at least seven distinct trematode families.

### Type 1. Virgulate xiphidiocercariae cercariae

### Lecithodendriidae Lühe, 1901 (sensu Odhner 1910)

**1.1 *Loxogenoides bicolor* (Krull, 1933) (sensu Kaw 1945)** (Fig. 3)

Body oval; throughout with granules. Oral sucker bigger than ventral sucker; globular in shape and with stylet. Virgulate organ in the anterior part of the body. Pharynx small; an esophagus was not observed. Three pairs of penetration glands present located at about two thirds of the body, two anterior pairs with fine granules and a posterior pair with rather coarse, dark granules. Genital primordial C-shaped; excretory bladder U-shaped. Tail shorter than body; spinose at its tip.

**Table 1.** List of ITS2 sequences used for the phylogenetic analysis. For SUT numbers, see the material lists in the main part of the text.

| Species of cercariae | Type of cercariae | Locality | GenBank accession number | Reference |
|---|---|---|---|---|
| *Angiostrongylus cantonensis* | – | – | HQ540551 | C. Y. Liu (unpubl.) |
| *Lecithodendrium spathulatum* | Xiphidiocercariae | – | JF784192 | Lord et al. (2012) |
| *Lecithodendrium linstowi* | Xiphidiocercariae | – | KJ934792 | Kudlai et al. (2015) |
| *Loxogenoides bicolor* | Xiphidiocercariae | SUT 0515066 B | MH991970 | This study |
| | | SUT 0515067 B | MH991981 | This study |
| | | SUT 0515077 B | MH991985 | This study |
| | | SUT 0515079 C | MH991978 | This study |
| | | SUT 0515087 B | MH991983 | This study |
| | | SUT 0515090 B | MH991976 | This study |
| | | SUT 0516106 A | MH991982 | This study |
| | | SUT 0516109 B | MH991977 | This study |
| | | SUT 0516118 B | MH991984 | This study |
| | | SUT 0516121 A | MH991974 | This study |
| | | SUT 0516125 A | MH991980 | This study |
| | | SUT 0516128 B | MH991972 | This study |
| | | SUT 0516129 B | MH991979 | This study |
| | | SUT 0516130 B | MH991971 | This study |
| | | SUT 0516139 B | MH991973 | This study |
| | | SUT 0516145 B | MH991975 | This study |
| *Loxogenes liberum* | Xiphidiocercariae | SUT 0516109 B | MH991986 | This study |
| | | SUT 0516143 B | MH991987 | This study |
| *Maritreminoides obstipus* | Xiphidiocercariae | SUT 0516124 A | MH991988 | This study |
| | | SUT 0516138 B | MH991989 | This study |
| *Haplorchis pumilio* | Parapleurophocercous cercariae | – | KP165437 | Mei et al. (2015) |
| | | – | KX815125 | Le et al. (2017) |
| *Haplorchis taichui* | Parapleurophocercous cercariae | SUT 0515090 B | MH991968 | This study |
| | | SUT 0516125 A | MH991969 | This study |
| | | – | KX815126 | Le et al. (2017) |
| *Stictodora tridactyla* | Parapleurophocercous cercariae | SUT 0515058 A | MH991962 | This study |
| | | SUT 0515059 B | MH991960 | This study |
| | | SUT 0515071 A | MH991958 | This study |
| | | SUT 0515072 B | MH991953 | This study |
| | | SUT 0515074 B | MH991959 | This study |
| | | SUT 0515075 B | MH991954 | This study |
| | | SUT 0515078 B | MH991963 | This study |
| | | SUT 0515086 A | MH991957 | This study |
| | | SUT 0516138 B | MH991961 | This study |
| | | SUT 0516139 B | MH991956 | This study |
| | | SUT 0516142 B | MH991955 | This study |
| *Centrocestus formosanus* | Pleurophocercous cercariae | SUT 0516102 B | MH991964 | This study |
| | | SUT 0516109 B | MH991966 | This study |
| | | SUT 0516125 A | MH991967 | This study |
| | | SUT 0516142 B | MH991965 | This study |
| *Centrocestus* sp. | Pleurophocercous cercariae | – | JQ390547 | M. Karamian, S. M. Sadjjadi and B. Farhangmehr (unpubl.) |
| *Centrocestus* sp. | Pleurophocercous cercariae | – | AY245699 | Dzikowski et al. (2004) |
| *Opisthorchis viverrini* | Pleurophocercous cercariae | – | AY584735 | Parvathi et al. 2008 |
| *Opisthorchis felineus* | Pleurophocercous cercariae | – | EF688141 | Katokhin et al. (2008) |
| *Philophthalmus gralli* | Megarulous cercariae | SUT 0515058 A | MH991965 | This study |
| | | – | KF986200 | Heneberg et al. (2014) |
| Echinostome cercariae | Echinostome cercariae | SUT 0515086 A | MH991991 | This study |
| Gymnocephalous cercariae | Gymnocephalous cercariae | SUT 0515059 B | MH991990 | This study |
| *Fasciola hepatica* | Gymnocephalous cercariae | – | AM900370 | Ali et al. (2008) |
| *Fasciola gigantica* | Gymnocephalous cercariae | – | AJ853848 | M. D. Bargues and S. Mas-Coma (unpubl.) |
| | | – | AM850108 | Ali et al. (2008) |

The cercariae develop within sporocysts.

The infection rate was 3.84% (579/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 53–88 µm (mean: 72 µm) × 105–138 µm (mean: 117 µm) |
| Stylet | 5–8 µm (mean: 6 µm) × 20–40 µm (mean: 30 µm) |
| Oral sucker | 23–40 µm (mean: 33 µm) × 23–33 µm (mean: 29 µm) |
| Pharynx | 8–12 µm (mean: 10 µm) × 5–8 µm (mean: 8 µm) |
| Ventral sucker | 13–25 µm (mean: 18 µm) × 8–20 µm (mean: 16 µm) |
| Excretory bladder | 18–55µm (mean: 33 µm) × 10–35 µm (mean: 20 µm) |
| Tail | 10–28 µm (mean: 21 µm) × 25–88 µm (mean: 44 µm) |

### 1.2 *Loxogenes liberum* Seno, 1907

(Fig. 4)

Body oval. Oral sucker at the anterior end of body, with stylet. Virgulate organ present. Ventral sucker roundish, smaller than oral sucker. Pharynx very small, a prepharynx, an esophagus and ceca were not observed. Four pairs of penetration glands present, located near the middle of the body; the two anterior pairs with fine granules and the two posterior pairs with coarse granules. Excretory bladder V-shaped. Tail shorter than body, rather slender and spinose at its tip.

The cercariae develop within sporocysts.

The infection rate was 0.15% (23/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 65–93 µm (mean: 81 µm) × 95–120 µm (mean: 108 µm) |

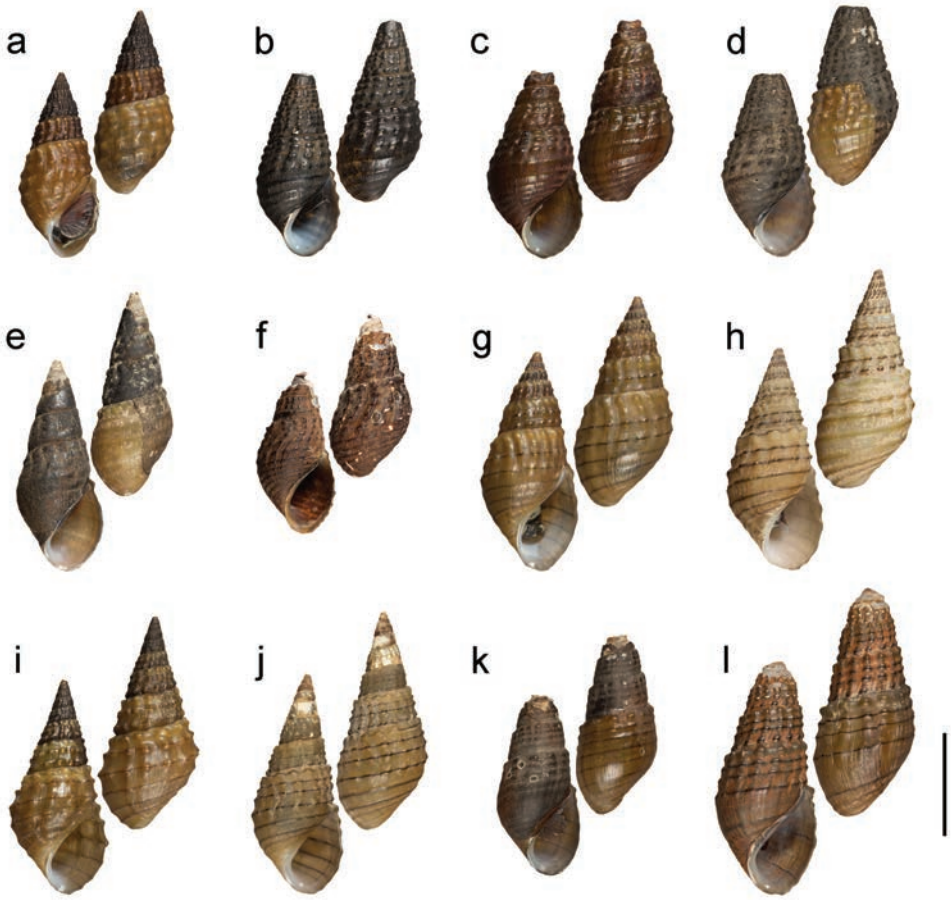
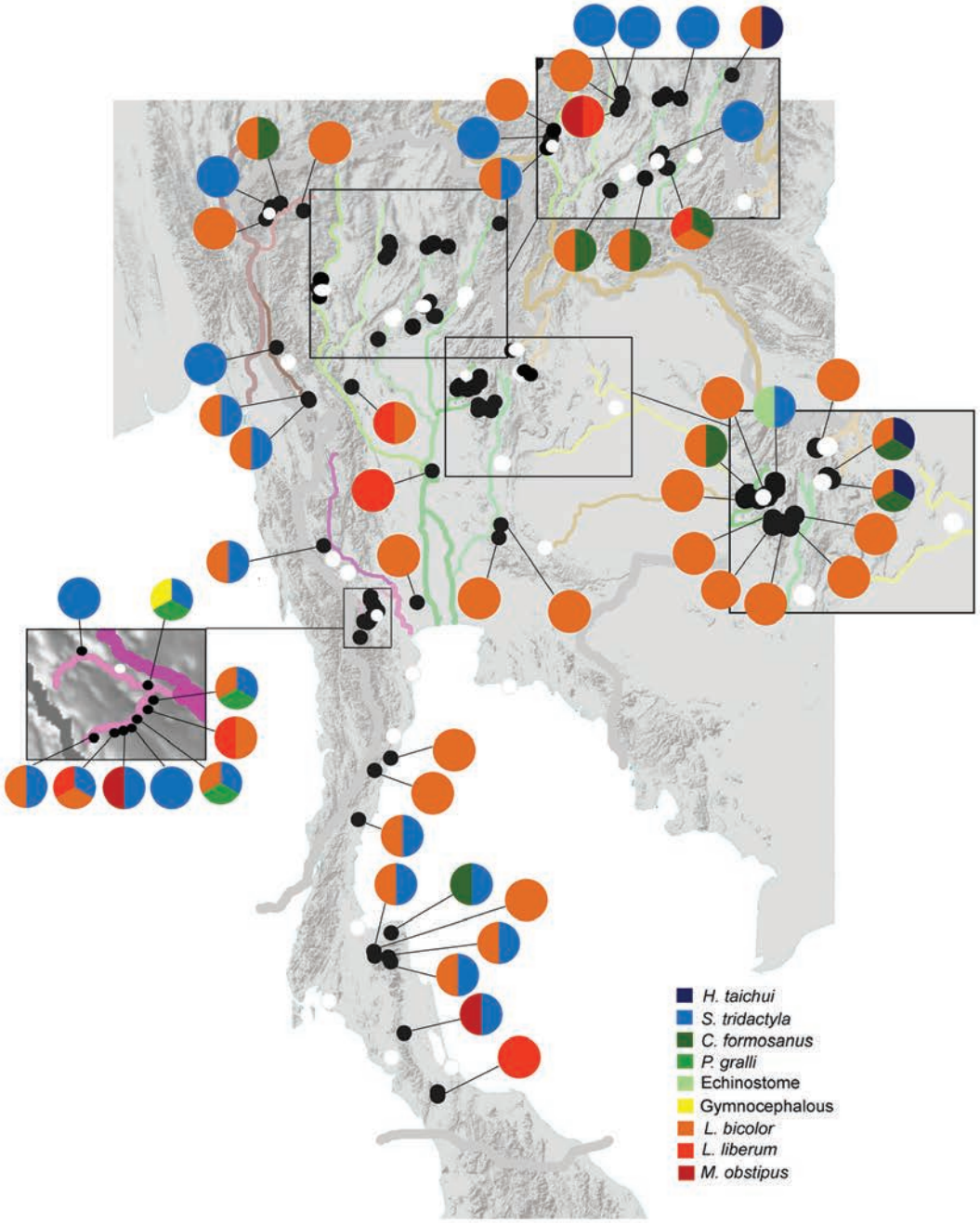



**Figure 1.** Shells of *Tarebia granifera* (Lamarck, 1816) from representative populations in Thailand. **a**. Ban Thung Hang stream, Lampang Province (SUT 0514044); **b**. Huai Sa Dao Pong, Phetchabun Province (SUT 0516123); **c**. Kaeng Bang Ra Chan, Phetchabun Province (SUT 0515088); **d**. Pla Ba waterfall, Loei Province (SUT 0515068); **e**. Ban Nong Phai, Kanchanaburi Province (SUT 0515059); **f**. Khlong Sathing Mo, Songkhla Province (SUT 0516144); **g**. Huay Nam Kong, Mae Hong Son Province (SUT 0515081); **h**. Huay MaeYuak, Lampang Province (SUT 0514046); **i**. Sam Sip Khot waterfall, Phetchabun Province (SUT 0516129); **j**. Sai Yok Yai waterfall, Kanchanaburi Province (SUT 0515092); **k**. Khlong Palian, Trang Province (SUT 0515095); **l**. Khlong Cham Rai reservoir, Songkhla Province (SUT 0516143). For more details on locality data, see Table 2. Scale bar: 10 mm.




| River system | Salween | | Chao Phraya | | | | | | Mae Klong | | Mekong | Gulf of Thailand |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Drainage system | Pai | Moei | Ping | Wang | Yom | Nan | Khek | Pa Sak | Khwae | Phachi | Loei | |
| Species of cercariae: *H. taichui* | | | | | | ■ | | | | | ■ | |
| *S. tridactyla* | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *C. formosanus* | ■ | | | | ■ | ■ | ■ | | | | ■ | |
| *P. gralli* | | | | | | | | | | ■ | | |
| Echinostome | | | | | | | ■ | | | | | |
| Gymnocephalous | | | | | | | | | | ■ | | |
| *L. bicolor* | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *L. liberum* | | | ■ | ■ | ■ | ■ | | | | ■ | | ■ |
| *M. obstipus* | | | | ■ | ■ | | | | | ■ | | ■ |

**Figure 2.** Distribution of *Tarebia granifera* and trematodes in different river systems in Thailand. **a**. Distribution map. **b**. Comparative table of the occurrence of trematode cercariae in different river systems in Thailand. Black dots with attached pie charts in the map represent sampling sites where trematode infected specimens of *T. granifera* were found; white dots represent sampling sites where no infections were observed. Colors in the pie charts and the comparative table refer to trematode species/types (see legend inset).

**Table 2.** Localities, number of collected snails, number of infected snails and trematodes obtained from collected snails; sampling periods: 2004–2009 and 2014–2016.

| NO. | VOUCHER NUMBER | LOCATION | GPS | 2004–2009 | | | 2014–2016 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. of collected snails | No. of infected snails | Infection rates (%) | No. of collected snails | No. of infected snails | Infection rates (%) |
| **THE NORTH** | | | | | | | | | |
| **N1** | SUT 0515083 | Huai Pa Hung (Pai drainage, Salween river system), Pang Mapha District, Mae Hong Son Province | 19°22‘19.6”N, 098°26‘35.9”E, Altitude 437 m | * | * | * | 179 | 1: *L. bicolor* (1) | 0.56 |
| **N2** | SUT 0515081 | Huay Nam Kong (Salween river system), Muang District, Mae Hong Son Province | 19°28‘33.6”N, 098°07‘02.4”E, Altitude 425 m | * | * | * | 24 | 0 | 0 |
| **N3** | SUT 0515077 | Tham Pla (Pai drainage, Salween river system), Muang District, Mae Hong Son Province | 19°25‘31.1”N, 097°59‘27.2”E, Altitude 300 m | 185 | 144: *L. bicolor* (34), *A. hitaense* (25), *H. pumilio* (68), *C. formosanus* (7), *C. alseae* (5), *T. laruei* (5) | 77.84 | 179 | 8: *L. bicolor* (3), *H. pumilio* (5) | 4.47 |
| **N4** | SUT 0515078 | Pai river (Pai drainage, Salween river system), Muang District, Mae Hong Son Province | 19°21’54.8”N, 097°58’10.7”E, Altitude 217 m | * | * | * | 64 | 1: *S. tridactyla* (1) | 1.56 |
| **N5** | SUT 0515079 | Huay Sua Tao (Pai drainage, Salween river system), Muang District, Mae Hong Son Province | 19°15’31.6”N, 097°54’44.6”E, Altitude 237 m | 574 | 98: *L. bicolor* (52), *A. hitaense* (38), *H. pumilio* (5), *T. laruei* (3) | 17.07 | 153 | 2: *L. bicolor* (2), | 1.31 |
| **N6** | SUT 0514052 | Ban Mai Saraphi (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province | 18°16’26.1”N, 098°38’54.0”E, Altitude 277 m | * | * | * | 162 | 11: *L. bicolor* (6), *S. tridactyla* (5) | 6.79 |
| **N7** | SUT 0514051 | Ban Mae Suai Luang (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province | 18°17’04.4”N, 098°39’15.0”E, Altitude 268 m | * | * | * | 23 | 2: *S. tridactyla* (2) | 8.70 |
| **N8** | SUT 0514054 | Mae Soy bridge (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province | 18°17’23.0”N, 098°39’3.6”E, Altitude 271 m | * | * | * | 70 | 5: *L. bicolor* (5) | 7.14 |
| **N9** | SUT 0514050 | Ban Huay Phang (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province, | 18°17’08.5”N, 098°39’16.9”E, Altitude 263 m | * | * | * | 103 | 0 | 0 |
| **N10** | SUT 0516119 | Thansawan waterfall (Yom drainage, Chao Phraya river system), Chiang Muan District, Phayao Province | 18°51’22.2”N, 100°11’09.1”E, Altitude 415 m | 219 | 2: *A. hitaense* (1), *A. mustelae* (1) | 0.91 | 17 | 1: *S. tridactyla* (1) | 5.88 |
| **N11** | SUT 0516117 | Yom river (Yom drainage, Chao Phraya river system), Chiang Muan District, Phayao Province | 18°54’39.7”N, 100°16’27.7”E, Altitude 266 m | * | * | * | 30 | 0 | 0 |
| **N12** | SUT 0516108 | Mae Nam Saai kg 9 +457 bridge (Yom drainage, Chao Phraya river system), Muang District, Phrae Province | 18°05’03.1”N, 100°13’00.1”E, Altitude 171 m | * | * | * | 143 | 0 | 0 |
| **N13** | SUT 0516113 | Mae Marn reservoir (Yom drainage, Chao Phraya river system), Sung Men District, Phrae Province | 18°00’50.6”N, 100°08’22.6”E, Altitude 205 m | * | * | * | 52 | 0 | 0 |
| **N14** | SUT 0514045 | Wang river (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province | 18°56’00.5”N, 099°38’54.6”E, Altitude 376 m | * | * | * | 49 | 12: *S. tridactyla* (12) | 24.49 |
| **N15** | SUT 0514044 | Ban Thung Hang stream (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province | 18°52’47.5”N, 099°40’01.0”E, Altitude 373 m | * | * | * | 165 | 11: *S. tridactyla* (11) | 6.67 |
| **N16** | SUT 0514046 | Huay MaeYuak (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province | 18°46’39.8”N, 099°38’38.7”E, Altitude 352 m | * | * | * | 44 | 1: *L. bicolor* (1) | 2.27 |
| **N17** | SUT 0516124 | km. 40+075 bridge (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province | 18°42’14.8”N, 099°35’31.7”E, Altitude 330 m | * | * | * | 59 | 4: *L. liberum* (3), *M. obstipus* (1) | 6.78 |
| **N18** | SUT 0515090 | Wa river (Nan drainage, Chao, Phraya river system), Bo Kluea District, Nan Province | 19°11’30.4”N, 101°12’13.2”E, Altitude 713 m | * | * | * | 159 | 16: *L. bicolor* (6), *H. taichui* (10) | 10.06 |
| **N19** | SUT 0516114 | Huay Si Pun reservoir (Nan drainage, Chao Phraya river system), Ban Luang District, Nan Province | 18°51’45.1”N, 100°28’37.1”E, Altitude 430 m | * | * | * | 108 | 0 | 0 |

| NO. | VOUCHER NUMBER | LOCATION | GPS | 2004–2009 | | | 2014–2016 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. of collected snails | No. of infected snails | Infection rates (%) | No. of collected snails | No. of infected snails | Infection rates (%) |
| **N20** | SUT 0516109 | Mae pool waterfall (Nan drainage, Chao Phraya river system), Laplae District, Uttaradit Province | 17°43'42.3"N, 099°58'49.6"E, Altitude 123 m | 137 | 43: *L. bicolor* (29), *A. hitaense* (5), *H. pumilio* (6), *C. formosanus* (3) | 31.39 | 91 | 10: *L. bicolor* (4), *L. liberum* (4), *C. formosanus* (2) | 10.99 |
| **N21** | SUT 0516112 | Kaeng Sai Ngam (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province | 17°52'19.5"N, 100°18'02.1"E, Altitude 257 m | * | * | * | 32 | 0 | 0 |
| **N22** | SUT 0513019 | Kaeng Wangwua (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province | 17°52'29.5"N, 100°18'25.6"E, Altitude 231 m | * | * | * | 292 | 4: *S. tridactyla* (4) | 1.37 |
| **N23** | SUT 0513023 | Huai Nam Re Noi (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province | 17°52'51.3"N, 100°16'14.9"E, Altitude 269 m | * | * | * | 155 | 0 | 0 |
| **N24** | SUT 0516103 | Tat Duen waterfall (Yom drainage, Chao Phraya river system), Si Satchanalai District, Sukhothai Province | 17°33'16.2"N, 099°29'48.2"E, Altitude 135 m | 300 | 141: *L. bicolor* (71), *A. hitaense* (36), *H. pumilio* (8), *C. formosanus* (19), *A. mustelae* (7) | 47 | 137 | 0 | 0 |
| **N25** | SUT 0516102 | Si Satchanalai national park (Yom drainage, Chao Phraya river system), Si Satchanalai District, Sukhothai Province | 17°33'07.7"N, 099°29'28.8"E, Altitude 147 m | 749 | 262: *L. bicolor* (85), *A. hitaense* (35), *H. pumilio* (11), *C. formosanus* (116), *A. mustelae* (15) | 34.98 | 147 | 1: *C. formosanus* (1) | 0.68 |
| **N26** | SUT 0515075 | Cheek point near moei river (Moei drainage, Salween river system), Tha Song Yang District, Tak Province | 17°13'23.4"N, 098°13'34.2"E, Altitude 130 m | * | * | * | 55 | 9: *S. tridactyla* (9) | 16.36 |
| **N27** | SUT 0515076 | Mae Salit Luang harbour (Moei drainage, Salween river system), Tha Song Yang District, Tak Province | 17°26'04.8"N, 098°03'33.3"E, Altitude 109 m | * | * | * | 25 | 0 | 0 |
| **N28** | SUT 0515073 | Ban Wang Takhian (Moei drainage, Salween river system), Mae Sot District, Tak Province | 16°42'38.5"N, 098°30'22.2"E, Altitude 196 m | * | * | * | 17 | 0 | 0 |
| **N29** | SUT 0515072 | Thong Dee harbour (Moei drainage, Salween river system), Mae Sot District, Tak Province | 16°41'39.3"N, 098°31'04.4"E, Altitude 206 m | * | * | * | 304 | 21: *L. bicolor* (3), *S. tridactyla* (18) | 6.91 |
| **N30** | SUT 0515074 | Ban Huay Muang (Moei drainage, Salween river system), Mae Sot District, Tak Province | 16°40'58.4"N, 098°31'06.9"E, Altitude 199 m | * | * | * | 300 | 21: *L. bicolor* (1), *S. tridactyla* (20) | 7.00 |
| **N31** | SUT 0516126 | Ban Pak Huay Mae Tho (Ping drainage, Chao Phraya river system), Muang District, Tak Province | 16°52'29.3"N, 099°07'13.6"E, Altitude 106 m | * | * | * | 150 | 3: *L. bicolor* (1), *L. liberum* (2) | 2.00 |
| **N32** | SUT 0516121 | Kaeng Wang Nam Yen (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province | 16°37'23.8"N, 100°54'0.5"E Altitude 710 m | * | * | * | 9 | 8: *L. bicolor* (8) | 88.89 |
| **N33** | SUT 0516120 | Rajapruek resort (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province | 16°36'01.3"N, 100°54'29.9"E, Altitude 707 m | * | * | * | 52 | 28: *L. bicolor* (28) | 53.85 |
| **N34** | SUT 0516123 | Huai Sa Dao Pong (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province | 16°34'24.1"N, 100°59'23.6"E, Altitude 322 m | * | * | * | 31 | 0 | 0 |
| **N35** | SUT 0515088 | Kaeng Bang Ra Chan (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province | 16°32'51.7"N, 100°54'03.2"E, Altitude 599 m | * | * | * | 71 | 6: *L. bicolor* (6) | 8.45 |
| **N36** | SUT 0516129 | Sam Sip Khot waterfall (Pa Sak drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province | 16°32'25.6"N, 101°04'58.4"E, Altitude 386 m | * | * | * | 47 | 18: *L. bicolor* (18) | 38.30 |
| **N37** | SUT 0514041 | Ban Wang Ta Pak Moo 13 (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province | 15°47'54.2"N, 101°14'8.1"E, Altitude 120 m | * | * | * | 312 | 0 | 0 |
| **N38** | SUT 0514042 | Huai Leng (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province | 15°47'52.2"N, 101°13'54.4"E, Altitude 117 m | * | * | * | 84 | 0 | 0 |

| NO. | VOUCHER NUMBER | LOCATION | GPS | 2004–2009 | | | 2014–2016 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. of collected snails | No. of infected snails | Infection rates (%) | No. of collected snails | No. of infected snails | Infection rates (%) |
| **N39** | SUT 0514040 | Ban Wang Tian (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province | 15°47'29.7"N, 101°13'30.7"E, Altitude 121 m | * | * | * | 212 | 0 | 0 |
| **N40** | SUT 0514043 | Huay Range reservoir, Ban Wang Ta Pak (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province | 15°47'19.3"N, 101°15'07.4"E, Altitude 138 m | * | * | * | 128 | 0 | 0 |
| **N41** | SUT 0516130 | Than Thip waterfall (Pa Sak drainage, Chao Phraya river system), Lom Sak District, Phetchabun Province | 16°39'46.3"N, 101°08'09.8"E, Altitude 374 m | * | * | * | 41 | 16: *L. bicolor* (16) | 39.02 |
| **N42** | SUT 0515087 | Ban Kaeng Lat (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province | 16°57'21.3"N, 100°55'31.0"E, Altitude 324 m | * | * | * | 14 | 5: *L. bicolor* (5) | 35.71 |
| **N43** | SUT 0516118 | Kaeng Sopha (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province | 16°52'13.1"N, 100°50'17.4"E, Altitude 413 m | 282 | 72: *L. bicolor* (33), *A. hitaense* (24), *C. formosanus* (15) | 25.53 | 30 | 2: *L. bicolor* (2) | 6.67 |
| **N44** | SUT 0515067 | Poi waterfall (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province | 16°50'36.3"N, 100°45'16.1"E, Altitude 208 m | * | * | * | 83 | 9: *L. bicolor* (6), *M. caridinae* (1), *H. pumilio* (2) | 10.84 |
| **N45** | SUT 0516105 | Phunamkej Resort (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province | 16°51'02.2"N, 100°36'41.1"E, Altitude 208 m | * | * | * | 73 | 0 | 0 |
| **N46** | SUT 0516111 | Kaeng Nangkoi (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province | 16°53'09.0"N, 100°38'47.8"E, Altitude 180 m | * | * | * | 15 | 0 | 0 |
| **N47** | SUT 0516106 | Kaeng Hom (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province | 16°52'20.8"N, 100°50'46.8"E, Altitude 185 m | * | * | * | 95 | 0 | 0 |
| **N48** | SUT 0515086 | Huai Nam Sai (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province | 17°01'07.6"N, 100°55'36.0"E, Altitude 217 m | * | * | * | 93 | 38: *S. tridactyla* (28), Echinostome (10) | 40.86 |
| **THE NORTHEAST** | | | | | | | | | |
| **NE1** | SUT 0516128 | Tat Kok Tup waterfall (Loei drainage, Mekong river system), Phu Luang District, Loei Province | 17°03'03.9"N, 101°31'38.7"E, Altitude 688 m | * | * | * | 45 | 12: *L. bicolor* (10), *H. taichui* (1), *C. formosanus* (1) | 26.67 |
| **NE2** | SUT 0515068 | Pla Ba waterfall (Mekong river system), Phu Ruea District, Loei Province | 17°23'24.7"N, 101°22'27.3"E, Altitude 664 m | 53 | 1: *A. hitaense* (1) | 1.89 | 178 | 3: *L. bicolor* (3) | 1.69 |
| **NE3** | SUT 0516125 | km. 50+350 Loei river (Loei drainage, Mekong river system), Phu Luang District, Loei Province | 17°04'38.0"N, 101°29'20.6"E, Altitude 675 m | * | * | * | 55 | 13: *L. bicolor* (9), *H. taichui* (3), *C. formosanus* (1) | 23.64 |
| **NE4** | SUT 0515064 | Bueng Thung Sang (Chi drainage, Mekong river system), Muang District, Khon Kaen Province | 16°34'45.6"N, 102°50'22.5"E, Altitude 160 m | * | * | * | 20 | 0 | 0 |
| **NE5** | SUT 0516131 | Lamphraphloeng reservoir (Mun drainage, Mekong river system), Pak Thong Chai District, Nakhon Ratchasima Province | 14°35'32.3"N, 101°50'30.1"E, Altitude 259 m | * | * | * | 36 | 0 | 0 |
| **THE EAST** | | | | | | | | | |
| **E1** | SUT 0516135 | Mae Rumphueng Beach (Mae Rumphueng canal, Gulf of Thailand), Muang Rayong District, Rayong Province | 12°37'50.0"N, 101°20'35"E, Altitude 8 m | * | * | * | 150 | 0 | 0 |
| **THE CENTRAL** | | | | | | | | | |
| **C1** | SUT 0516127 | Bung Boraphet (Chao Phraya river system), Muang District, Nakhon Sawan Province | 15°40'59.6"N, 100°14'59.3"E Altitude 32 m | * | * | * | 42 | 1: *L. liberum* (1) | 2.38 |
| **C2** | SUT 0516133 | Dong Phaya Yen waterfall (Pa Sak drainage, Chao Phraya river system), Muak Lek District, Sara Buri Province | 14°44'06.4"N, 101°11'31.4"E, Altitude 156 m | 371 | 1: *L. bicolor* (1) | 0.27 | 27 | 1: *L. bicolor* (1) | 3.70 |
| **C3** | SUT 0516132 | Suanmaduea waterfall (Pa Sak drainage, Chao Phraya river system), Phatthana Nikhom District, Lop Buri Province | 14°55'12.3"N, 101°13'10.9"E, Altitude 136 m | 358 | 5: *L. bicolor* (5) | 1.40 | 48 | 0 | 0 |
| **C4** | SUT 0515055 | Pond of Silpakorn University (Tha Chin river system), Muang District, Nakhon Pathom Province | 13°49'01.2"N, 100°02'27.9"E, Altitude 79 m | 381 | 2: *L. bicolor* (2) | 0.52 | 30 | 0 | 0 |

| NO. | VOUCHER NUMBER | LOCATION | GPS | 2004–2009 | | | 2014–2016 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. of collected snails | No. of infected snails | Infection rates (%) | No. of collected snails | No. of infected snails | Infection rates (%) |
| **C5** | SUT 0515091 | Hin dad hot spring (Khwae Noi drainage, Mae Klong river system), Thong Pha Phum District, Kanchanaburi Province | 14°37'25.9"N, 098°43'40.5"E, Altitude 159 m | 39 | 5: *L. bicolor* (1), *H. pumilio* (3), *S. tridactyla* (1) | 12.82 | 2 | 0 | 0 |
| **C6** | SUT 0515092 | Sai Yok Yai waterfall (Khwae drainage, Mae Klong river system), Sai Yok District, Kanchanaburi Province | 14°26'03.0"N, 098°51'14.7"E, Altitude 104 m | * | * | * | 49 | 0 | 0 |
| **C7** | SUT 0515093 | Sai Yok Noi waterfall (Khwae drainage, Mae Klong river system), Sai Yok District, Kanchanaburi Province | 14°14'27.6"N, 099°03'55.9"E, Altitude 116 m | * | * | * | 29 | 0 | 0 |
| **C8** | SUT 0515061 | Ban Thung Makham Tia (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province | 13°54'18.1"N, 099°23'07.8"E, Altitude 45 m | * | * | * | 42 | 1: *S. tridactyla* (1) | 2.38 |
| **C9** | SUT 0515060 | Ban Ta Pu (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province | 13°51'17.7"N, 099°22'58.9"E, Altitude 56 m | * | * | * | 99 | 0 | 0 |
| **C10** | SUT 0515059 | Ban Nong Phai (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province | 13°46'44.8"N, 099°25'26.7"E, Altitude 72 m | * | * | * | 118 | 5: *S. tridactyla* (3), *P. gralli* (1), Gymnocephalous (1) | 4.24 |
| **THE SOUTH** | | | | | | | | | |
| **S1** | SUT 0515066 | Ban Purakom (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province | 13°19'29.2"N, 099°14'22.0"E, Altitude 277 m | * | * | * | 280 | 30: *L. bicolor* (29), *S. tridactyla* (1) | 10.71 |
| **S2** | SUT 0515069 | Huay Nueng (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province | 13°32'52.2"N, 099°17'33.7"E, Altitude 156 m | 832 | 94: *L. bicolor* (30), *S. tridactyla* (64) | 11.30 | 272 | 23: *L. liberum* (2), *S. tridactyla* (21) | 8.46 |
| **S3** | SUT 0515070 | Lum Nam Phachi (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province | 13°32'54.2"N, 099°21'42.3"E, Altitude 110 m | * | * | * | 242 | 5: *S. tridactyla* (5) | 2.07 |
| **S4** | SUT 0515057 | Ban Dan Thap Tako (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province | 13°41'28.1"N, 099°29'08.1"E, Altitude 82 m | * | * | * | 240 | 11: *L. bicolor* (3), *L. liberum* (8) | 4.58 |
| **S5** | SUT 0515058 | Phachi river Bridge (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province | 13°45'00.5"N, 099°26'27.4"E, Altitude 65 m | * | * | * | 292 | 16: *L. bicolor* (1), *M. caridinae* (10), *S. tridactyla* (4), *P. gralli* (1) | 5.48 |
| **S6** | SUT 0515056 | Ban Pa Wai (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province | 13°37'0.15"N, 099°24'36.9"E, Altitude 74 m | * | * | * | 111 | 11: *L. bicolor* (3), *M. caridinae* (4), *S. tridactyla* (3), *P. gralli* (1) | 9.91 |
| **S7** | SUT 0515071 | Huai Ban Bor (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province | 13°32'07.4"N, 099°20'31.8"E, Altitude 137 m | * | * | * | 196 | 21: *M. obstipus* (1), *S. tridactyla* (20) | 10.71 |
| **S8** | SUT 0513032 | Khlong Cha-am (Cha-am canal, Gulf of Thailand), Cha-am District, Phetchaburi Province | 12°48'02.7"N, 099°58'53.2"E, Altitude 22 m | * | * | * | 72 | 0 | 0 |
| **S9** | SUT 0516146 | Khlong Bueng reservoir (Bueng canal, Gulf of Thailand), Muang District, Prachuap Khiri Khan Province | 11°55'29.1"N, 099°42'40.9"E, Altitude 72 m | * | * | * | 92 | 0 | 0 |
| **S10** | SUT 0514037 | Khlong Huai Yang (Yang canal), Thap Sakae District, Prachuap Khiri Khan Province | 11°36'50.0"N, 099°40'07.9"E, Altitude 53 m | 961 | 1: *L. bicolor* (1) | 0.10 | 22 | 0 | 0 |
| **S11** | SUT 0514038 | Kar on waterfall (Nongyaplong canal), Bang Saphan District, Prachuap Khiri Khan Province | 11°26'14.4"N, 099°26'33.0"E, Altitude 53 m | 685 | 5: *L. bicolor* (5) | 0.73 | 39 | 0 | 0 |
| **S12** | SUT 0516149 | Krapo waterfall (Tha Sae canal), Tha Sae District, Chumphon Province | 10°44'28.8"N, 099°12'54.9"E, Altitude 74 m | 223 | 181: *L. bicolor* (32), *S. tridactyla* (149) | 81.17 | 30 | 0 | 0 |
| **S13** | SUT 0516137 | Khlong Klai (Nong Noi canal, Ta Pi river system), Ban Na San District, Surat Thani Province | 08°48'06.9"N, 099°26'45.1"E, Altitude 108 m | * | * | * | 104 | 4: *L. bicolor* (4) | 3.85 |
| **S14** | SUT 0514048 | Dat Fa waterfall (Lumpool canal, Ta Pi river system), Ban Na San District, Surat Thani Province | 08°52'18.8"N, 099°25'59.1"E, Altitude 79 m | * | * | * | 144 | 2: *L. bicolor* (1), *S. tridactyla* (1) | 1.39 |

| NO. | VOUCHER NUMBER | LOCATION | GPS | 2004–2009 | | | 2014–2016 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. of collected snails | No. of infected snails | Infection rates (%) | No. of collected snails | No. of infected snails | Infection rates (%) |
| **S15** | SUT 0516142 | Vibhavadi waterfall (Tha Thong canal), Don Sak District, Surat Thani Province | 09°08'07.2"N, 099°40'31.6"E, Altitude 26 m | * | * | * | 107 | 24: *S. tridactyla* (17), *C. formosanus* (7) | 22.43 |
| **S16** | SUT 0516147 | Khlong Tha Sai (Takhoei canal, Gulf of Thailand), Tha Chang District, Surat Thani Province | 09°12'39.8"N, 099°11'55.7"E, Altitude 8 m | * | * | * | 20 | 0 | 0 |
| **S17** | SUT 0516148 | Ban Tung Ao (Ta Khoei canal, Gulf of Thailand), Phunphin District, Surat Thani Province | 09°12'25.7"N, 099°12'25.7"E, Altitude 7 m | * | * | * | 35 | 0 | 0 |
| **S18** | SUT 0516145 | Krung Ching waterfall (Klai canal), Nopphitam District, Nakhon Si Thammarat Province | 08°43'17.3"N, 099°40'14.8"E, Altitude 195 m | 157 | 12: *L. bicolor* (5), *A. hitaense* (2), *S. tridactyla* (5) | 7.64 | 22 | 4: *L. bicolor* (4) | 18.18 |
| **S19** | SUT 0516139 | Khlong Prong (Klai canal), Nopphitam District, Nakhon Si Thammarat Province | 08°47'23.0"N, 099°38'13.2"E, Altitude 98 m | * | * | * | 50 | 11: *L. bicolor* (1), *S. tridactyla* (10) | 22.00 |
| **S20** | SUT 0515097 | Khlong Sai (Khlong Sai canal, Andaman sea), Muang District, Krabi Province | 08°10'20.8"N, 098°47'37.6"E, Altitude 23 m | * | * | * | 5 | 0 | 0 |
| **S21** | SUT 0515098 | Wang Than Thip (Wang Than Thip canal, Andaman sea), Muang District, Krabi Province | 08°09'49.2"N, 098°47'50.9"E, Altitude 21 m | * | * | * | 42 | 0 | 0 |
| **S22** | SUT 0515095 | Khlong Palian (Palian canal), Yan Ta Khao District, Trang Province | 07°22'11.0"N, 099°40'47.9"E, Altitude 19 m | 77 | 15: *L. bicolor* (2), *S. tridactyla* (11), *C. alseae* (2) | 19.48 | 15 | 4: *S. tridactyla* (4) | 26.67 |
| **S23** | SUT 0516138 | Khlong Tha Leung (Tha Nae canal), Si Banphot District, Phatthalung Province | 07°42'48.3"N, 099°51'33.6"E, Altitude 70 m | * | * | * | 36 | 14: *M. obstipus* (5), *S. tridactyla* (9) | 38.89 |
| **S24** | SUT 0516141 | Khlong La reservoir (Utaphao canal, Gulf of Thailand), Khlong Hoi Khong District, Songkhla Province | 06°52'29.3"N, 100°19'48.4"E, Altitude 60 m | * | * | * | 35 | 0 | 0 |
| **S25** | SUT 0516144 | Khlong Sathing Mo (Songkhla lake, Gulf of Thailand), Singhanakhon District, Songkhla Province | 07°13'36.6"N, 100°31'41.8"E, Altitude 10 m | * | * | * | 3 | 0 | 0 |
| **S26** | SUT 0516143 | Khlong Cham Rai reservoir (Utaphao canal), Khlong Hoi Khong District, Songkhla Province | 06°49'29.5"N, 100°19'49.7"E, Altitude 56 m | * | * | * | 139 | 3: *L. liberum* (3) | 2.16 |
| **TOTAL** | | | | 6,583 | 1,084 | 16.47 | 8,493 | 493 | 5.80 |

N = North, NE = Northeast, E = East, C = Central, S = South
* = no record.

| | |
|---|---|
| Stylet | 3–3 µm (mean: 3 µm) × 10–23 µm (mean: 16 µm) |
| Oral sucker | 13–30 µm (mean: 24 µm) × 10–28 µm (mean: 20 µm) |
| Pharynx | 5–15 µm (mean: 10 µm) × 8–10 µm (mean: 8 µm) |
| Ventral sucker | 8–33 µm (mean: 18 µm) × 13–28 µm (mean: 19 µm) |
| Excretory bladder | 13–35 µm (mean: 27 µm) × 13–48 µm (mean: 37 µm) |
| Tail | 15–25 µm (mean: 20 µm) × 40–90 µm (mean: 72 µm) |

### 1.3 *Acanthatrium histaense* Koga, 1953

(Fig. 5)

Body oval. Oral sucker with stylet, virgulate organ near oral sucker. Pharynx round and short, esophagus absent. Ventral sucker smaller than oral sucker. Two pairs of penetration glands present, one anterior pair with fine granules and one posterior pair with coarse granules. Excretory bladder near posterior end of body. Tail short, spinose at its end.

The cercariae develop within sporocysts.

The infection rate was 1.11% (167/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 54–93 µm (mean: 78 µm) × 80–110 µm (mean: 100 µm) |
| Stylet | 9–14 µm (mean: 11 µm) × 12–14 µm (mean: 12 µm) |
| Oral sucker | 26–33 µm (mean: 31 µm) × 35–41 µm (mean: 38 µm) |
| Pharynx | 11–16 µm (mean: 14 µm) × 13–25 µm (mean: 21 µm) |

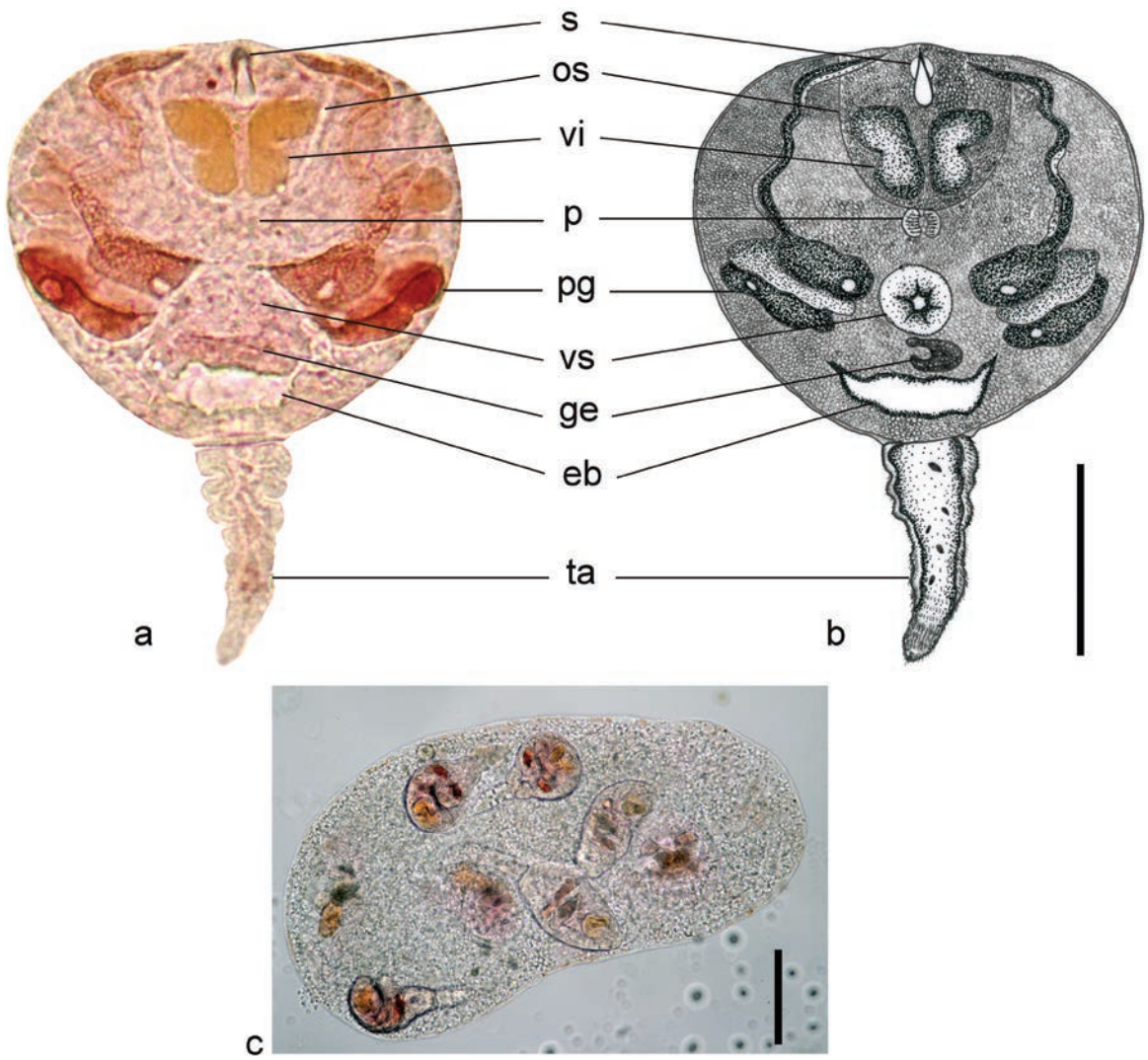



**Figure 3.** Images of *Loxogenoides bicolor* (Krull, 1933). **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ge: genital primordium; p: pharynx; pg: penetration gland; os: oral sucker; s: stylet; ta: tail; vi: virgulate organ; vs: ventral sucker. Scale bars: 50 µm.

| | |
|---|---|
| Ventral sucker | 15–17 µm (mean: 17 µm) × 16–19 µm (mean: 18 µm) |
| Excretory bladder | 9–13 µm (mean: 10 µm) × 21–47 µm (mean: 39 µm) |
| Tail | 18–26 µm (mean: 24 µm) × 27–76 µm (mean: 69 µm) |

**Type 2. Armatae xiphidiocercariae cercariae**

**Microphallidae Ward, 1901 (sensu Travassos 1921)**

**2.1 *Maritreminoides caridinae* (Yamaguti & Nisimura, 1944) (sensu Chen 1957)**
(Fig. 6)

Body oval, rather small. Stylet present, but virgulate organ absent. Pharynx small, esophagus Y-shaped. Ventral sucker poorly developed. Two pairs penetration glands present, located near the middle of the body. Excretory bladder thin-walled, located in the posterior part of the body. Tail long and round.

The cercariae develop within sporocysts.

The infection rate was 0.10% (15/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 78–98 µm (mean: 89 µm) × 105–133 µm (mean: 113 µm) |
| Stylet | 3–3 µm (mean: 3 µm) × 10–18 µm (mean: 15 µm) |
| Oral sucker | 18–30 µm (mean: 25 µm) × 20–30 µm (mean: 23 µm) |
| Pharynx | 5–10 µm (mean: 8 µm) × 5–10 µm (mean: 9 µm) |
| Ventral sucker | 15–20 µm (mean: 19 µm) × 15–20 µm (mean: 18 µm) |
| Excretory bladder | 30–40 µm (mean: 34 µm) × 15–18 µm (mean: 16 µm) |

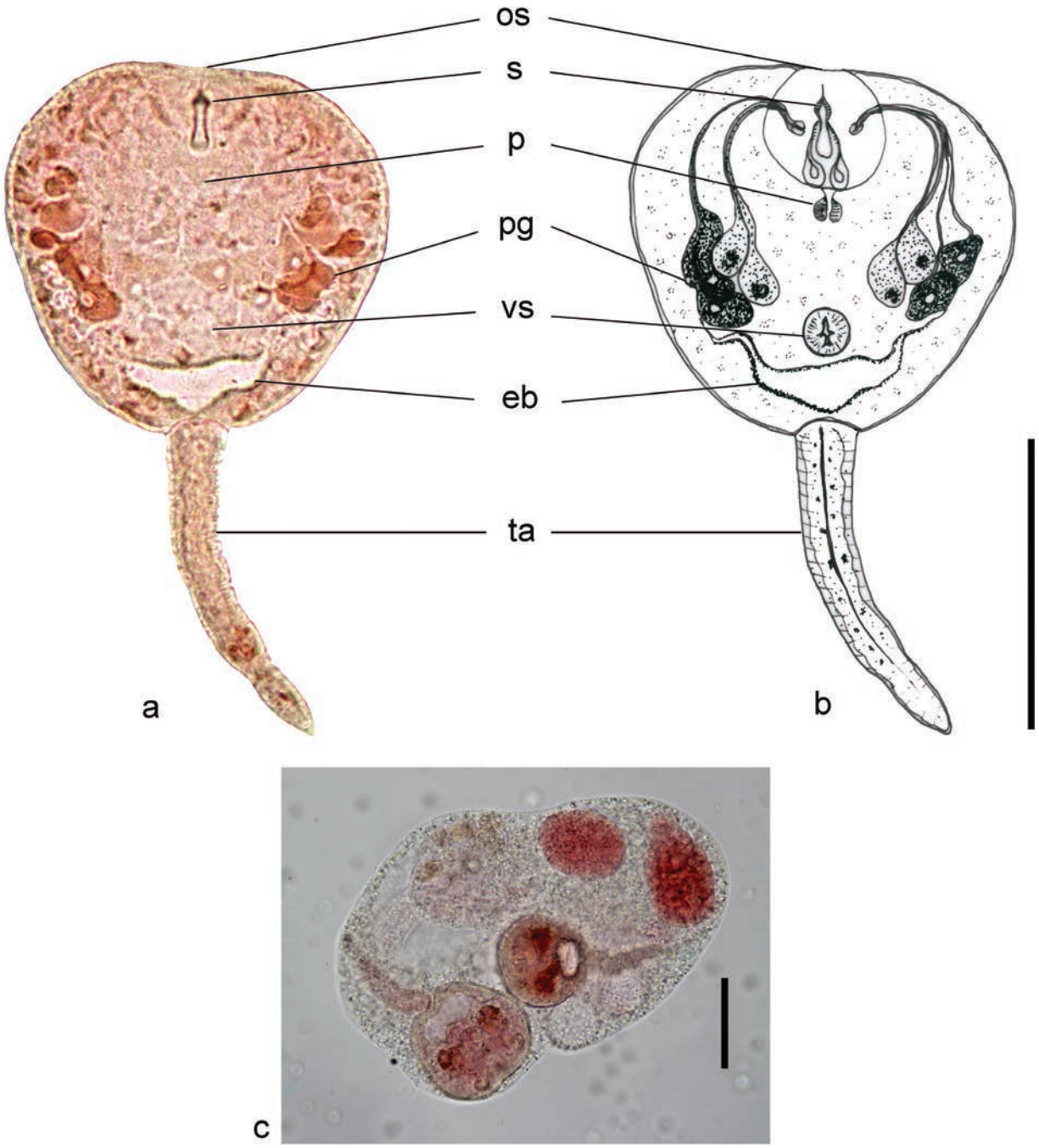



**Figure 4.** *Loxogenes liberum* Seno, 1907. **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; os: oral sucker, p: pharynx, pg: penetration gland, s: stylet; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

| | |
|---|---|
| Tail | 13–20 µm (mean: 16 µm) × 85–125 µm (mean: 106 µm) |

### 2.2 *Maritreminoides obstipus* (Van Cleave & Mueller, 1932) (sensu Rankin 1939)

(Fig. 7)

Body oval, rather small. Oral and ventral sucker of approximately equal in size. Oral sucker with long stylet, virgulate organ absent. Pharynx rather large, esophagus short and slender, bifurcating, located between oral and ventral sucker. Genital primordium located just posterior of ventral sucker. Four pairs of penetration glands grouped together near anterior margin of ventral sucker. Excretory bladder thin-walled. Tail shorter than body and round, not spinose at its tip.

The cercariae develop within sporocysts.

The infection rate was 0.05% (7/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

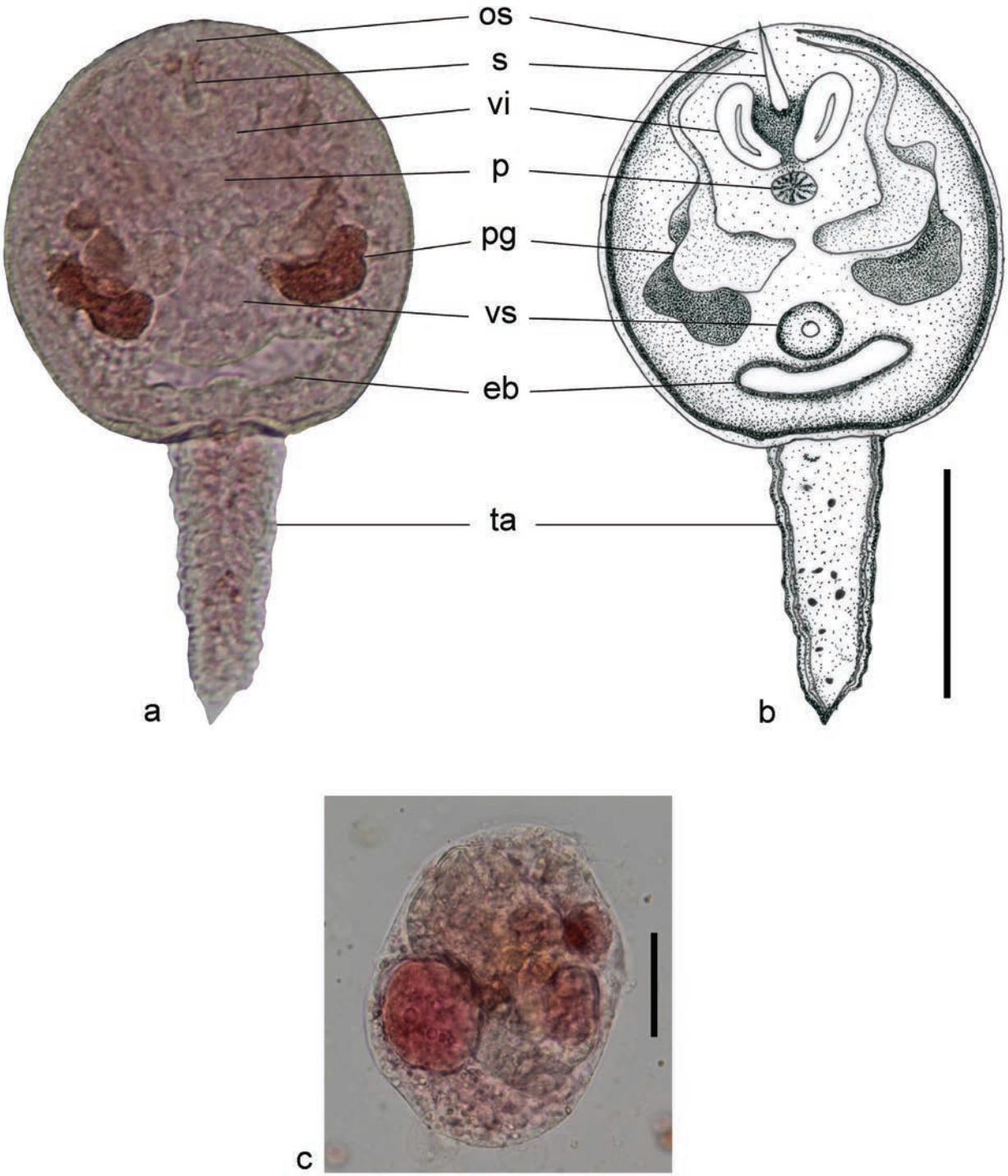



**Figure 5.** *Acanthatrium histaense* Koga, 1953. **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; os: oral sucker; s: stylet; p: pharynx; pg: penetration gland; ta: tail; vi: virgulate organ; vs: ventral sucker. Scale bars: 50 µm.

| | | | |
|---|---|---|---|
| Body | 73–103 µm (mean: 89 µm) × 85–128 µm (mean: 106 µm) | Ventral sucker | 13–20 µm (mean: 16 µm) × 10–20 µm (mean: 15 µm) |
| Stylet | 3–3 µm (mean: 3 µm) × 13–18 µm (mean: 16 µm) | Excretory bladder | 18–35 µm (mean: 28 µm) × 13–23 µm (mean: 16 µm) |
| Oral sucker | 20–30 µm (mean: 25 µm) × 13–30 µm (mean: 24 µm) | Tail | 15–28 µm (mean: 20 µm) × 65–113 µm (mean: 82 µm) |
| Pharynx | 8–13 µm (mean: 9 µm) × 5–13 µm (mean: 9 µm) | | |

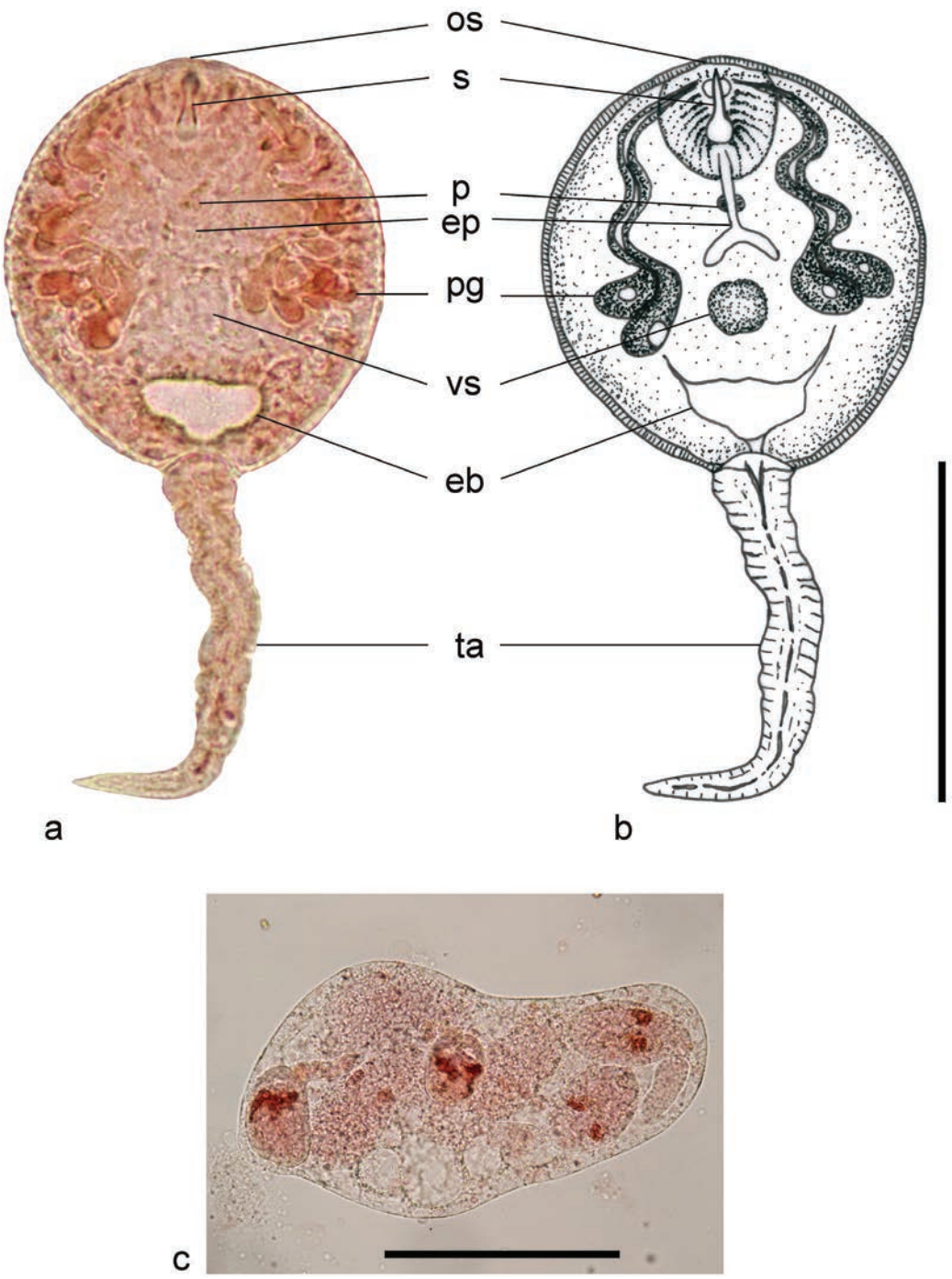



**Figure 6.** *Maritreminoides caridinae* (Yamaguti & Nisimura, 1944). **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; os: oral sucker; p: pharynx; pg: penetration gland; s: stylet; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

### Type 3. Parapleurophocercous cercariae

### Heterophyidae (Leiper, 1909) (sensu Odhner 1914)

### 3.1 *Haplorchis pumilio* (Looss, 1896) (sensu Looss 1899)

(Fig. 8)

The cercarial body is pear-shaped. It has a circular oral sucker that is located near the proximal end of the body. The mouth is equipped with transverse rows of spines. The small ventral sucker is located approximately at two-thirds of the body length measured from the front. The small pharynx is situated in the anterior part of the body just distal of the oral sucker between the two distinct eyespots; an esophagus is absent. There are seven pairs of penetration glands, which are arranged laterally in two longitudinal rows in the posterior two thirds of the body. The excretory bladder has an oval shape and is

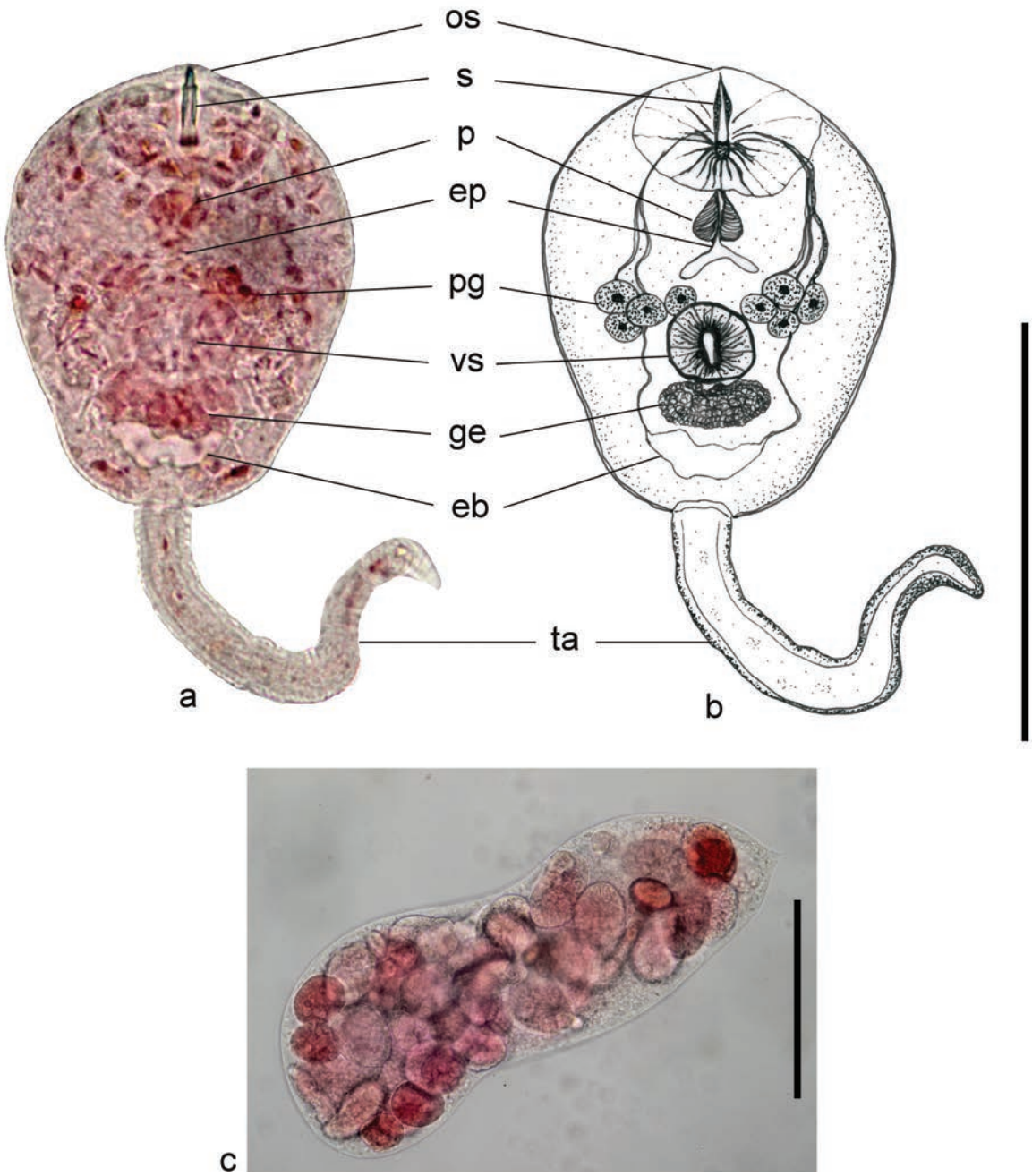



**Figure 7.** *Maritreminoides obstipus* (Van Cleave & Müller, 1932). **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; ge: genital primordium; os: oral sucker; p: pharynx; pg: penetration gland; s: stylet; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

dark pigmented. A genital primordium is present, located between the ventral sucker and the excretory bladder. The tail is longer than the body and rather slender, and is equipped with lateral finfolds proximally and a dorso-ventral finfold along the longer distal portion.

The cercariae develop within rediae.

The infection rate was 0.72% (108/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 91–141 µm (mean: 125 µm) × 169–296 µm (mean: 258 µm) |
| Oral sucker | 28–49 µm (mean: 37 µm) × 28–49 µm (mean: 36 µm) |
| Pharynx | 9–11 µm (mean: 10 µm) × 13–20 µm (mean: 16 µm) |
| Ventral sucker | 15–25 µm (mean: 19 µm) × 15–24 µm (mean: 18 µm) |
| Excretory bladder | 29–41 µm (mean: 35 µm) × 29–41 µm (mean: 35 µm) |
| Tail | 11–37 µm (mean: 31 µm) × 466–529 µm (mean: 491 µm) |
| Lateral finfolds | 9–18 µm (mean: 14.75 µm) × 70–129 µm (mean: 111 µm) |

**Table 3.** Distribution of trematodes obtained from *Tarebia granifera* (A total of 15,076 snails) in Thailand (N = North, NE = Northeast, E = East, C = Central, S = South).

| Type and species of trematodes | 2004–2009 No. infected snails | | | | | 2014–2016 No. infected snails | | | | | Total | Infection rate (%) (infected snail / no. of the total collected snails = 15,076) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | NE | E | C | S | N | NE | E | C | S | | |
| **Type 1. Virgulate xiphidiocercariae cercariae** | | | | | | | | | | | | |
| **1. *Loxogenoides bicolor*** | 304 | 0 | 0 | 9 | 75 | 122 | 22 | 0 | 1 | 46 | 579 | 3.84 |
| **2. *Loxogenes liberum*** | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 1 | 13 | 23 | 0.15 |
| **3. *Acanthatrium histaense*** | 164 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 167 | 1.11 |
| **Total** | **468** | **1** | **0** | **9** | **77** | **131** | **22** | **0** | **2** | **59** | **769** | **5.10** |
| **Type 2. Armatae xiphidiocercariae cercariae** | | | | | | | | | | | | |
| **1. *Maritreminoides caridinae*** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 14 | 15 | 0.10 |
| **2. *Maritreminoides obstipus*** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 6 | 7 | 0.05 |
| **Total** | **0** | **0** | **0** | **0** | **0** | **2** | **0** | **0** | **0** | **20** | **22** | **0.15** |
| **Type 3. Parapleurophocercous cercariae** | | | | | | | | | | | | |
| **1. *Haplorchis pumilio*** | 98 | 0 | 0 | 3 | 0 | 7 | 0 | 0 | 0 | 0 | 108 | 0.72 |
| **2. *Haplorchis taichui*** | 0 | 0 | 0 | 0 | 0 | 10 | 4 | 0 | 0 | 0 | 14 | 0.09 |
| **3. *Stictodora tridactyla*** | 0 | 0 | 0 | 1 | 229 | 111 | 0 | 0 | 4 | 95 | 440 | 2.92 |
| **Total** | **98** | **0** | **0** | **4** | **229** | **128** | **4** | **0** | **4** | **95** | **562** | **3.73** |
| **Type 4. Pleurophocercous cercariae** | | | | | | | | | | | | |
| **1. *Centrocestus formosanus*** | 160 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 7 | 172 | 1.14 |
| **Total** | **160** | **0** | **0** | **0** | **0** | **3** | **2** | **0** | **0** | **7** | **172** | **1.14** |
| **Type 5. Megarulous cercariae** | | | | | | | | | | | | |
| **1. *Philophthalmus gralli*** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 0.02 |
| **Total** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **1** | **2** | **3** | **0.02** |
| **Type 6. Furcocercous cercariae** | | | | | | | | | | | | |
| **1. *Cardicola alseae*** | 5 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 7 | 0.05 |
| **2. *Alaria mustelae*** | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0.15 |
| **3. *Transversotrema laruei*** | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0.05 |
| **Total** | **36** | **0** | **0** | **0** | **2** | 0 | 0 | 0 | 0 | 0 | **38** | **0.25** |
| **Type 7. Echinostome cercariae** | | | | | | | | | | | | |
| **1. Echinostome cercariae** | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 10 | 0.07 |
| **Total** | **0** | **0** | **0** | **0** | **0** | **10** | **0** | **0** | **0** | **0** | **10** | **0.07** |
| **Type 8. Gymnocephalous cercariae** | | | | | | | | | | | | |
| **1. Gymnocephalous cercariae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0.01 |
| **Total** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **1** | **0** | **1** | **0.01** |

### 3.2 *Haplorchis taichui* (Nishigori, 1924) (sensu Witenberg 1930)

(Fig. 9)

Body is oval in shape. The oral sucker is located at the anterior of body. The mouth aperture is equipped with transverse rows of spines. A pair of pigmented eyespots and pharynx are present. Seven pairs of penetration glands extend from the pharynx to the posterior end of the body. Cystogenous cells are arranged in lateral fields from the level of the pharynx to the posterior end of the body. The excretory bladder is saccular and thick-walled. The tail is longer than the body. There are lateral finfolds at one-third of tail tunk and a dorso-ventral finfold at the distal portion.

The cercariae develop within rediae.

The infection rate was 0.09% (14/15,076) (Table 3)

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 43–83 µm (mean: 61 µm) × 105–140 µm (mean: 120 µm) |
| Oral sucker | 20–30 µm (mean: 25 µm) × 23–35 µm (mean: 28 µm) |
| Ventral sucker | 15–33 µm (mean: 23 µm) × 18–30 µm (mean: 25 µm) |
| Pharynx | 8–20 µm (mean: 14 µm) × 8–25 µm (mean: 12 µm) |
| Excretory bladder | 10–50 µm (mean: 26 µm) × 20–35 µm (mean: 26 µm) |
| Tail | 20–30 µm (mean: 26 µm) × 263–355 µm (mean: 311 µm) |
| Lateral finfolds | 8–15 µm (mean: 13 µm) × 75–125 µm (mean: 103 µm) |
| Dorsal finfolds | 5–23 µm (mean: 13 µm) × 183–253 µm (mean: 218 µm) |

### 3.3 *Stictodora tridactyla* Martin & Kuntz, 1955

(Fig. 10)

The body is oval in shape. The oral sucker is located at the anterior end of the body. There are three transverse rows of oral spines present. Seven pairs of penetration glands in

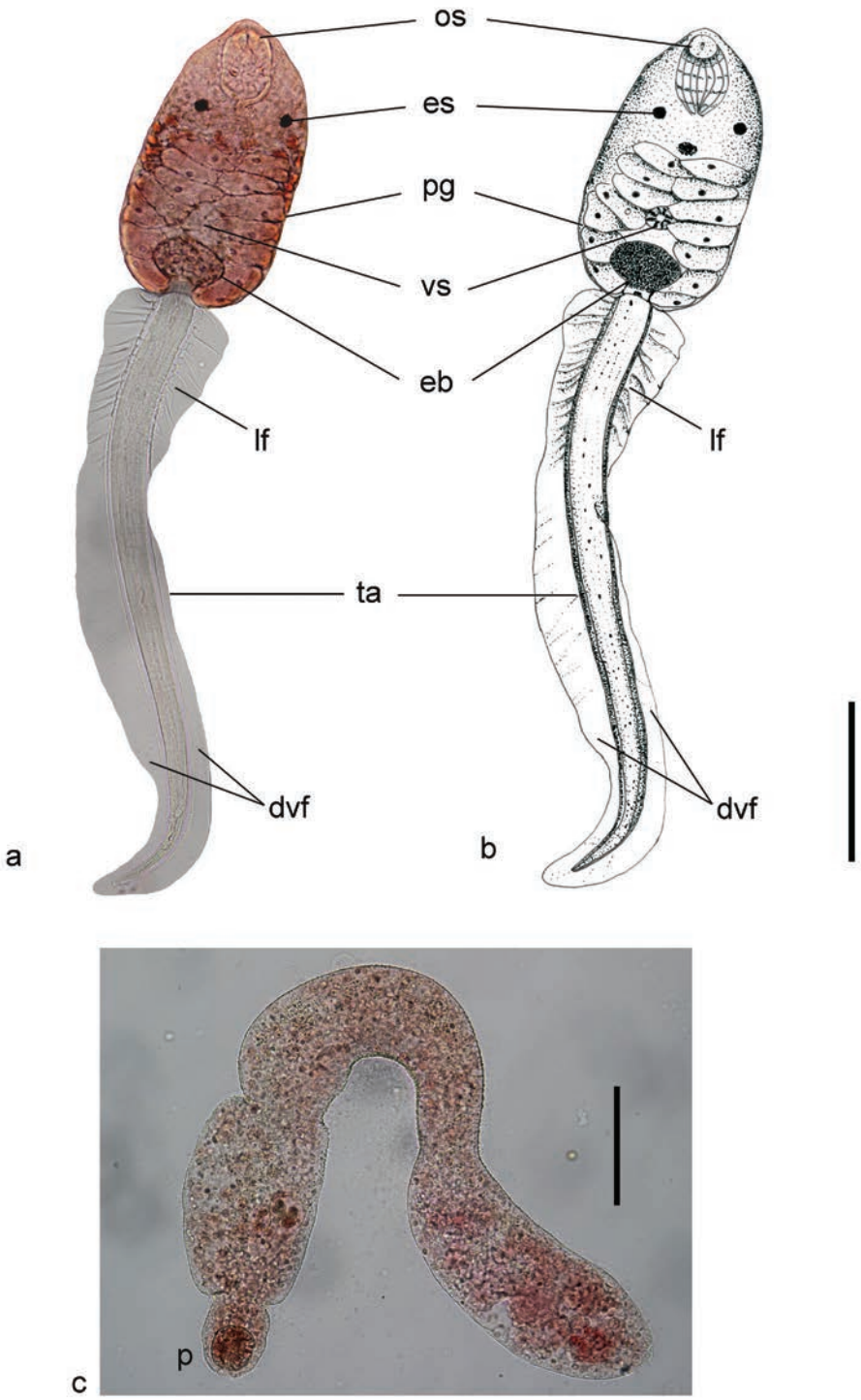



**Figure 8.** *Haplorchis pumilio* (Looss, 1896). **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. redia stained with 0.5% neutral red. Abbreviations – dvf: dorsoventral finfold; eb: excretory bladder; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta – tail; vs: ventral sucker. Scale bars: 50 µm.

four groups of 3:4:4:3 are present that are situated between the pharynx and the excretory bladder. A pair of pigmented eyespots and a pharynx are present. The ventral sucker is poorly developed. The excretory bladder is V-shaped and thick-walled. The tail is longer than the body. There is a bilateral finfold and a dorso-ventral finfold on the tail.

The cercariae develop within rediae.

The infection rate was 2.92% (440/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 80–118 µm (mean: 99 µm) × 168–207 µm (mean: 202 µm) |
| Oral sucker | 28–38 µm (mean: 34 µm) × 30–50 µm (mean: 41 µm) |

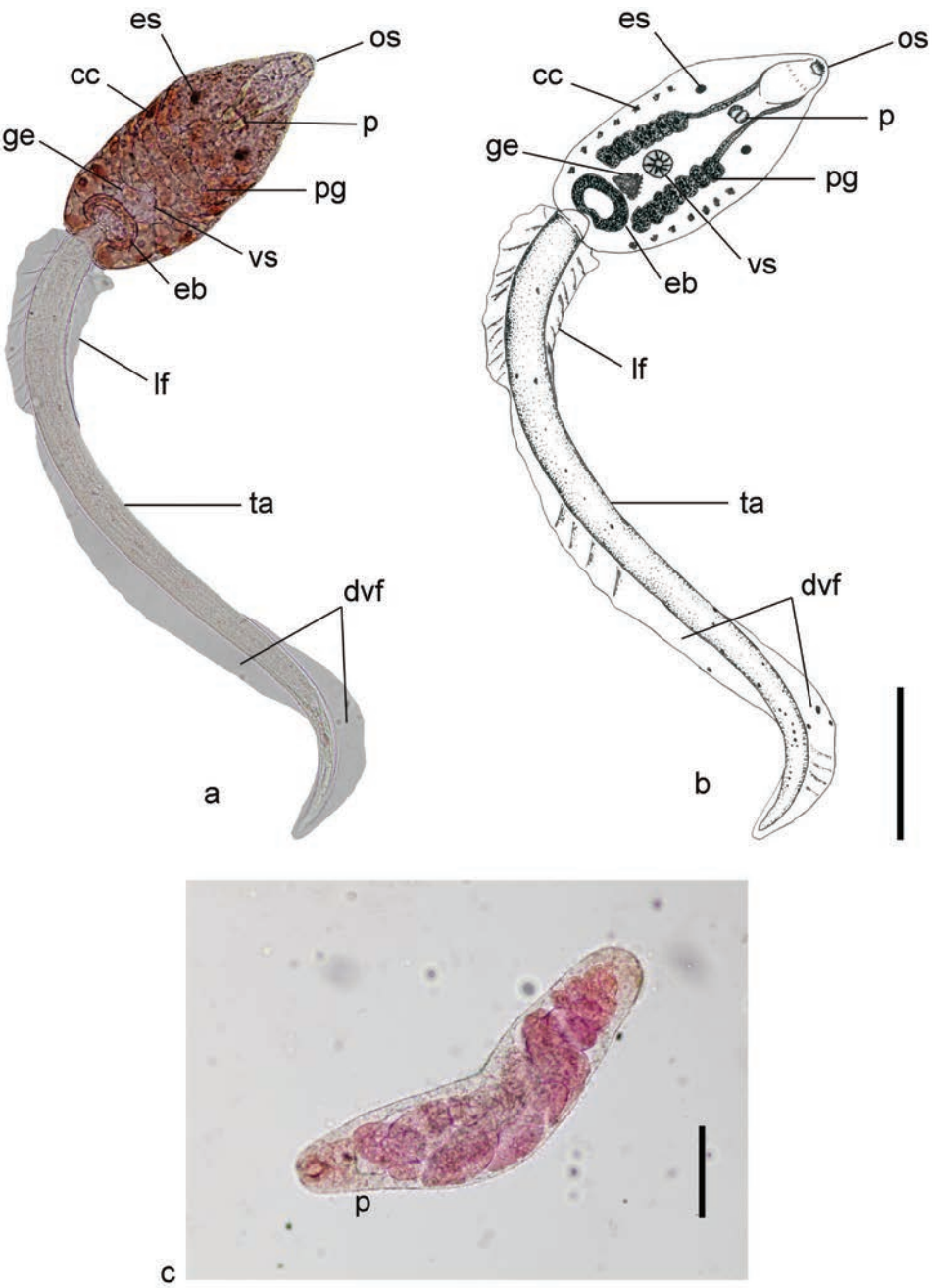



**Figure 9.** *Haplorchis taichui* (Nishigori, 1924). **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. redia stained with 0.5% neutral red. Abbreviations – cc: cystogenous cells; dvf: dorsoventral finfold; eb: excretory bladder; es: eyespot; ge: genital primordium; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

| | |
|---|---|
| Eye spots | 5–15 µm (mean: 9 µm) × 5–15 µm (mean: 9 µm) |
| Pharynx | 10–22 µm (mean: 17 µm) × 10–28 µm (mean: 19 µm) |
| Ventral sucker | 13–35 µm (mean: 23 µm) × 15–45 µm (mean: 27 µm) |
| Excretory bladder | 43–90 µm (mean: 64 µm) × 20–55 µm (mean: 39 µm) |
| Tail | 20–33 µm (mean: 26 µm) × 405–495 µm (mean: 458 µm) |
| Lateral finfold | 10–25 µm (mean: 18 µm) × 74–148 µm (mean: 108) |

**Type 4. Pleurophocercous cercariae**

**Heterophyidae (Leiper, 1909) (sensu Odhner 1914)**

**4.1 *Centrocestus formosanus* (Nishigori, 1924) (sensu Price 1932)**
(Fig. 11)

The body is oval in shape. The oral sucker has oral spines or rostellar hooks like a tapeworm on the dorsal wall of the mouth aperture. A pair of eyespots is located above the prenetration glands at the same level as the pharynx. There are seven pairs of penetration

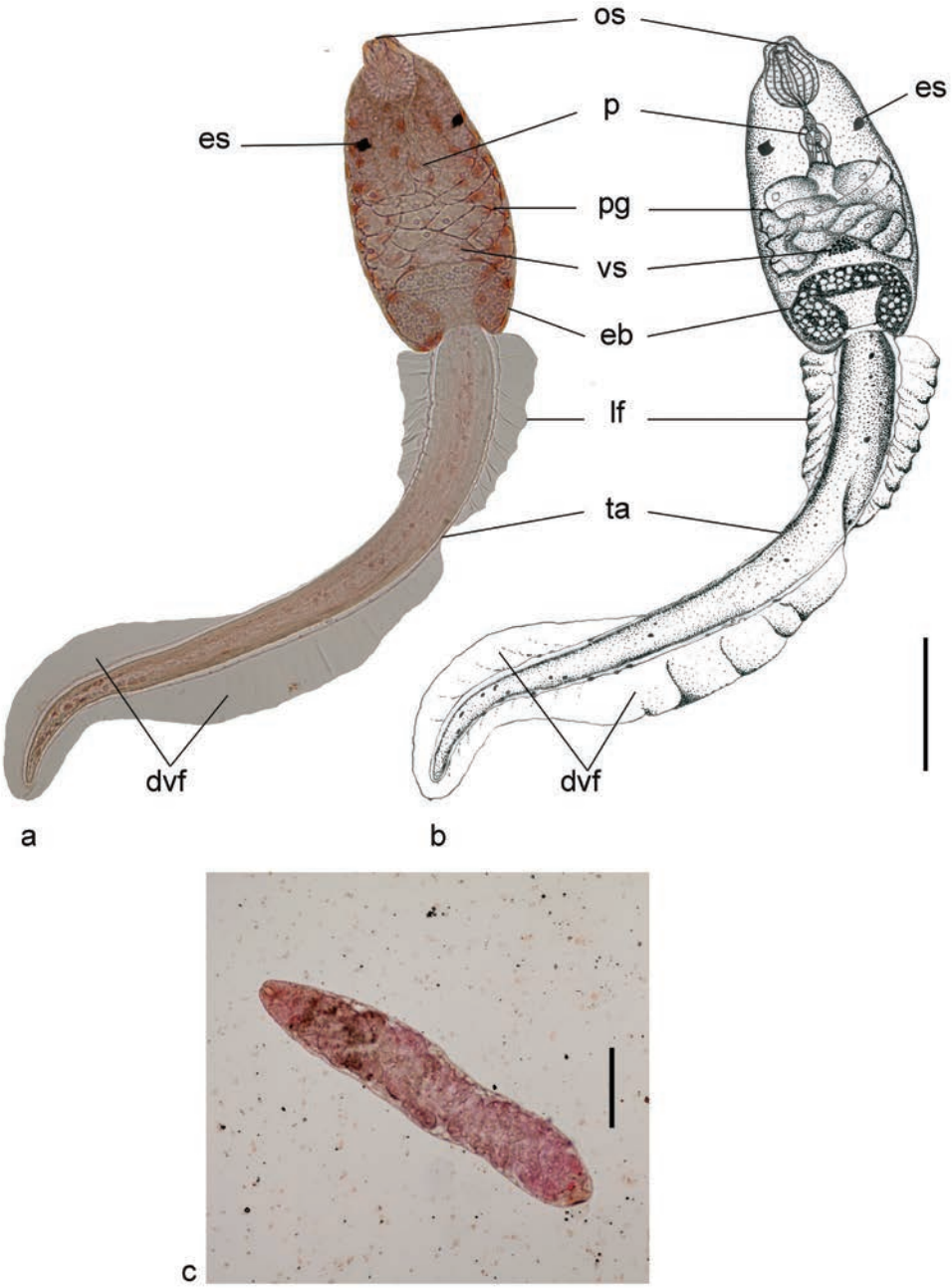



**Figure 10.** *Stictodora tridactyla* Martin & Kuntz, 1955. **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. redia stained with 0.5% neutral red. Abbreviations – dvf: dorsal finfold; eb: excretory bladder; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

glands. The genital primordial is elongated-triangular and located between the ventral sucker and the excretory bladder. The excretory bladder has dark granules and is thin-walled. The tail is slender and longer than the body. It is equipped with very narrow finfolds.

The cercariae develop within rediae.

The infection rate was 1.14% (172/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 45–73 µm (mean: 65 µm) × 83–121 µm (mean: 118 µm) |
| Oral sucker | 17–27 µm (mean: 25 µm) × 18–30 µm (mean: 26 µm) |
| Pharynx | 8–10 µm (mean: 9 µm) × 9–11 µm (mean: 10 µm) |
| Ventral sucker | 13–17 µm (mean: 15 µm) × 14–18 µm (mean: 16 µm) |
| Excretory bladder | 25–31 µm (mean: 29 µm) × 39–53 µm (mean: 46 µm) |
| Tail | 15–18 µm (mean: 15 µm) × 70–93 µm (mean: 83 µm) |

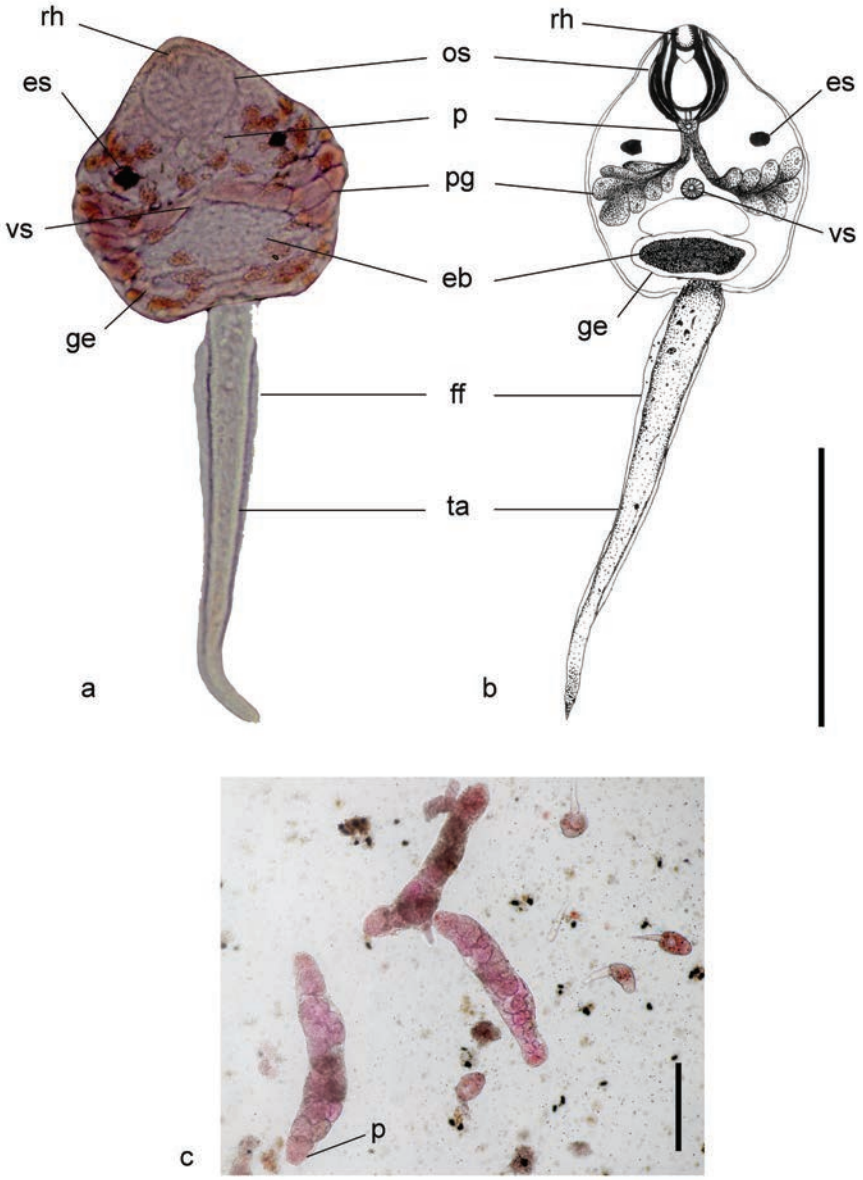



**Figure 11.** *Centrocestus formosanus* (Nishigori, 1924). **a**. Specimen stained with 0.5% neutral red. **b**. Drawing of cercaria. **c**. Redia stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; es: eyespot; ff: finfold; ge: genital primordium; os: oral sucker; p: pharynx; pg: penetration gland; rh: rostellar hooks; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

**Type 5. Megarulous cercariae**

**Philophthalmidae (Looss, 1899) (sensu Travassos 1918)**

**5.1 *Philophthalmus gralli* Mathis & Léger, 1910**
(Fig. 12)

The body is elongate pear-shaped and distinctly granulose. Eyespots are absent. The pharynx is large and extends into an esophagus that is bifurcating (Y-shape) into two blind ending intestinal caeca that almost reach the posterior end of the body. The ventral sucker is bigger than the oral sucker. The excretory bladder is rather small. The tail is about as long as the body and relatively slender. There is an adhesive gland present at its tip.

The cercariae encyst rapidly after developing within rediae.

The infection rate was 0.02% (3/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 143–175 µm (mean: 153 µm) × 438–470 µm (mean: 453 µm) |
| Oral sucker | 50–68 µm (mean: 60 µm) × 63–73 µm (mean: 68 µm) |
| Pharynx | 15–23 µm (mean: 20 µm) × 28–38 µm (mean: 34 µm) |
| Ventral sucker | 60–78 µm (mean: 67 µm) × 48–80 µm (mean: 6 µm) |
| Excretory bladder | 43–48 µm (mean: 45 µm) × 33–40 µm (mean: 36 µm) |
| Tail | 40–50 µm (mean: 45 µm) × 463–475 µm (mean: 469 µm) |

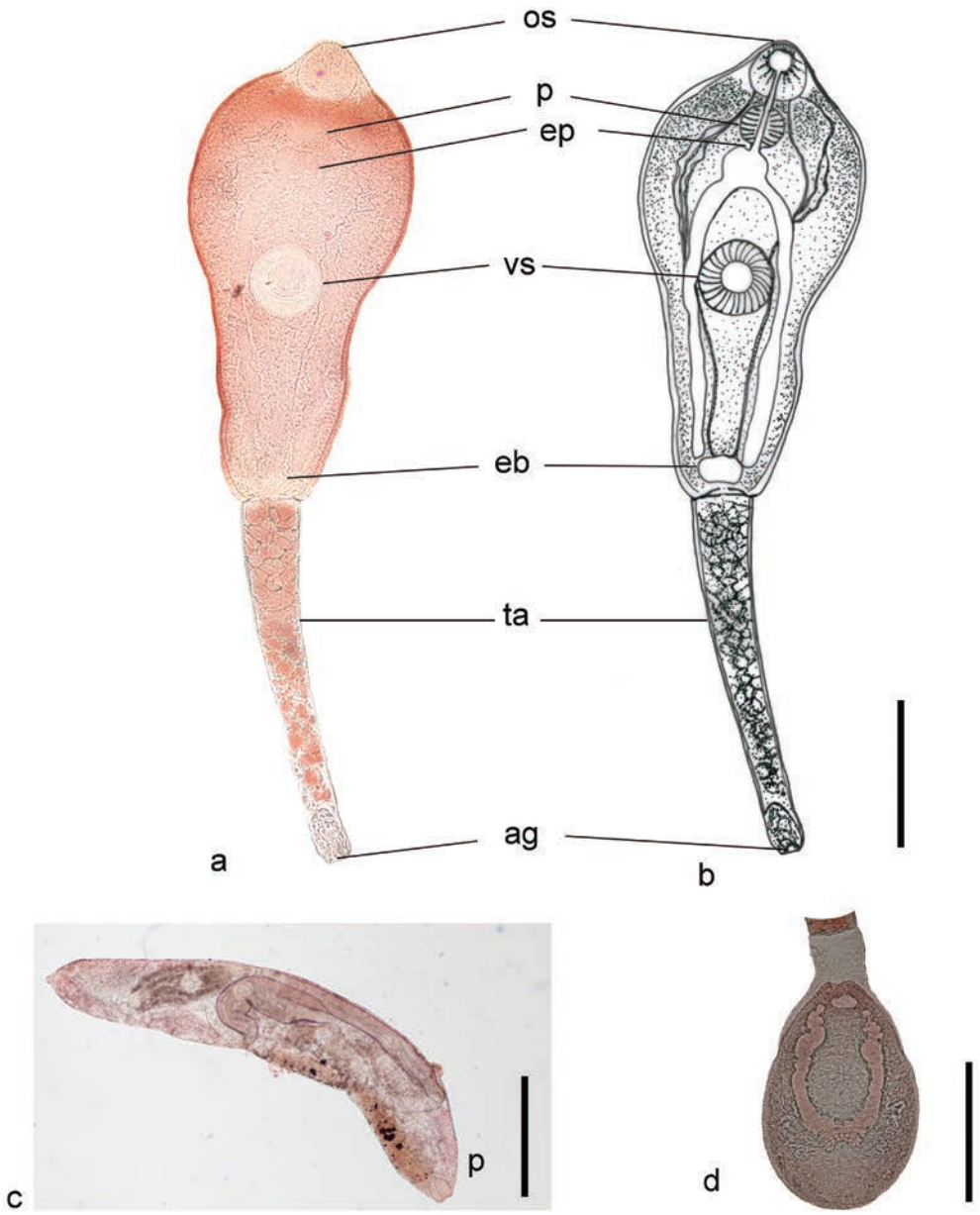



**Figure 12.** *Philophthalmus gralli* Mathis & Léger, 1910 **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. redia stained with 0.5% neutral red. **d**. metacercaria stained with 0.5% neutral red. Abbreviations – ag: adhesive gland; eb: excretory bladder; ep: esophagus; os: oral sucker; p: pharynx; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

**Type 6. Furcocercous cercariae**

**Sanguinicolidae Graff, 1907**

**6.1 *Cardicola alseae* Meade & Pratt, 1965**
(Fig. 13)

The body is elongate-oval, slightly bent. Eyespots, a pharynx, an esophagus, intestinal caeca and a ventral sucker are absent. There is a narrow dorsal finfold in the middle part of the body. The penetration gland is located in the anterior part of the body. The excretory bladder is small and thin-walled, located at the posterior end of the body. The tail is forked. The stem of the tail is rather thick and longer than the furcae. Finfolds are present along the margins of the furcae.

The cercariae develop within sporocysts.

The infection rate was 0.05% (7/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 19–40 µm (mean: 30 µm) × 73–112 µm (mean: 96 µm) |
| Anterior organ | 12–16 µm (mean: 14 µm) × 15–22 µm (mean: 19 µm) |
| Excretory bladder | 4–8 µm (mean: 6 µm) × 12–37 µm (mean: 23 µm) |
| Tail stem | 16–32 µm (mean: 28 µm) × 155–199 µm (mean: 187 µm) |
| Tail furcal | 8–12 µm (mean: 10 µm) × 29–56 µm (mean: 52 µm) |
| Dorso-median finfold | 6–15 µm (mean: 11 µm) |

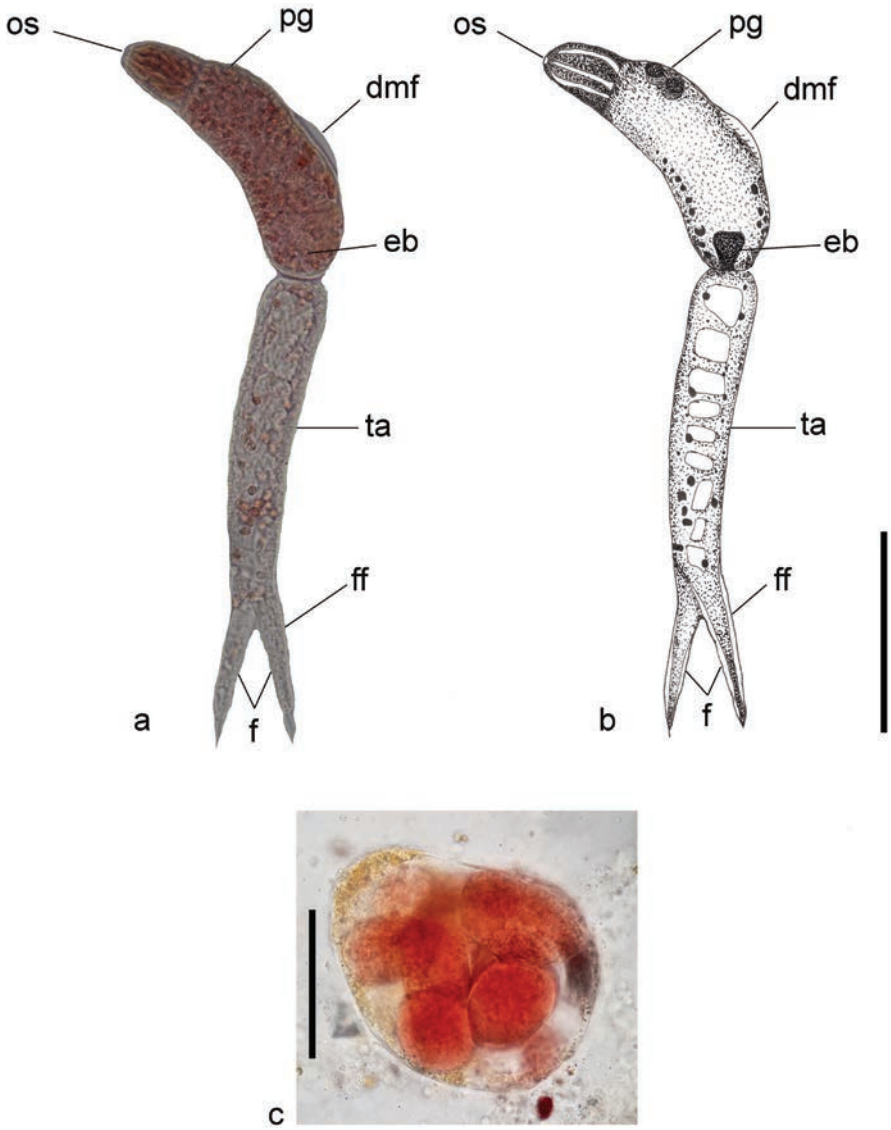



**Figure 13.** *Cardicola alseae* Meade & Pratt, 1965. **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. sporocyst stained with 0.5% neutral red. Abbreviations – dmf: dorso-median finfold; eb: excretory bladder; f: furca; ff: furcal finfold; os: oral sucker; pg: penetration gland; ta: tail. Scale bars: 50 µm.

**Diplostomidae Poirier, 1886**

**6.2 *Alaria mustelae* Bosma, 1931**
(Fig. 14)

The body is elongate-oval in shape. A pair of unpigmented eyespots is present. A prepharynx is present but rather short. The pharynx is small and roundish in shape. The esophagus is long, bifurcating into two intestinal caeca that are shorter than half the length of the esophagus. The oral sucker is larger than the ventral sucker. There are two pairs of penetration glands, filled with dark granules that are located around the ventral sucker. There is a Y-shaped excretory bladder located medially close to the posterior end of the body. The tail is longer than the body and divided into two furcae. The tail stem is slender and about as long as the furcae.

The cercariae develop within sporocysts.

The infection rate was 0.15% (23/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 106–155 µm (mean: 139 µm) × 186–282 µm (mean: 257 µm) |
| Oral sucker | 29–41 µm (mean: 37 µm) × 29–42 µm (mean: 38 µm) |
| Pharynx | 12–16 µm (mean: 14 µm) × 15–20 µm (mean: 17 µm) |
| Ventral sucker | 16–38 µm (mean: 26 µm) × 16–32 µm (mean: 23 µm) |
| Tail | 49–62 µm (mean: 57 µm) × 221–311 µm (mean: 275 µm) |
| Fork-tail | 40–65 µm (mean: 61 µm) × 241–321 µm (mean: 286 µm) |

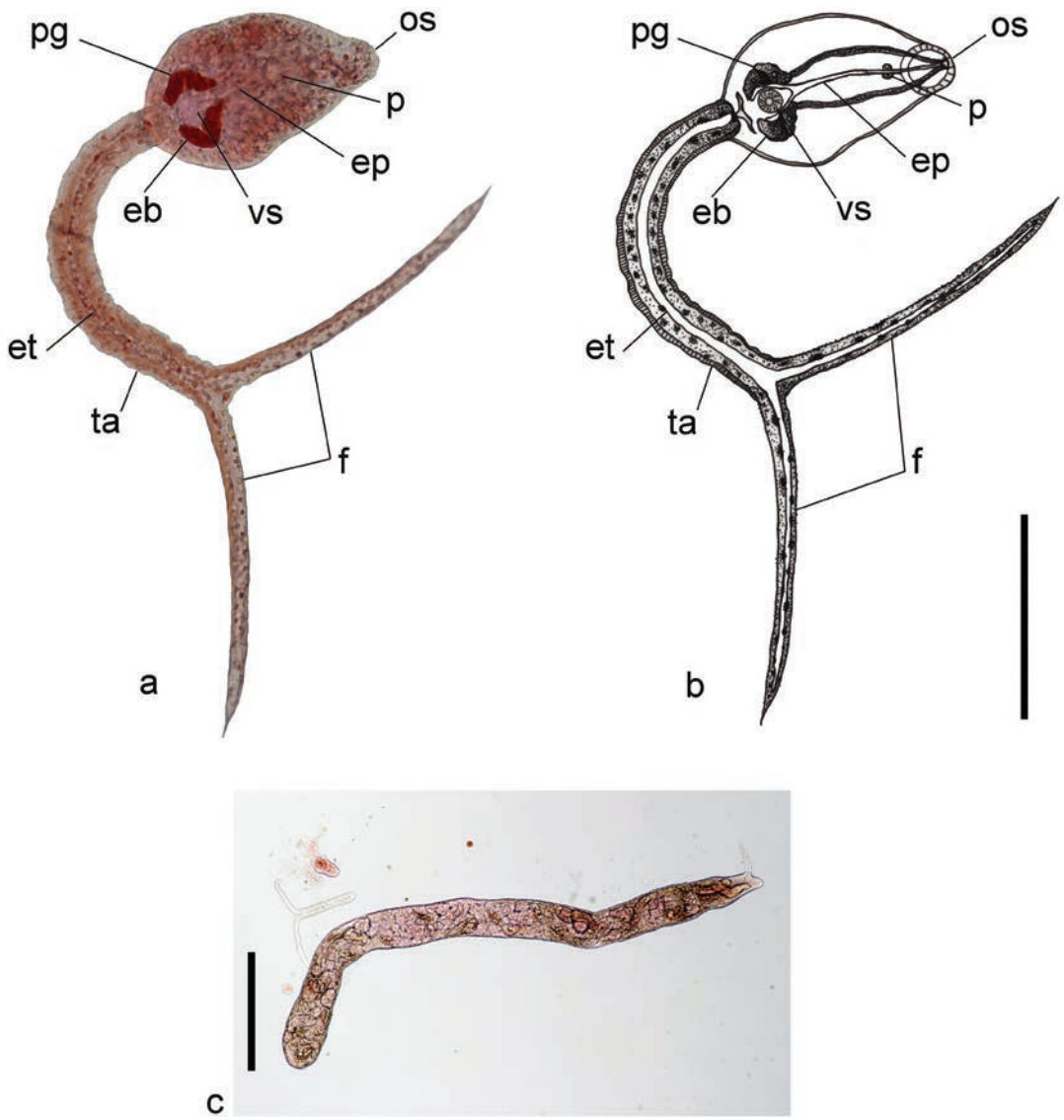



**Figure 14.** *Alaria mustelae* Bosma, 1931. **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. redia stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; et: excretory tubule; f: furca; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

**Transversotrematidae Yamaguti, 1954**

**6.3 *Transversotrema laruei* Velasquez, 1958**
(Fig. 15)

The body is of a bowl-like shape. The surface of the body is covered with spines that have the appearance of fish scales. The genital pore of the seminal vesicle is located in the anterior part of the body. Eyespots are present. The mouth is located near the ventral sucker. The esophagus is narrow and the intestinal caeca form a ring. There is one pair of testes present, and an ovary is located anterolateral to the left of the testes. The excretory bladder is small and short, and is situated close to the posterior end of the body. The tail is longer than the body and possesses spatulate furcae. At the base of the tail a pair of bilaterally symmetrical appendages is present, each equipped with an adhesive pad at its distal end.

The cercariae develop within rediae.

The infection rate was 0.05% (8/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 460–600 µm (mean: 533 µm) × 280–430 µm (mean: 362 µm) |
| Genital pore | 20–40 µm (mean: 31 µm) × 20–50 µm (mean: 34 µm) |
| Ventral sucker | 50–110 µm (mean: 76 µm) × 50–120 µm (mean: 77 µm) |
| Testis | 30–120 µm (mean: 88 µm) × 40–120 µm (mean: 85 µm) |
| Excretory bladder | 20–70 µm (mean: 40 µm) × 40–90 µm (mean: 57 µm) |
| Tail | 120–180 µm (mean: 146 µm) × 620–800 µm (mean: 686 µm) |

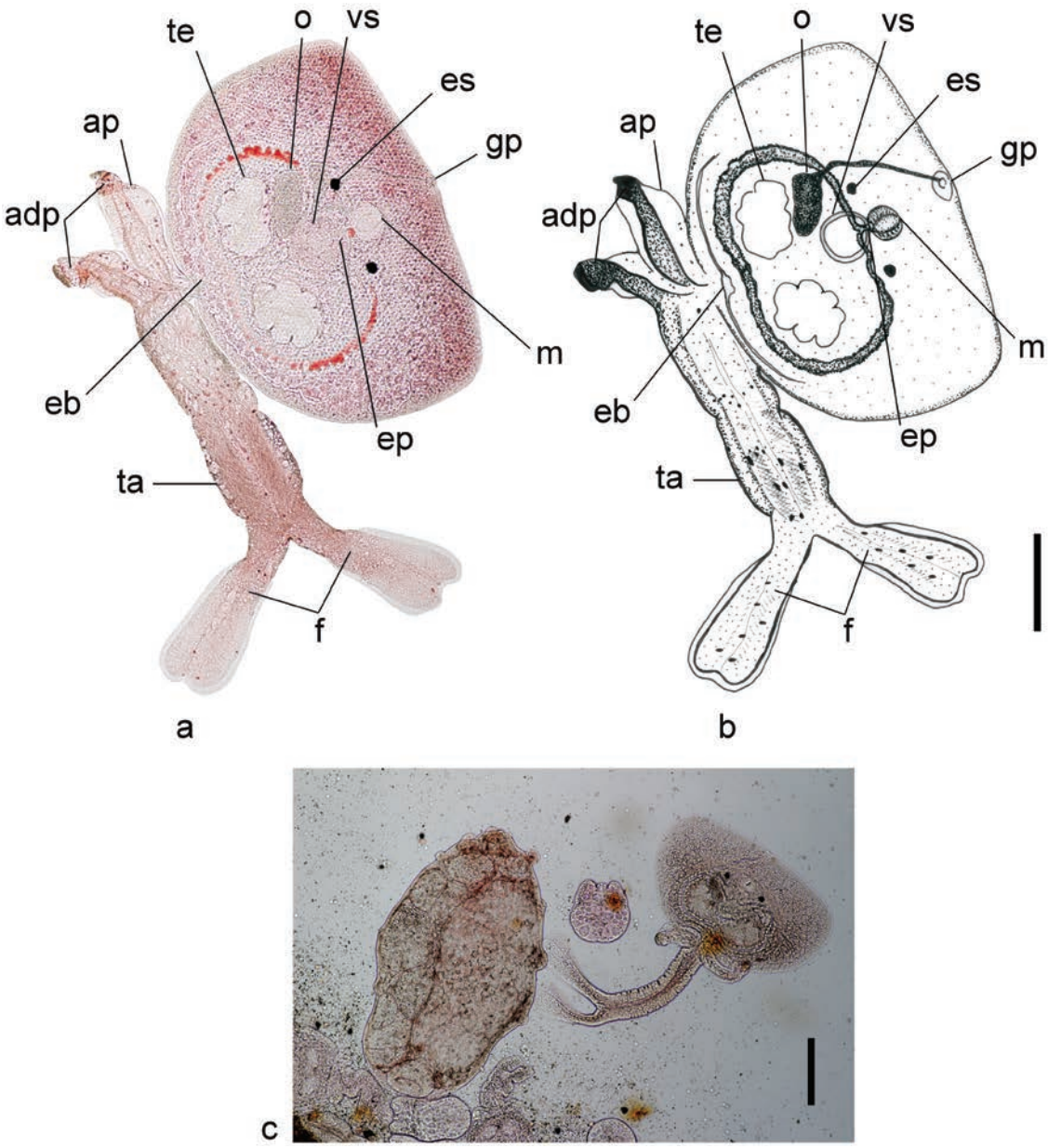



**Figure 15.** *Transversotrema laruei* Velasquez, 1958. **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. redia (left) and cercaria (right) stained with 0.5% neutral red. Abbreviations – adp: adhesive pad; ap: appendages; eb: excretory bladder; ep: esophagus; es: eyespot; f: furca; gp: genital pore; m: mouth; ov: ovary; ta: tail; te: testes; vs: ventral sucke. Scale bars: 50 µm.

| | |
|---|---|
| Tail stem | 120–180 µm (mean: 146 µm) × 390–530 µm (mean: 467 µm) |
| Tail furcal | 80–150 µm (mean: 111 µm) × 180–290 µm (mean: 219 µm) |
| Appendages | 40–70 µm (mean: 58 µm) × 120–150 µm (mean: 138 µm) |

**Type 7. Echinostome cercariae**
(Fig. 16)

The body is elongate pear-shaped. Eyespots are absent. The oral sucker is circular in shape and is equipped with collar spines. The prepharynx is long. The esophagus is shorter than the prepharynx, bifurcating into two intestinal caeca that almost reach to the posterior end of the body. The relatively large ventral sucker is located approximately at two-thirds of the body length measured from the front. Penetration glands are absent. The excretory bladder is small and triangular in shape, its two main collecting tubes beginning at the level of the esophagus. The tail is slender and almost of the same length as the body.

The cercariae develop within rediae.

The infection rate was 0.07% (10/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 150–163 µm (mean: 151 µm) × 243–325 µm (mean: 270 µm) |

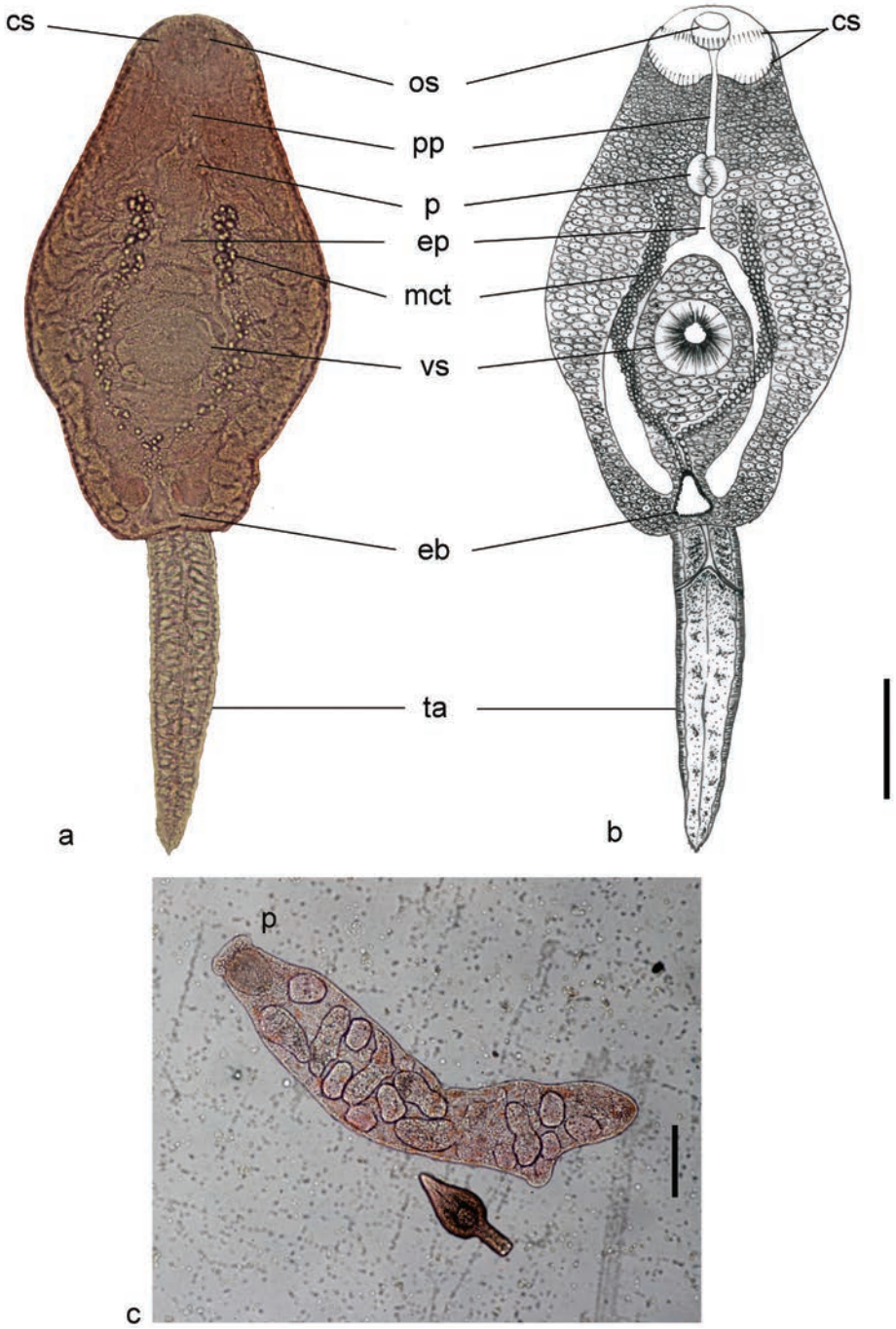



**Figure 16.** Echinostome cercaria. **a**. specimen stained with 0.5% neutral red. **b**. drawing of cercaria. **c**. redia stained with 0.5% neutral red. Abbreviations – cs: collar spines; eb: excretory bladder; ep: esophagus; mct: main collecting tube; os: oral sucker; p: pharynx; pp: prepharynx; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

| | |
|---|---|
| Oral sucker | 38–48 µm (mean: 44 µm) × 38–48 µm (mean: 44 µm) |
| Ventral sucker | 40–73 µm (mean: 62 µm) × 55–63 µm (mean: 60 µm) |
| Pharynx | 13–18 µm (mean: 14 µm) × 20–30 µm (mean: 24 µm) |
| Excretory bladder | 18–55 µm (mean: 38 µm) × 18–55 µm (mean: 33 µm) |
| Tail | 28–40 µm (mean: 34 µm) × 195–313 µm (mean: 240 µm) |

**Type 8. Gymnocephalous cercariae**
(Fig 17)

The body is oval and covered with spines. The terminal oral sucker is equipped with minute spines. Eyespots are absent. The prepharynx is long and thin. The pharynx is rather large and of a round shape. The esophagus is short but rather wide, bifurcating into two intestinal caeca that extend towards the posterior part of the body. There are 4–5 penetration glands present that are located laterally of the caeca between the

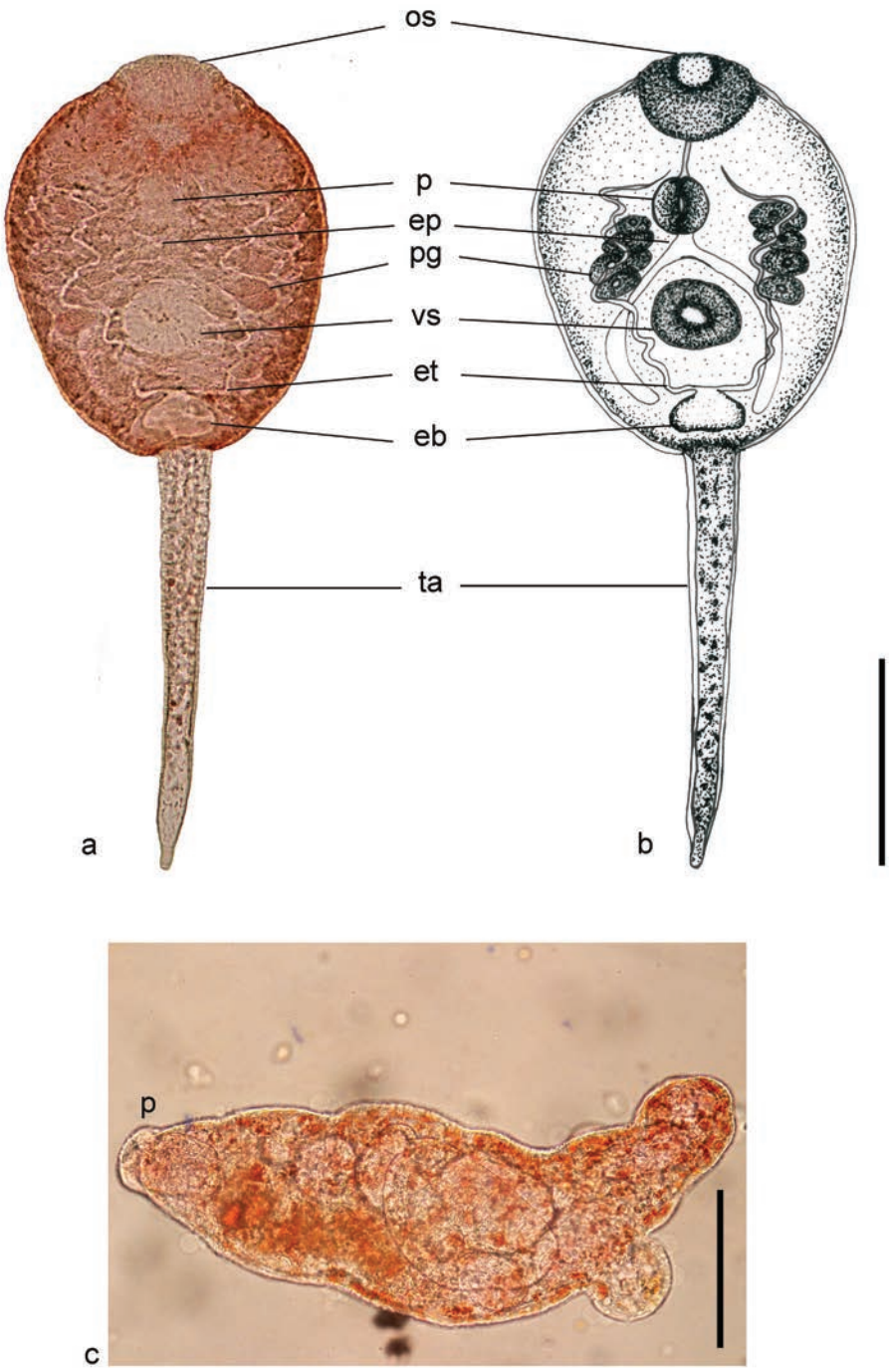



**Figure 17.** Gymnocephalous cercaria. **a**. Specimen stained with 0.5% neutral red. **b**. Drawing of cercaria. **c**. Redia stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; et: excretory tubule; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

level of the pharynx and the ventral sucker. The ventral sucker is of about the same size as the oral sucker. The excretory bladder is roundish, with a thin wall and located medially near the posterior end of the body. Two thin, undulating excretory tubules that begin just anterior of the pharynx insert into the excretory bladder. The tail is longer than the body, with the opening duct of the excretory bladder located at its end. There are groups of of 3–5 distinct pigment granules present in the tail but flame cells could not observed.

The cercariae develop within rediae.

The infection rate was 0.01% (1/15,076) (Table 3).

Size range and average size (in micrometers, calculated from 10 cercariae):

| | |
|---|---|
| Body | 115–160 µm (mean: 134 µm) × 150–195 µm (mean: 176 µm) |
| Oral sucker | 30–40 µm (mean: 33 µm) × 28–40 µm (mean: 36 µm) |
| Pharynx | 8–20 µm (mean: 13 µm) × 13–28 µm (mean: 22 µm) |

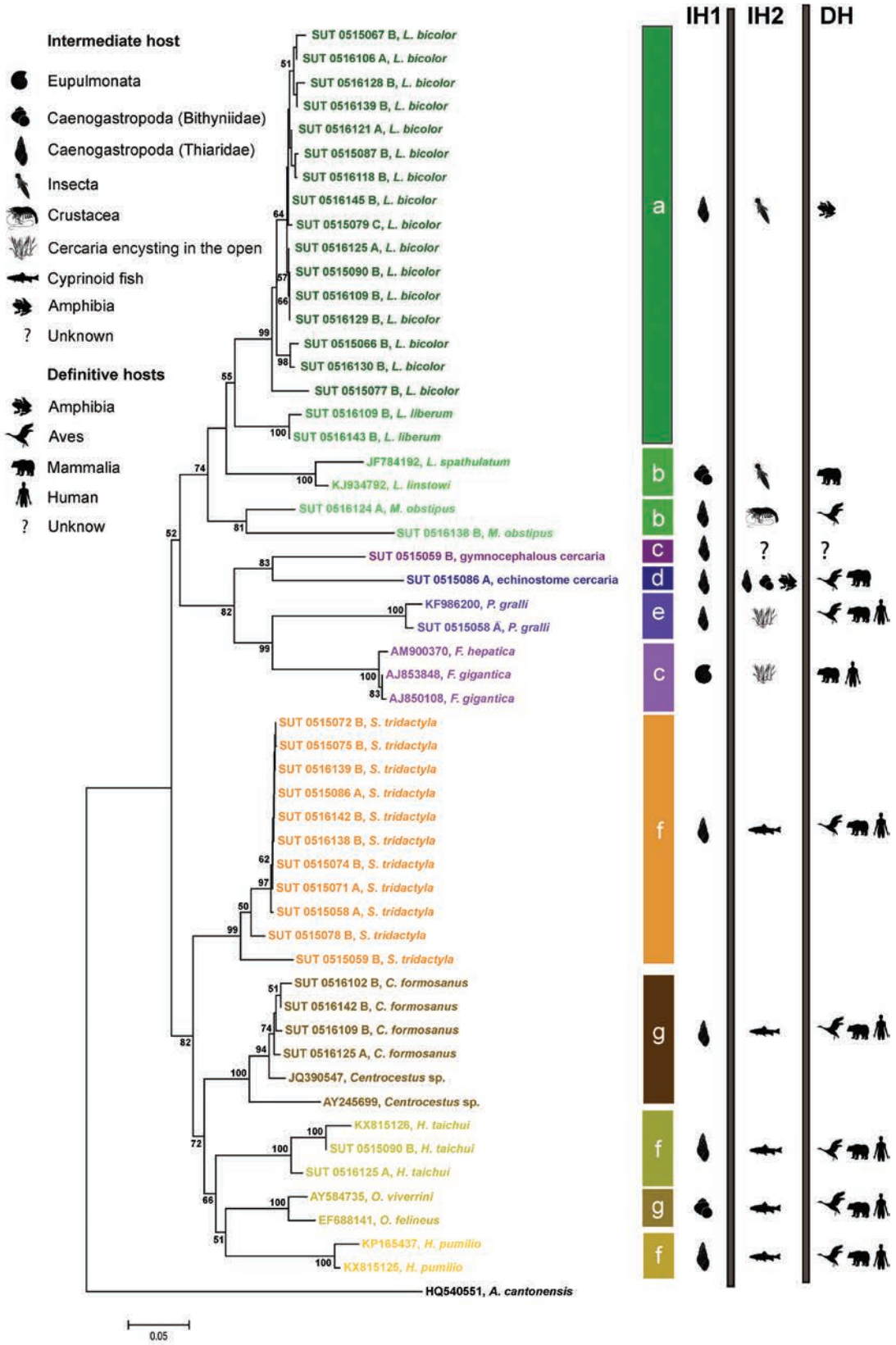



**Figure 18.** Neighbor-joining tree on the basis of ITS2 sequences of cercarial species obtained from Thai populations of *Tarebia granifera* (Lamarck, 1816) and several published sequences obtained from GenBank. Nodes are annotated with bootstrap support values ≥ 50. Taxon names and voucher or GenBank accession numbers are provided at the tips of the tree (see also Table 1). First and second intermediate hosts and definitive hosts are indicated (see legend). Abbreviations – DH: definitive host: IH1: first intermediate host; IH2: second intermediate host. Cercarial types – **a**: virgulate xiphidiocercariae; **b**: armatae xiphidiocercariae; **c**: gymnocephalous cercariae; **d**: echinostome cercariae; **e**: megarulous cercariae; **f**: parapleurophocercous cercariae; **g**: pleurophocercous cercariae.

| | |
|---|---|
| Ventral sucker | 35–48 µm (mean: 41 µm) × 33–45 µm (mean: 41 µm) |
| Excretory bladder | 28–45 µm (mean: 39 µm) × 25–43 µm (mean: 31 µm) |
| Tail | 23–35 µm (mean: 27 µm) × 183–223 µm (mean: 199 µm) |

## Molecular analysis

In the present study, ITS2 sequences from nine distinct cercarial types (collected during the second period of this study) of a total of fifteen trematode species found in Thai populations of *Tarebia granifera* could be amplified by PCR and sequenced. The ITS2 sequences of the virgulate xiphidiocercariae and the armatae xiphidiocercariae had a length of approximately 320 bp, while the ITS2 sequences of the parapleurophocercous cercariae and the pleurophocercous cercariae had a length of approximately 380 bp. The ITS2 sequences of the remaining cercarial types, i.e. megarulous cercariae, echinostome cercariae and gymnocephalous cercariae, had a length of approximately 500 bp.

The phylogenetic tree obtained from the neighbor-joining analysis (Fig. 18) was rooted with the nematode *Angiostrongylus cantonensis* (Chen, 1935) (GenBank accession number: HQ540551.1). All trematode species from Thai populations of *T. granifera* that were distinguished on the basis of cercarial morphology and for which more than one sequence was obtained, formed well supported groups in the phylogenetic analysis. These are highlighted in the following:

- Specimens of *S. tridactyla*, *C. formosanus*, *Centrocestus* sp., *H. taichui*, *O. viverrini*, *O. felineus* (Rivolta, 1884) and *H. pumilio*, which all have cyprinoid fish as a second intermediate host, were grouped together with relatively high support.
- The sequences of the echinostome cercaria and the gymnocephalous cercaria obtained from *T. granifera* were grouped together with relatively high support.
- This latter clade in turn formed a well-supported clade together with *P. gralli* and *Fasciola hepatica* Linnaeus, 1758 and *Fasciola gigantica* Cobbold, 1856 (for which we obtained data from previously published sequences).
- A group of species with arthropods as second intermediate hosts, i.e. *L. bicolor*, *L. liberum*, *Lecithodendrium spathulatum* (Ozaki, 1929), *Lecithodendrium linstowi* Dollfus, 1931 and *M. obstipus*, formed a moderately supported group in the phylogenetic analysis. The relationships of species within this clade, however, could not be resolved robustly.

## Discussion

Thiarid gastropods, that transmit parasites of native birds, fishes or mammals, have frequently been reported as first intermediate hosts of trematodes affecting the respiratory, intestinal and hepatic systems not only in some domestic animals but also in humans. As outlined in the Introduction, this represents a serious threat to public health. For example, thiarid snails such as *Melanoides tuberculata*, *T. granifera*, *Mieniplotia scabra* and *Sermyla riqueti* have been reported as the intermediate hosts of a wide array of diverse trematodes, such as *Haplorchis pumilio*, *H. taichui*, *Loxogenoides bicolor*, *Centrocestus formosanus*, *Acanthatrium hitaense*, *Haematoloechus similes*, *Cloacitrema philippinum*, *Transversotrema laruei*, *Stictodora tridactyla*, *Apatemon gracilis*, *Mesostephanus appendicalatus*, *Cardicola alseae* and *Alaria mustelae* (Dechruksa et al. 2007, Ukong et al. 2007, Krailas et al. 2011, 2014). Furthermore, the phenotypically highly diverse and, thus, systematically problematic thiarid snails are widely distributed in Southeast Asia and Australasia (e.g. Glaubrecht 1996, 2009, 2011, Glaubrecht et al. 2009). This not only renders them most suitable objects for various systematic, biogeographical and evolutionary studies but also brings them into special focus from a parasitological perspective.

The present study aimed at bringing together the classical parasitological approach of the morphological characterization of the cercariae stages of trematodes obtained from their snail host, with a molecular parasitology approach, presenting a phylogenetic analyses of the minute intestinal flukes identified from their thiarids host, exemplified here for the first time with *Tarebia granifera* from Thailand. This particular snails host is common in many Thai freshwater systems, inhabiting rivers, lakes, streams and ponds (Hyslop 2003). Pillay and Perissinotto (2008) recorded that *T. granifera* was also able to colonize moderately saline habitats (brackish water). Without doubt, therefore, this thiarid is well established as an intermediate host for several species of trematodes.

We here focussed on the larval trematode infections found in this snail collected in various regions in Thailand during two periods of field work. When we started the research in the first period (2004–2009), *T. granifera* was found in 18 sampling sites. In the second period (2014–2016), we not only went back to the same sampling sites but also added samples from new locations. Thus, snails from a total of 72 locations could be analysed from all over Thailand, covering for the first time most of the distributional range of the snail host *T. granifera* in this country. In more than two-thirds of these locations, i.e. in populations at 51 sampling sites, infected snails were found, indicating the wide prevalence of these trematodes in Thailand.

As we mentioned above, only three species of trematodes, viz. *L. bicolor*, *S. tridactyla*, *C. formosanus*, were found to commonly occur in *Tarebia granifera* from most river systems and regions in Thailand. They were also found during all seasons, thus independent of the time of the year the snails were collected. By re-visiting during the years 2014 to 2016 the same locations of the first collecting period five to ten years earlier, and recording infected snails in 18 of these sampling sites, we also found that

these trematode infections are apparently long-lasting, in the sense of a permanent phenomenon of these snail host populations, despite seasonal variation in the abundances of plants and animals in general (Shimadzu et al. 2013). Among the total of 15 species from 8 types of cercariae recorded in our study, we found only half of them (i.e. 8 species from 4 types) during the first period; whereas 11 species from 7 types were found in the second period. Thus, with the new study period and with collecting at various other and thus new locations all over Thailand we were able to expand our knowledge with respect to the taxonomical and geographical aspects of this analysis.

In the following we discuss in more details various aspects for the distinct trematode species found in their Thai thiarid snail host *Tarebia granifera*:

Parapleurophocercous cercariae and pleurophocercous cercariae were reported to be commonly found also in other freshwater snails in Thailand, such as e.g. *Melanoides tuberculata* (Krailas et al. 2014). In this study, three species of parapleurophocercous cercariae and one species of pleurophocercous cercariae were found in *T. granifera*.

Various reports have indicated the presence of parapleurophocercous cercariae and some species of pleurophocercous cercariae of the intestinal trematodes Heterophyidae, such as *H. taichui, H. pumilio, S. tridactyla* and *C. formosanus* (e.g. Chontananarth and Wongsawad 2013, Waikagul and Thaekham 2014). These parasites have an aquatic life cycle, using freshwater snails as the first and cyprinid fish as the second intermediate host, with definitive hosts being fish-eating mammals and humans (Nithikathkul and Wongsawad 2008, Krailas et al. 2011, 2014, 2016).

In this study, we found human trematodes, viz. *H. taichui, H. pumilio, S. tridactyla* and *C. formosanus*. Especially the snail infections by the minute intestinal fluke of *S. tridactyla* (2.92%) and *C. formosanus* (1.14%) showed a high level of prevalence in Thailand. In addition, *H. taichui* is important for public health, as was shown in several studies. For example, Kumchoo et al. (2005) reported high prevalence of fish as being the second intermediate host (91.4%) of *H. taichui* from Mae Taeng district of Chiang Mai province. Also, in the PDR Laos many patients have been infected by *H. taichui*, as cases were reported with mucosal ulceration, chronic inflammation and fibrosis of submucosa (Sukontason et al. 2005, Sohn et al. 2014). Chai et al. (2013) reported for seven patients who were infected by *C. formosanus* in Laos that they had abdominal pain, indigestion and diarrhea. Chung et al. (2011) reported the first case in Korea for patients being infected by *H. pumilio*. This heterophyid trematode is an important and continuing public health problem in many countries, as there are case reports not only from Southeast Asia but also from other Asian countries.

Therefore, it is from this perspective that for the epidemiology of zoonosis in general we recommend the study of snail intermediate hosts of human and animal trematode infections. It would be interesting to study whether there are geographically related higher or lower incidences of human infections, perhaps also correlated to infected fishes in these areas.

In contrast, known as parasites to animals only, xiphidiocercariae can be distinguished by their stylet organ in the mouth part of the cercariae. They can be divided into two morphological types, the first type being the virgulate xiphidiocercariae, and the second type the armatae xiphidiocercariae (see e.g. Frandsen and Christensen 1984). The virgulate xiphidiocercariae have a virgular organ present in the region of the oral sucker. For this group, the present study reported three species of parasites from the Lecithodendriidae, viz. *L. bicolor, L. liberum* and *A. histaense*, for which the hosts are amphibians (Brooks et al. 1985). It should be noted that we found *L. bicolor* to have the highest prevalence, with an infection rate of 3.84 %, and to be distributed in every water body, river system and region of Thailand. In the second group, i.e. the armatae xiphidiocercariae, the cercariae do not possess a virgular organ. For this group, we here reported two species, viz. *M. caridinae* and *M. obstipus* of the Microphallidae, which are parasites in birds as definite hosts. For the time being, we refrain from speculating on what might cause these differences before more detailed studies will be done.

Megarulous cercariae have been morphologically characterized as belonging to *Philophthalmus*. This parasite is commonly known as the oriental avian eyefluke and it had been reported in connection with human accidental infections (Waikagul et al. 2006, Derraik 2008). Nollen and Murray (1978) reported that *P. gralli* parasitized the conjunctival sac of various galliform and anseriform birds. This fluke was also found in ostriches, causing conjunctivitis. In earlier studies the cercariae of this trematode were found in the thiarid snail *Melanoides tuberculata* as intermediate host (Kalatan et al. 1997, Pinto and de Melo 2010, Krailas et al. 2014). In this study, we found *P. gralli* now also in the thiarid *Tarebia granifera* from the Phachi River in western Thailand. This river system originates in the Tenasserim mountain range and tributes to the Mae Klong river system to the east of it.

Furcocercous cercariae are generally from trematodes of the Sanguinicolidae; they develop to cercariae in brackish-water and freshwater snails, while the definitive hosts were found in fishes. In this study, we found cercariae of three species, viz. *C. alseae, A. mustelae* and *T. laruei*, to parasitize *Tarebia granifera* as intermediate host. Cercariae of all three trematode species were also found in other thiarid snails, as they were reported in *Melanoides tuberculata* (Krailas et al. 2014, Anucherngchai et al. 2017).

Echinostome cercariae are distributed throughout Southeast Asia (Chai 2009). Most species mainly parasitize avian hosts, such as migratory birds, but sometimes also infect mammals including humans. The echinostome trematodes are associated with the ingestion of raw snails and amphibians that transmit metacercariae as the infective stage (Esteban and Antoli 2009). In the present study, echinostome cercariae were found in *Tarebia gran-*

*ifera* populations from the north of Thailand only; which corroborates the report by Nithikathkul and Wongsawad (2008) that echinostomiasis cases have been commonly found in the north and northeast of Thailand.

Gymnocephalous cercariae are small larval stages of trematodes, in general attributed to the Fasciolidae (e.g. Schell 1970). In this study, we found only one snail infection with cercariae that morphologically are obviously attributable to *Fasciola* cercariae. However, the molecular identification showed that these cercariae were actually neither *F. gigantica* nor *F. hepatica*. Instead, the phylogentic analyses indicate a closer affinity of these sequences to those from cercariae with echinostoma type. By morphology the echinostome cercariae are clearly distinguishable by being elongated spinose with a reniform collar, armed with a single or double row of spines surrounding the dorsal and lateral margins of the oral sucker (Anucherngchai et al. 2016, Ayoub et al. 2017). Thus, our study here revealed one case of obvious conflict between the morphologically based identification and the molecular indication of affinity, which clearly is in need to be studied further. We cannot exclude the possibility of a simple laboratory mix-up, but should also keep in mind e.g. hybrid effects.

In a previous report, the gymnocephalous cercariae were produced by trematodes of the Fasciolidae. They were found in *Biomphalaria* sp., *Bulinus* sp., *Ceratophallus* sp., *Gabbiella* sp., *Gyraulus* sp., *Lymnaea* sp., and in *Melanoides* sp. (Frandsen and Christensen 1984). However, thiarid snails were never reported with fasciolid trematode infections in Thailand. Even though, the morphology of gymnocephalous cercariae was obviously to be *Fasciola* cercariae. The sequence of DNA was shown to match with that from the group of echinostome cercariae.

## Molecular analyses of cercaria and their host correlations

In general, morphological as well as molecular studies of cercariae were able to confirm the specific identity and prevalence of various infectious trematodes in Thai freshwater snails of *Tarebia granifera*. In this study, we found that the ITS2 marker allowed to distinguish a total of nine trematode species, with the cercariae attributable to seven of the morphologically distinguishable types, viz. the parapleurophocercous cercariae, pleurophocercous cercariae, the virgulate xiphidiocercariae and armatae xiphidiocercariae, megarulous cercariae, as well as echinostome cercariae and gymnocephalous cercariae; only the furcocercous cercariae were not available for molecular studies. And in one case only we found a conflict insofar, as the sequence data revealed that cercariae of the morphologically distinguished gymnocephalous type grouped closely with those of the echinostome type.

We also used the ITS2 marker for a phylogenetic reconstruction (Fig. 18), in which two characteristics are most noteworthy: First, while the relationships of species within molecular clades found could not be resolved robustly, our analyses revealed, second, two well-supported major molecular clusters or groups. These clusters can be well interpreted in context of their respective zoonotic parasites and human pathogens.

The first group with parapleurophocercous and pleurophocercous cercariae, respectively (marked f and g in Fig. 18), i.e. *S. tridactyla*, *C. formosanus*, *Centrocestus* sp., *H. taichui*, *H. pumilio*, *O. viverrini*, *O. felineus*, all have cyprinoid fish as second intermediate host, while birds and mammals, including in particular humans, are the definite host. Note that the latter two trematode species have a bithyniid instead a thiarid snail as first intermediate host.

In a second group cluster trematode species with virgulate xiphidiocercariae and armatae xiphidiocercariae, respectively (marked a and b in Fig. 18), i.e. *L. bicolor*, *L. liberum*, *Lecithodendrium spathulatum*, *L. linstowi* and *M. obstipus*), which all have arthropods (Insecta or Crustacea) as second intermediate hosts while amphibians, birds and mammals, but with the exclusion of humans, are the definite hosts.

In addition, also the sequences of trematode species with echinostome cercaria and the gymnocephalous cercaria obtained from *T. granifera* grouped together with relatively high support. However, no clear picture as to a correlation with their second intermediate hosts and definitive hosts is visible to date, as we lack knowledge on the latter in particular for the gymnocephalous cercaria. Nevertheless, the latter two form a well-supported clade together with *P. gralli*, *F. hepatica* and *F. gigantica*, which all have gymnocephalous, echinostome or megarulous cercariae (Fig. 18, c,d,e). Note that the latter two trematode species are known to have an eupulmonate instead of a thiarid snail host. Interestingly, in this latter monophyletic clade, formed by *P. gralli* together with *F. hepatica* and *F. gigantica*, only those trematodes are known to be human pathogens (as definite hosts) when the cercaria are encysting in the open instead of parasitizing a second intermediate host; see Fig. 18.

We anticipate that more detailed studies, based on molecular phylogenetic analyses, looking into these and other correlations of intermediate hosts, their regional occurrences and ecological specifics will shed more light on the evolutionary potential of trematode parasites from thiarid snails.

## Conclusion and outlook

To date, studies on freshwater snails and their interactions with parasitic trematodes are under-represented worldwide (Adema et al. 2012). There is an urgent need for collaboration bringing together deeper understanding on the basic biology, biodiversity, and evolutionary associations of parasitic trematodes on the one hand and their snail hosts on the other, i.e. those studying parasitology and

malacology, taking advantage of their respective expertise in host-parasite interactions and evolutionary systematics.

Accordingly, the aims of this approach presented here were to establish reliable and reproducible data for the morphological identification as well as the methodology for the extraction of high quality DNA from preserved trematode cercariae in specifically known populations of their thiarid snails hosts (including museum samples collected several years ago). It was also the aim to conduct a phylogenetic analysis of the minute intestinal flukes. In addition, the present paper adds to a more in-depth evolutionary systematic analysis with data on reproductive biology, geographical distribution, morphology and molecular phylogenies of *T. granifera*.

Using this combinational approach, it will eventually be possible to identify in more details the host-parasite relationships of thiarid snails as first intermediate host populations not only in Thailand, and also to determine the role of parasitic infections in these gastropods and as human pathogens.

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## References

Abbott RT (1952) A study of an intermediate snail host (*Thiara granifera*) of the Oriental lung fluke (*Paragonimus*). Proceedings of the United States National Museum 102: 71–116. https://doi.org/10.5479/si.00963801.102-3292.71

Adema CM et al. (2012) Will all scientists working on snails and the diseases they transmit please stand up? PLoS Neglected Tropical Diseases 6(12): e1835. https://doi.org/10.1371/journal.pntd.0001835

Ali H, Ai L, Song HQ, Ali S, Lin RQ, Seyni B, Lssa G, Zhu XQ (2008) Genetic charaterisation of *Fasciola* samples from different host species and geographical localities revealed the existence of *F. hepatica* and *F. gigantica* in Niger. Parasitology Research 102: 1021–1024. https://doi.org/10.1007/s00436-007-0870-7

Anucherngchai S, Tejangkura T, Chontananarth T (2016) Epidemiological situation and molecular identification of cercarial stage in freshwater snails in Chao-Phraya Basin, Central Thailand. Asian Pacific Journal of Tropical Biomedicine 6: 539–545. https://doi.org/10.1016/j.apjtb.2016.01.015

Anucherngchai S, Tejangkura T, Chontananarth T (2017) Molecular confirmation of trematodes in the snail intermediate hosts from Ratchaburi Province, Thailand. Asian Pacific Journal of Tropical Disease 7: 286–292. https://doi.org/10.12980/apjtd.7.2017D6-399

Appleton CC (2002) First report of *Tarebia granifera* (Lamarck, 1816) (Gastropoda: Thiaridae) from Africa. Journal of Molluscan Studies 68: 399–402. https://doi.org/10.1093/mollus/68.4.399

Appleton CC, Forbes AT, Demetriades NT (2009) The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa. Zoologische Mededelingen 83: 525–536.

Ayoub M, Tadrosi M, Bardicy SE (2017) *Echinochasmus*, new species (Trematoda: Echinostomatidae) from Egypt. Journal of the Egyptian Society of Parasitology 47: 159–165.

Brandt AM (1974) The non-marine aquatic Mollusca of Thailand. Archiv fȕr Molluskenkunde 105: 1–423.

Brooks DR, O'Grady RT, Glen DR (1985) Phylogenetic analysis of the Digenea (Platyhelminthes: Cercomeria) with comments on their adaptive radiation. Canadian Journal of Zoology 63: 411–443. https://doi.org/10.1139/z85-062

Chai JY (2009) Echinostomes in humans. In: Fried B, Toledo R (Eds) The Biology of Echinostomes: From the Molecule to the Community. Springer Science + Business Media LLC, New York, 147–183. https://doi.org/10.1007/978-0-387-09577-6_7

Chai JY, Murrell KD, Lymbery AJ (2005) Fish-borne parasitic zoonoses: status and issues. International Journal of Parasitology 35: 1233–1254. https://doi.org/10.1016/j.ijpara.2005.07.013

Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Lee MY, Lim H, Insisiengmay B, Phommasack B, Rim HJ (2013) *Centrocestus formosanus* (Heterophyidae): human infections and the infection source in Lao PDR. Journal Parasitology 99: 531–536. https://doi.org/10.1645/12-37.1

Chaniotis BN, Butler JM, Ferguson FF, Jobin WR (1980) Bionomics of *Tarebia granifera* (Gastropoda: Thiaridae) in Puerto Rico, an Asian vector of *Paragonimiasis westermani*. Caribbean Journal of Science 16: 81–89.

Chontananarth T, Wongsawad C (2013) Epidemiology of cercarial stage of trematodes in freshwater snails from Chiang Mai Province, Thailand. Asian Pacific Journal of Tropical Biomedicine 3: 237–243. https://doi.org/10.1016/S2221-1691(13)60058-1

Chuboon S, Wongsawad C, Ruamsuk A, Nithikathkul C (2005) Survival of *Haplorchis taichui* metacercariae in Lab-pla, Thai traditional food preparation. Southeast Asian Journal of Tropical Medicine and Public Health 36 (Supplement 4): 110–111.

Chung OS, Lee HJ, Kim YM, Sohn WM, Kwak SJ, Seo M (2011) First report of human infection with *Gynaecotyla squatarolae* and first Korean record of *Haplorchis pumilio* in a patient. International Journal for Parasitology 60: 227–229. https://doi.org/10.1016/j.parint.2010.11.003

Davies D, Davies C, Lauthier JJ, Hamann M, Ostrowski de Núñez M (2015) Morphological and ITS2 molecular characterization of *Ribeiroia* cercariae (Digenea: Psilostomidae) from *Biomphalaria* spp. (Gastropoda: Planorbidae) in Northern Argentina. Journal of Parasitology 101:549–555. https://doi.org/10.1645/13-350.1

Dechruksa W, Krailas D, Glaubrecht M (2013) Evaluating the status and identity of "*Melania*" *jugicostis* Hanley & Theobald, 1876 – an enigmatic thiarid gastropod in Thailand (Caenogastropoda, Cerithioidea). Zoosystematics and Evolution 89(2): 293–310. https://doi.org/10.1002/zoos.201300015

Dechruksa W, Glaubrecht M, Krailas D (2017) Natural trematode infections of freshwater snail *Melanoides jugicostis* Hanley & Theobald, 1876 (Family Thiaridae), the first intermediate host of animal and human parasites in Thailand. Silpakorn University Science and Technology Journal 11: 9–16.

Dechruksa W, Krailas D, Ukong S, Inkapatanakul W, Dangprasert T (2007) Trematode infections of freshwater snails family Thiaridae in Khek River. Southeast Asian Journal of Tropical Medicine and Public Health 38: 1016–1028.

Derraik JGB (2008) The potential significance to human health associated with the establishment of the snail *Melanoides tuberculata* in New Zealand. The New Zealand Medical Journal 121: 25–32.

Dzikowski R, Levy MG, Poore MF, Flowers JR, Paperna L (2004) Use of rDNA polymorphism for identification of Heterophyidae infecting freshwater fishes. Diseases of Aquatic Organisms 59: 35–41. https://doi.org/10.3354/dao059035

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Esteban JG, Antoli CM (2009) Echinostomes: Systematics and life cycle. In: Fried B, Toledo R (Eds) The Biology of Echinostomes: From the Molecule to the Community. Springer Science + Business Media LLC, New York, 1–34.

Facon BT, David P (2006) Metapopulation dynamics and biological invasions: a spatially explicit model applied to a freshwater snail. The American Naturalist 168: 769–783. https://doi.org/10.1086/508669

Fernández LD, Casalis AE, Masa AM, Perez MV (1992) Estudio preliminar de la variación de *Tarebia granifera* (Lamarck), Río Hatibonico, Camagüey. Revista Cubana de Medicina Tropical 44: 66–70.

Frandsen F, Christensen NQ (1984) An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. Acta Tropica 41: 181–202.

Galaktionov KV, Dobrovolskij AA (2003) The biology and evolution of trematodes: an essay on the biology, morphology, life cycles, transmissions, and evolution of digenetic trematodes. Kluwer Academic Publisher, Dordrecht, 592 pp. https://doi.org/10.1007/978-94-017-3247-5

Glaubrecht M (1996) Evolutionsökologie und Systematik am Beispiel von Süβ- und Brackwasserschnecken (Mollusca: Caenogastropoda: Cerithioidea): Ontogenese-Strategien, paläontologische Befunde und Historische Zoogeographie. Backhuys Publishers, Leiden, 499 pp. [25 pls]

Glaubrecht M (1999) Systematics and the evolution of viviparity in tropical freshwater gastropods (Cerithioidea: Thiaridae sensu lato) – an overview. Courier Forschungs-Institut Senckenberg 215: 91–96.

Glaubrecht M (2009) On "Darwinian Mysteries" or molluscs as models in evolutionary biology: from local speciation to global radiation. American Malacological Bulletin 27: 3–23. https://doi.org/10.4003/006.027.0202

Glaubrecht M (2011) Towards solving Darwin's "mystery": Speciation and radiation in lacustrine and riverine freshwater gastropods. American Malacological Bulletin 29: 187–216. https://doi.org/10.4003/006.029.0211

Glaubrecht M, Brinkmann N, Pöppe J (2009) Diversity and disparity 'down under': Systematics, biogeography and reproductive modes of the 'marsupial' freshwater Thiaridae (Caenogastropoda, Cerithioidea) in Australia. Zoosystematics and Evolution 85: 199–275. https://doi.org/10.1002/zoos.200900004

Gutierrez A, Perera G, Yong M, Fernandez JA (1997) Relationships of the prosobranch snails *Pomacea paludosa*, *Tarebia granifera* and *Melanoides tuberculata* with the abiotic environment and freshwater snail diversity in the central region of Cuba. Malacologial Review 30: 39–44.

Heneberg P, Rojas A, Bizos J, Kockova L, Mala M, Rojas D (2014) Focal *Philophthalmus gralli* infection possibly persists in *Melanoides tuberculata* over two years following the definitive hosts' removal. Parasitology International 63: 802–807. https://doi.org/10.1016/j.parint.2014.07.012

Hyslop EJ (2003) Additions to the freshwater malacofauna of Jamaica. Revista de Biologîa Tropical 51: 262–263.

Ito J (1980) Studies on cercariae in Japan. Shizuoka University, Oya, Surugaku, Shizuoka, 376 pp.

Kalatan AMN, Arfin M, Al-Arefi HA, Bobshait HI, Hamadah SA, Al-Thawab FH, Al-Shamrani AA (1997) Occurrence of larval *Philophthalmus gralli* (Mathis & Leger, 1910) in freshwater snail *Melanoides tuberculatus* (Muller) from Al-Hafuf, Saudi Arabia and its development into adult in various experimental hosts. Parasitology International 46: 127–136. https://doi.org/10.1016/S1383-5769(97)00019-6

Katokhin AV, Shekhovtsov SV, Konkow S, Yurlova NI, Serbina EA, Vodianitskai SN, Fedorov KP, Loktev VB, Muratov IV, Ohyama F, Makhnev TV, Pel'tek SE, Mordvinov VA (2008) Assessment of the genetic distinctions of *Opisthorchis felineus* from *O. viverrini* and *Clonorchis sinensis* by ITS2 and CO1 sequences. Doklady Biochemistry and Biophysics 421: 214–230. https://doi.org/10.1134/S1607672908040133

Kaw BL (1945) On the present status of the genus Loxogenes. Proceedings of the Indian Academy of Siences. Section B.

Krailas D, Chotesaengsri S, Pattaradussadee N, Notesiri N, Dechruksa W (2008) Bucephalid (Gasterostome) cercariae obtained from freshwater clams in Thailand. The Journal of Tropical Medicine and Parasitology 31: 70–76.

Krailas D, Dechruksa W, Ukong S, Janecharut T (2003) Cercarial infection in *Paludomus petrosus*, Freshwater snail in Pa La-U Waterfall. The Southeast Asian Journal of Tropical Medicine and Public Health 34: 286–290.

Krailas D, Namchote S, Rattanathai P (2011) Human intestinal flukes *Haplorchis taichui* and *Haplorchis pumilio* in their intermediate hosts, freshwater snails of the families Thiaridae and Pachychilidae, in southern Thailand. Zoosystematics and Evolution 87(2): 349–360. https://doi.org/10.1002/zoos.201100012

Krailas D, Namchote S, Koonchornboon T, Dechruksa W, Boonmekarm D (2014) Trematodes obtained from the thiarid freshwater snail *Melanoides tuberculata* (Müller, 1774) as vector of human infections in Thailand. Zoosystematics and Evolution 90: 57–86. https://doi.org/10.3897/zse.90.7306

Krailas D, Veeravechsukij N, Chuanprasit C, Boonmekam D, Namchote S (2016) Prevalence of fish-borne trematodes of the family Heterophyidae at Pasak Cholasid Reservoir, Thailand. Acta Tropica 156: 79–86. https://doi.org/10.1016/j.actatropica.2016.01.007

Kudlai O, Stunzenas V, Tkach V (2015) The taxonomic identity and phylogenetic relationships of *Cercaria pugnax* and *C. helvetica* XII (Digenea: Lecithodendriidae) based on morphological and molecular data. Folia Parasitologica 62: 2–7. https://doi.org/10.14411/fp.2015.003

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Kumchoo K, Wongsawad C, Chai JY, Vanittanakom P, Rojanapaibul A (2005) High prevalence of *Haplorchis taichui* metacercariae in cyprinoid fish from Chiang Mai Province, Thailand. Southeast Asian Journal of Tropical Medicine Public Health 36: 451–455.

Lamarck JBPA de Monet de (1816) Tableau encyclopédique et méthodique des trois règnes de la nature. 33 partie, Mollusques et polypes divers. Paris, Agasse. 2, 16, 98 pp.

Le TH, Nguyen KT, Nguyen NT, Doan HT, Dung DT, Blair D (2017) The ribosomal transcription units of *Haplorchis pumilio* and *H. taichui* and the use of 28S rDNA sequences for phylogenetic identification of common heterophyids in Vietnam. Parasites Vectors 10: 1–9. https://doi.org/10.1186/s13071-017-1968-0

Lord JS, Parker S, Parker F, Brooks DR (2012) Gastrointestinal helminths of pipistrelle bats (*Pipistrellus pipistrellus*/*Pipistrellus pygmaeus*) (Chiroptera: Vespertilionidae) of England. Parasitology 139: 366–374. https://doi.org/10.1017/S0031182011002046

Lydeard C, Holznagel WE, Glaubrecht M, Ponder WF (2002) Molecular phylogeny of a circum-global, diverse gastropod superfamily (Cerithioidea: Mollusca: Caenogastropoda): pushing the deepest phylogenetic limits of mitochondrial LSU rDNA sequences. Molecular Phylogenetics and Evolution 22: 399–406. https://doi.org/10.1006/mpev.2001.1072

Malek EA, Cheng TC (1974) Medical and Economic malacology. Academic Press, New York, 398 pp.

Mei X-F, Li S-Q, Hu C-H, Huang T-F, Chen Z-F, Huang W-Y (2015) ITS2 Sequencing and phylogenetic analysis of *Haplorchis pumilio and H. taichui*. China Animal Husbandry & Vetinary Medicine 42: 1943–1949.

Miranda NAF, Perissinotto R, Appleton CC (2010) Salinity and temperature tolerance of the invasive freshwater gastropod *Tarebia granifera*. South African Journal of Science 106: 156. https://doi.org/10.4102/sajs.v106i3/4.156

Miranda NAF, Perissinotto R, Appleton CC (2011) Population structure of an invasive parthenogenetic gastropod in coastal lakes and estuaries of northern KwaZulu-Natal, South Africa. PLoS ONE 6: e24337. https://doi.org/10.1371/journal.pone.0024337

Miranda NAF, Perissinotto R (2012) Stable isotope evidence for dietary overlap between alien and native gastropods in coastal lakes of northern KwaZulu-Natal, South Africa. PLoS ONE 7: e31897. https://doi.org/10.1371/journal.pone.0031897

Mukaratirwa S, Hove T, Cindzi ZM, Maononga DB, Taruvinga M, Matenga E (2005) First report of an outbreak of the oriental eyefluke *Philophthalmus gralli* (Mathis & Ledger, 1910), in commercially reared ostriches (*Struthio camelus*) in Zimbabwe. Onderstepoort Journal of Veterinary Research 72: 203–206. https://doi.org/10.4102/ojvr.v72i3.197

Nithikathkul C, Wongsawad C (2008) Prevalence of *Haplorchis taichui* and *Haplorchoides* sp. metacercariae in freshwater fish from water reservoirs, Chiang Mai, Thailand. Korean Journal Parasitology 46: 109–112. https://doi.org/10.3347/kjp.2008.46.2.109

Nithiuthai S, Suwansaksri J,Wiwanitkit V, Chaengphukeaw P (2002) A survey of metacercariae in cyprinoid fish in Nakhon Ratchasima, northeast Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health 33: 103–105.

Nollen PM, Murray HD (1978) *Philophthalmus gralli*: Identification, growth characteristics, and treatment of an Oriental eyefluke of Birds introduced into the continental United States. Journal of Parasitology 64: 178–180. https://doi.org/10.2307/3279646

Odhner T (1910) Nordostafrikanische Trematoden, grösstenteils vom Weissen Nil (von der schwedischen zoologischen Expedition gesammelt). Results of the Swedish Zoological Expedition to Egypt and the White Nile 4: 1–166.

Olivier LC, Schneiderman M (1956) Method for estimating the density of aquatic snail population. Experimental Parasitology 5: 109–117. https://doi.org/10.1016/0014-4894(56)90008-X

Parvathi A, Umesha KR, Kumar S, Sithithaworn P, Karunasagar I, Karunasagar I (2008) Development and evaluation of a polymerase chain reaction (PCR) assay for the detection of *Opisthorchis viverrini* in fish. Acta Tropica 107: 13–16. https://doi.org/10.1016/j.actatropica.2008.04.001

Pillay D, Perissinotto R (2008) The Benthic macrofauna of the St. Lucia Estuary during the 2005 drought year, Estuarine. Coastal and Shelf Science 77: 35–46. https://doi.org/10.1016/j.ecss.2007.09.004

Pinto HA, de Melo AL (2010) *Melanoides tuberculata* (Mollusca: Thiaridae) as an intermediate host of *Centrocestus formosanus* (Trematoda: Heterophyidae) in Brazil. Revista do Instituto de Medicina Tropical de São Paulo 52: 207–210. https://doi.org/10.1590/S0036-46652010000400008

Pointier JP, Jourdane J (2000) Biological control of the snail hosts of schistosomiasis in areas of low transmission: the example of the Caribbean area. Acta Tropica 77: 53–60. https://doi.org/10.1016/S0001-706X(00)00123-6

Pointier JP, Samadi S, Jarne P, Delay B (1998) Introduction and spread of *Thiara granifera* (Lamarck, 1822) in Martinique, French West Indies. Biodiversity and Conservation 7: 1277–1290. https://doi.org/10.1023/A:1008887531605

Prasad PK, Goswami LM, Tandon V, Chatterjee A (2011) PCR-based molecular characterization and in *silico* analysis of food-borne trematode parasites *Paragonimus westermani, Fasciolopsis buski and Fasciola gigantica* from Northeast India using ITS2 rDNA. Bioinformation 6: 64–68. https://doi.org/10.6026/97320630006064

Prentice MA (1983) Displacement of *Biomphalaria glabrata* by the snail *Thiara granifera* in field habitats in St. Lucia, West Indies. Annals of Tropical Medicine and Parasitology 77: 51–59. https://doi.org/10.1080/00034983.1983.11811672

Pungpak S, Radomyos P, Radomyos B, Schelp FP, Jongsuksuntigul P, Bunnag D (1998) Treatment of *Opisthorchis viverrini* and intestinal fluke infections with praziquentel. Southeast Asian Journal of Tropical Medicine and Public Health 29: 246–249.

Radomyos B, Wongsaroj T, Wilairatana P, Radomyos P, Praevanich R, Meesomboon V, Jongsuksuntikul P (1998) Opisthorchiasis and intestinal fluke infections in northern Thailand. Southeast Asian Journal of Tropical Medicine and Public Health 29: 123–127.

Sato M, Thaenkham U, Dekumyoy P, Waikagul J (2009) Discrimination of *Opisthorchis viverrini, Clonorchis sinensis, Haplorchis pumilio* and *Haplorc*his taich*ui* using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. Acta Tropica 109: 81–83. https://doi.org/10.1016/j.actatropica.2008.09.015

Schell SC (1970) How to know the trematodes. WC Brown Co., Dubuque, 355 pp.

Shimadzu H, Dornelas M, Henderson PA, Magurran AE (2013) Diversity is maintained by seasonal variation in species abundance. BMC Biology 11: 98. https://doi.org/10.1186/1741-7007-11-98

Shin HR et al. (2010) Epidemiology of cholangiocarcinoma: An update focusing on risk factors. Cancer Science 101(3): 579–585. https://doi.org/10.1111/j.1349-7006.2009.01458.x

Skov J, Kania PW, Dalsgaard A, Jørgensen TR, Buchmann K (2009) Life cycle stages of heterophyid trematode in Vietnamese freshwater fishes traced by molecular and morphometric methods. Veterinary Parasitology 160: 66–75. https://doi.org/10.1016/j.vetpar.2008.10.088

Sohn WM, Yong TS, Eom KS, Min DY, Lee D, Jung BK, Banouvong V, Insisiengmay B, Phoommasack B, Rim HJ, Chai JK (2014) Prevalence of *Haplorchis taichui* among humans and fish in Luang Prabang Province, Lao PDR. Acta Tropica 136: 74–80. https://doi.org/10.1016/j.actatropica.2014.04.020

Sri-aroon P, Lohachit C, Harada M (2005) Brackish-water mollusks of Surat Thani province, Southern Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health 36 (Supplement 4): 180–188.

Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ (2007). Liver fluke induces cholangiocarcinoma. PLoS Medicine 4(7): e201. https://doi.org/10.1371/journal.pmed.0040201

Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ (2010) Food-borne trematodiases in Southeast Asia: epidemiology, pathology, clinical manifestation and control. Advances in Parasitology 72: 305–350. https://doi.org/10.1016/S0065-308X(10)72011-X

Srisawangwong T, Sithithaworn P, Tesana S (1997) Metacercariae isolated from cyprinoid fishes in Khon Kaen district by digestion technic. Southeast Asian Journal of Tropical Medicine and Public Health 28 (Supplement 1): 224–226.

Strong EE, Colgan DJ, Healy JM, Lydeard C, Ponder WF, Glaubrecht M (2011) Phylogeny of the gastropod superfamily Cerithioidea using morphology and molecules. Zoological Journal of the Linnean Society 162: 43–89. https://doi.org/10.1111/j.1096-3642.2010.00670.x

Sukontason K, Piangjai S, Muangyimpong Y, Sukontason K, Methanitikorn R, Chaithong U (1999) Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao district, Chiang Mai Province, northern Thailand. Southeast Asian Journal of Tropical Medicine and Public Health 30: 365–370.

Sukontason K, Unpunyo P, Sukontason KL, Piangjai S (2005) Evidence of *Haplorchis taichui* infection as pathogenic parasite: Three case reports. Scandinavian Journal of Infection Diseases 37: 388–390. https://doi.org/10.1080/00365540510034473

Torgerson PR et al. (2015) World Health Organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis. PLoS Medicine 12(12): e1001920. https://doi.org/10.1371/journal.pmed.1001920

Ukong S, Krailas D, Dangprasert T, Channgarm P (2007) Studies on the morphology of cercariae obtained from freshwater snails at Erawan Waterfall, Erawan National Park, Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health 38: 302–312.

Upatham ES, Kruatrachue M, Chitramwong Y, Jantataemae S (1995) Malacology. Suksopa Publishing, Bangkok, 517 pp.

Upatham ES, Sornmai S, Thirachantra S, Sitaputra P (1980) Field studies on the bionomics of alpha and gamma races of *Tricula aperta* in the Mekong River at Khemmarat, Ubol Ratchathani Province, Thailand. In: Bruce JI, Sornmani S, Asch HL, Crawford KA (Eds) The Mekong schistosome. Malacological Review 1980 (Supplement 2): 239–261.

Vargas M, Gomez J, Perera G (1991) Geographic expansion of *Marisa cornuarietis* and *Tarebia granifera* in the Dominican Replublic. Journal of Medical and Applied Malacology 3: 69–72.

Veeravechsukij B, Krailas D, Namchote S, Wiggering B, Neiber MT, Glaubrecht M (2018) Phylogeography and reproductive biology of the freshwater snail *Tarebia granifera* in Thailand and Timor (Cerithioidea, Thiaridae): morphological disparity versus genetic diversity. Zoosystematics and Evolution 94(2). https://doi.org/10.3897/zse.94.28981

Waikagul J, Dekumyoy P, Yoonuan T, Praevanit R (2006) Conjunctiva philophthalmosis: A case report in Thailand. The American Society of Tropical Medicine and Hygiene 74: 848–849. https://doi.org/10.4269/ajtmh.2006.74.848

Waikagul J, Thaekham U (2014) Approaches to research on the systematics of fish-borne trematodes (1st edn). Academic Press, Cambridge, 130 pp.

Watson JM (1960) Medical Helminthology. Bailliere Tindall & Cox, London, 487 pp.

Watthanakulpanich D, Waikagul J, Maipanich W, Nuamtanong S, Sanguankiat S, Pubampen S, Praevanit R, Mongkhonmu S, Nawa Y (2010) *Haplorchis taichui* as a possible etiologic agent of irritable bowel syndrome-like symptoms. Korean Journal of Parasitology 48: 225–229. https://doi.org/10.3347/kjp.2010.48.3.225

Wongratanacheewin S, Pumidonming W, Sermswan RW, Maleewong W (2001) Development of a PCR-based method for the detection of *Opisthorchis viverrini* in experimentally infected hamsters. Parasitology 122: 175–180. https://doi.org/10.1017/S0031182001007235

Yamaguti S (1975) A synoptical review of life histories of digenetic trematodes of vertebrates. Keigaku Publishing Co., Tokyo, 590 pp. [219 pls]

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# Molecular phylogeography and reproductive biology of the freshwater snail *Tarebia granifera* in Thailand and Timor (Cerithioidea, Thiaridae): morphological disparity versus genetic diversity

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## Abstract

The freshwater thiarid gastropod *Tarebia granifera* (Lamarck, 1816), including taxa considered either congeneric or conspecific by earlier authors, is widespread and abundant in various lentic and lotic water bodies in mainland and insular Southeast Asia, with its range extending onto islands in the Indo-West-Pacific. This snail is, as one of the most frequent and major first intermediate host, an important vector for digenic trematodes causing several human diseases. As a typical thiarid *T. granifera* is viviparous and parthenogenetic, with various embryonic stages up to larger shelled juveniles developing within the female's subhemocoelic (i.e non-uterine) brood pouch. Despite the known conchological disparity in other thiarids as well as this taxon, in Thailand *Tarebia* has been reported with the occurrence of one species only. In light of the polytypic variations found in shell morphology of freshwater snails in general and this taxon in particular, the lack of a modern taxonomic-systematic revision, using molecular genetics, has hampered more detailed insights to date, for example, into the locally varying trematode infection rates found in populations of *Tarebia* from across its range in Thailand as well as neighboring countries and areas. Here, we integrate evidence from phylogeographical analyses based on phenotypic variation (shell morphology, using biometry and geometric morphometrics) with highly informative and heterogeneous mtDNA sequence data (from the gene fragments cytochrome c oxidase subunit 1 and 16 S rRNA). We evaluate both the morphological and molecular genetic variation (using several phylogenetic analyses, including haplotype networks and a dated molecular tree), in correlation with differences in the reproductive biology among populations of *Tarebia* from various water bodies in the north, northwest, central, and south of Thailand, supplementing our respective analyses of parasite infections of this thiarid by cercaria of 15 trematode species, reported in a parallel study. Based on the comparison of topotypical material from the island of Timor, with specimens from 12 locations as reference, we found significant, albeit not congruent variation of both phenotype and genotype in *Tarebia granifera*, based on 1,154 specimens from 95 Thai samples, representing a geographically wide-ranging, river-based cross-section of this country. Our analyses indicate the existence of two genetically distinct clades and hint at possible species differentiation within what has been traditionally considered

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as *T. granifera*. These two lineages started to split about 5 mya, possibly related to marine transgressions forming what became known as biogeographical barrier north of the Isthmus of Kra. Grounded on the site-by-site analysis of individual *Tarebia* populations, our country-wide chorological approach focussing on the conchologically distinct and genetically diverse lineages of *Tarebia* allows to discuss questions of this either reflecting subspecific forms versus being distinct species within a narrowly delimited species complex. Our results, therefore, provide the ground for new perspectives on the phylogeography, evolution and parasitology of Thai freshwater gastropods, exemplified here by these highly important thiarids.

## Introduction

Thailand is situated in one of the most biodiverse areas of the world (e.g. Baimai 2010). Located in the center of mainland Southeast Asia, it is situated in a hot and humid climatic zone of the wet tropics, which supports complex ecosystems as varied as rainforests and coral reefs, with numerous life forms. Although Thailand is a relatively small country, there are various kinds of limnic systems providing aquatic habitats that have gained little attention. Thailand is bordered to the north by Myanmar and Laos, to the east by Laos and Cambodia, to the south by the Gulf of Thailand and Malaysia, and to the west by the Andaman Sea and the southern extremity of Myanmar. Its maritime boundaries include Vietnam in the Gulf of Thailand to the southeast, and Indonesia and India on the Andaman Sea to the southwest. Biologists divide Thailand into two regions, viz. the Indochinese region and the Sundaic region separated by the Isthmus of Kra, a biogeographical barrier believed to be affected by sea level change in the past (e.g. Bruyn et al. 2005, Parnell 2013, Dejtaradol et al. 2016). For example, in contrast to those species among birds of the Northern Highland with Chinese affinities, a number of species in the Southern Peninsula are related to those from the Sundaic region (e.g. Lekagul and Round 1991). However, the Thai peninsula not only forms a barrier to the distribution of several groups, but is also an important bridge in the biogeography of Southeast Asia, connecting taxa of northern and southern biotas.

In addition, Thailand can be divided into geographical regions based on distinct drainage basins; with those in the north, for example, forming the Chao Phraya drainage flowing into the Gulf of Thailand, those in the northeast as part of the Mekong river basin which eventually drains into the South China Sea, or the north-western region as part of the Salween river system. In contrast to these and other major river systems, in the south there are shorter rivers that either run east to the Gulf of Thailand or west to the Andaman Sea. These water bodies in Thailand form hotspots of aquatic biodiversity with various local endemism.

Among the aquatic biota, limnic molluscs are diverse, and include about 280 species of fresh and brackish water gastropods (Brandt 1974). Studies trying to elucidate the origin of biodiversity and mechanisms of speciation in diverse systems have focused primarily on vertebrates (mostly birds and fishes), while other groups, particularly invertebrates, remained widely untested. As shown by Schwenk et al. (2008) and Glaubrecht (1993, 1996, 2009, 2010, 2011, and literature therein), molluscs and in particular freshwater gastropods hold the same promise for studying evolutionary phenomena as other groups. Speciation should not only be reflected in the taxonomic description of any speciose group, but instead by the actual study of causation and underlying mechanisms of how species arise. Thus, instead of merely referring to "speciation", "adaptive radiation" or any "megadiverse" species assemblage for each and every speciose taxonomic group we should strive to investigate, with adequate methods and founded on solid theoretical ground, the underlying mechanisms of anagenetic versus cladogenetic change; see e.g. the discussion of freshwater gastropods as model of speciation and evolutionary systematics in Glaubrecht (2006, 2009, 2010, 2011).

Accordingly, non-marine molluscs in Thailand should receive more attention and focus on studies looking into species diversity and contributing to solving fundamental questions and the evolution of faunal diversity. However, biological information on gastropods in Thai river systems and lakes is generally scarce and often lacks recently collected material or available former museum collections which hampers more in-depth studies. This is problematic, as several freshwater snails with their main occurrence in Southeast Asia have a considerable importance as first intermediate hosts for infections in humans and animals. Despite their proven medical importance, in particular the faunistic and systematic knowledge on cerithioidean freshwater snails of the various families acting as one of the most important vectors for digenic human pathogens, is precarious. The Cerithioidea is an ecologically and phylogenetically important, albeit essentially marine caenogastropod group, with its freshwater members in Southeast Asia acting as first intermediate hosts of a wide array of diverse trematodes (see details and references e.g. in Dechruksa et al. 2007, Ukong et al. 2007, Krailas et al. 2011, 2012, 2014, Veeravechsukij et al. 2018).

Cerithioidean freshwater taxa were long subsumed under the historical concept of "melaniids", which was later uncritically replaced by the family assignment to the Thiaridae (see e.g. Brandt 1974, Brown 1994). For a

discussion of a more up-to-date concept of the freshwater Cerithioidea see reviews by Glaubrecht (1996, 1999, 2010, 2011), supplemented by comparative morphological and molecular phylogenetic studies corroborating these earlier findings (Lydeard et al. 2002, Strong et al. 2011). For example, molecular phylogenic analysis now supports the inclusion of the Thai genera *Brotia*, *Paracrostoma* and *Adamietta* into the Pachychilidae from members of the Thiaridae sensu stricto, representing two independent invasions and colonisations of freshwater habitats in the tropics worldwide (e.g. Glaubrecht 1996, 2011, Köhler and Glaubrecht 2001, 2006, Glaubrecht and Köhler 2004, Lydeard et al. 2002, Glaubrecht et al. 2009, Strong et al. 2011).

Thiaridae which are found mostly in tropical to subtropical regions worldwide, inhabit virtually all freshwater and brackish-water bodies, both in lotic (including springs, creeks, rivers and streams) and lentic habitats (lakes and ponds). They are essentially, and presumably originally, widely distributed throughout Southeast Asia and in Australia (see Glaubrecht 1996, 2011, Glaubrecht et al. 2009). With an estimated 60 to 200 species in about 12 genera, but many more named taxa (Glaubrecht unpublished data), the thiarids are yet in need of a comprehensive phylogenetic analysis as well as thorough systematic revision. The largely unresolved taxonomy of thiarids is characterized by a high frequency of redundancy (in the order of up to 70 % of all named species) due to the typological approach of naming each and every phenotype as distinct (morpho-)species, as was exemplarily shown for the Australian thiarid taxa (see e.g. Glaubrecht 2010, 2011, Glaubrecht et al. 2009).

To complicate matters, Thiaridae are both parthenogenetic, with many populations essentially representing clones of individual females, and viviparous, with various typical embryonic stages developing within the female's non-uterine, i.e. subhemocoelic brood pouch, and with distinct reproductive strategies to be found, viz. eu-viviparous vs. ovo-viviparous modes that are correlated with the amount of nourishment provided by the female (Glaubrecht 1996, 1999, 2006, 2011, Glaubrecht et al. 2009, Maaß and Glaubrecht 2012).

In Thailand, the Thiaridae are represented by several described species, mostly being conchologically highly variable, such as e.g. *Melanoides tuberculata* (O. F. Müller, 1774), *Mieniplotia scabra* (O. F. Müller, 1774) or *Tarebia granifera* (Lamarck, 1816), the latter being commonly referred to as the "Quilted Melania" in the aquarium industry. Accordingly, as is typical in thiarids, a plethora of species names has been applied, irrespective of the fact that their known polymorphic phenotype, in combination with their viviparity and mainly parthenogenetic reproduction, renders unequivocal species delimitation quite problematic; see for detailed discussion e.g. Glaubrecht 2009, 2010, 2011, Glaubrecht et al. 2009, Maaß and Glaubrecht 2012, Dechruksa et al. 2013).

This holds true especially for species assigned to *Tarebia* H. & A. Adams, 1854, which are found in rivers, streams and lakes as well as canals and ponds throughout its authochthonous distributional range. It extends, according to literature records (e.g. Rensch 1934, Benthem-Jutting 1937, 1959, Brandt 1974, Starmühlner 1976) and our analyses here (see Fig. 1), from India through the mainland and insular Southeast Asia, with northern occurrences in South China and Taiwan, to the Philippine Islands in the east, and further south and east throughout the Indonesian Archipelago (including Sumatra, Java, Bali and Lombok, Sumbawa, Sumba and Flores, as well as Borneo, Sulawesi and the Moluccas) and from New Guinea onto numerous islands of the Western Pacific; with the type locality of the nominal species *T. granifera* being Timor.

In addition, this snail has become widely invasive in the tropics outside its native range, the spreading being attributed to the aquarium trade. As early as the 1950s, though, Abbott (1952) noted that the snail has been introduced in North, Central and South America. *T. granifera* was also first reported in South Africa in 1999, established in a concrete lined reservoir in Mandeni, northern KwaZulu-Natal (Appleton and Nadasan 2002). It has since become widespread in the eastern part of South Africa, particularly in the provinces of KwaZulu-Natal and Mpumalanga (Appleton et al. 2009). Kruger National Park, South Africa's flagship national park, has also seen recent invasions with spread of *T. granifera* increasing substantially between 2001 and 2006 (Wolmarans and de Kock 2006).

That way, this snail exhibits its potency as neozoon, in combination with its role as important vector for several diseases, supporting the life cycles of digenic parasites infecting humans as well as other animals. Throughout Southeast Asia and in particular in Thailand, *T. granifera* is known as major first intermediate host and thus transmission vector for trematode parasites dangerous to humans, livestock and wild animals; among which are most prominently several species of the Heterophyidae and Opisthorchiidae reported as causing opportunistic infections in people (e.g. Dechruksa et al. 2007, Krailas et al. 2011, 2012, 2014). As we show in a parallel study (see Veeravechsukij et al. 2018), these trematodes with their larval stage (i.e. the cercariae) found in *T. granifera* occur in nearly every limnic habitat and ecological circumstance, including next to more or less natural streams, rivers, and lakes also those water bodies that are subject to rapid environmental change in an increasingly human-dominated world.

Therefore, being able to ecologically adopt apparently to a broad range of different freshwater habitats, *Tarebia* is highly diverse, with quite polymorphic shells, which are mostly elongately ovate, turreted and strongly sculptured, with both spiral grooves and ridges formed by nodules or tubercles, resulting in a plethora of named shell phenotypes (see Fig. 2). In Thailand, this snail has been reported with only a single species by Brandt (1974), though, who assigned all forms to *T. granifera*. However, as we will show here specimens from various locations in Thailand traditionally identified as of this species exhibit

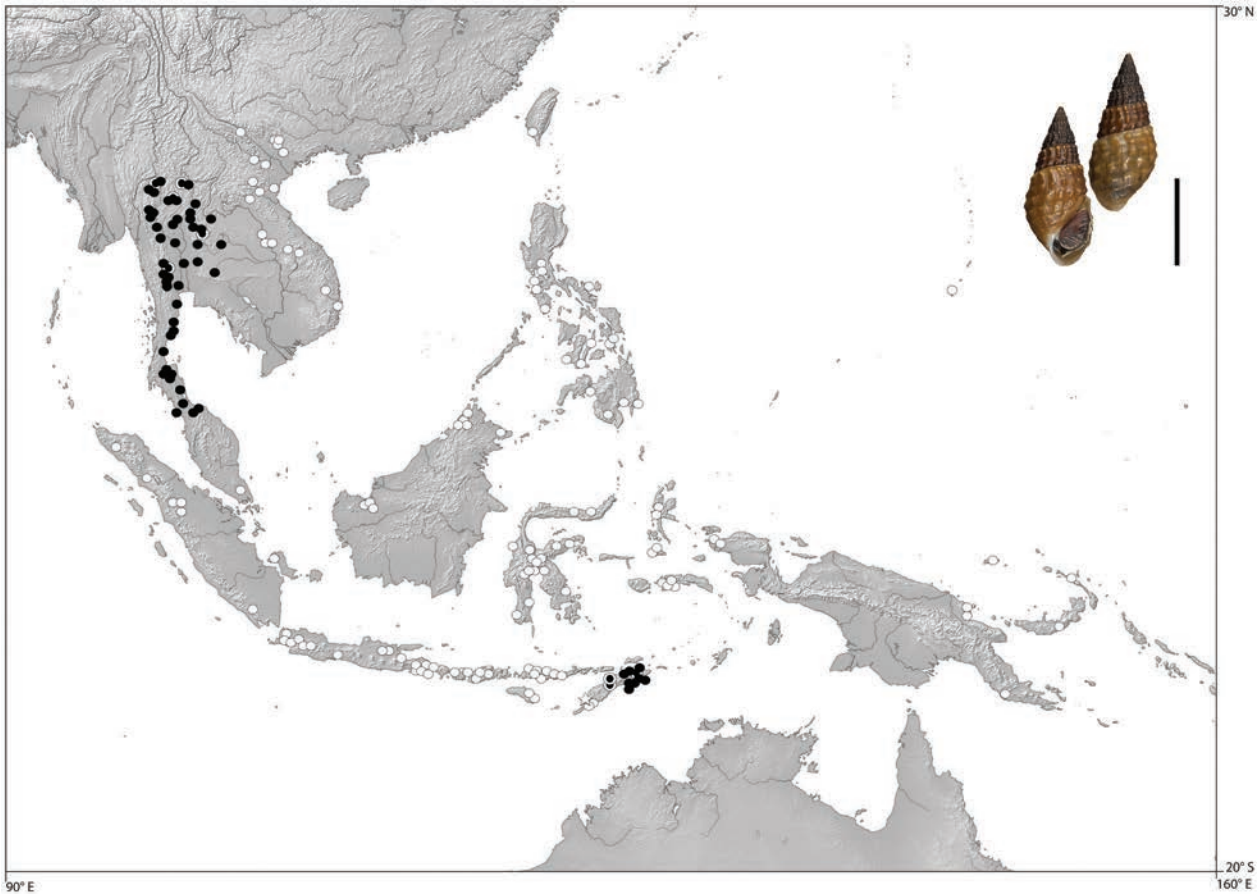


**Figure 1.** Distribution of the freshwater thiarid snail *Tarebia granifera* (Lamarck, 1816) across its range in Southeast Asia, with the focus on occurrences in Thailand, contrasted with type and topotypical material from the island of Timor. Asteriks: type locality of "*Melania*" *granifera* Lamarck, 1816, reconstructed to originate from near Kupang in western Timor (see text for more details); black dots: sequenced material used in this study; white dots: shell material from museum collections analysed and literature records; white dots with black dot inside: wet material preserved in ethanol.

a considerably high degree of variation in shell morphology, particularly in size, shape, sculpture, and colouration. Basically, there are two conchologically variable phenotypes or morphs: (i) with light brown to dark brown body whorls ornamented with tubercles, resembling quite closely the shells described and depicted as *granifera* by Lamarck (1816, 1822) and similar to the syntypes from Timor (MHNG 1093/72/1-4) (see Fig. 2a–g); (ii) with characteristic rows of nodules or tubercles most distinctly arranged in undulating spiral ridges and often with brown to dark brown spiral lines, similarly to those in typical *lineata* as described by Gray (1828) (see Fig. 2h–m).

In light of these phenotypical variations found in the shell morphology of *Tarebia*, a modern taxonomic-systematic revision, utilizing evidence from molecular phylogenetics and phylogeographical analyses, becomes desirable. However, as it is the case for most thiarids this taxon also has not found more attention yet as to intra- and interspecific species diversity, neither in Thailand nor elsewhere in adjacent regions. Here, we present results from our study of the morphological and molecular genetic variation in combination with the distributional and phylogenetic relationships as well as differences in the reproductive biology of thiarids, in particular in populations from the North, Central, Northeast and South of Thailand. We focus on the two phylogenetically highly informative and heterogeneous mitochondrial gene fragments cytochrome c oxidase subunit 1 and 16 S rRNA genes. In addition, we have studied the progeny and ontogeny of representatives from populations throughout the geographical distribution in Thailand, i.e. the frequency of various ontogenetic stages of embryos and shelled juveniles in the females' brood pouch. Combining the study of morphological variation (using biometry and geometric morphometrics) with molecular genetic variation and reproductive biology analyses, we compared the populations of Thailand as our special focus to topotypical samples recently collected from the type locality Timor as reference.

Viewed from the background of a molecular backbone phylogeny we are, finally, able to analyse a suite of questions concerning the nature of cladogenesis, phylogeography and reproductive biology in these snails, in context with the infections by various trematodes, eventually hoping to elucidate the interrelationship and co-existence of human-infectious trematode parasites and their first intermediate snail hosts.

**Figure 2.** Shells of *Tarebia granifera* (Lamarck, 1816) from Timor and Thailand. **a**. Syntypes (MHNG 1093/72/1-4) from Timor. **b–g**. Morph A, i.e. specimens from Thailand corresponding to *T. granifera* (SUT 0514044, SUT 0516123, SUT 0515088, SUT 0515068, SUT 0515059, SUT 0516144). **h–m**. Morph B, i.e. specimens from Thailand corresponding to named *T. lineata* (Gray, 1828) (SUT 0515081, SUT 0514046, SUT 0516129, SUT 0515092, SUT 0515095, SUT 0516143). **n–s**. Morph C from Thailand (SUT 0515079, SUT 0516126, SUT 0515055, SUT 0515091, SUT 0516147, SUT0516142). **t–y**. Shells of *T. granifera* from Timor Leste (ZMH 119364, ZMH 119359, ZMH 119357, ZMH 119353, ZMH 119363, ZMH 119361). For locality data, see the material list in the main part of the text. Scale bar: 10 mm.

## Material and methods

### Drainage and river systems of Thailand

The National Committee on Hydrology separates Thailand into 25 distinct hydrological units or river basins, which are used in this study as an established geographical reference. These units comprise the following rivers and drainage systems: Salween, Mekong, Kok, Shi, Moon, Ping, Wang, Yom, Nan, Chao Phraya, Sakaekrang, Pasak, Tha Chin, Mae Klong, Prachinburi, Bang Pakong, Tonle Sap, Peninsular East Coast, Phetchaburi, Peninsular West Coast, Southeast Coast, Tapi, Songkhla Lake, Pattani and Southwest Coast. These catchment and drain-

age systems are re-grouped here into seven areas, each with specific characteristics; refer to Figs 1, 4a, 8 for a graphical overview:

1 *Central area:* This is the most important area for Thailand, as it is an area without large water sources. The region, therefore, depends heavily on water from river basins upstream, such as Chao Phraya River as the main river of Thailand. The Chao Phraya begins at the confluence of the Ping and Nan rivers (Northern area) at Nakhon Sawan province. It flows from north to south from the central plains through Bangkok to the Gulf of Thailand.
2 *Northern area:* This area is a rich source of water for the central area (see above). For example, water of the Wang River flowing from north to south has its source in the Chiang Rai province. One of the principal cities along the river is Lampang, which is on the north bank of a curve in the river. From Lampang, the river flows southwards passing into Tak province. It joins the Ping River near Mae Salit north of the town of Tak. The Ping River originates in the Chiang Mai province, flowing through the provinces of Lamphun, Tak, and Kamphaeng Phet. The Nan River originates in the Nan province, subsequently draining the provinces Uttaradit, Phisanulok and Phichit. The Yom River joins the Nan River in the Chumsaeng district, Nakhon Sawam province. When the Nan River joins the Ping River it forms the Chao Phraya.
3 *North-western area:* This is a part of the drainage system of the Salween River, which flows into the neighbouring country of Myanmar.
4 *Western area:* This is part of the basin formed by the Me Klong River, which runs into the Gulf of Thailand.
5 *North-eastern area:* This is part of the Mekong river basin's catchment area, which drains into the South China Sea.
6 *Eastern area:* An area characterized by many short rivers.
7 *Southern area:* Many short rivers and high annual rainfall characterize this area. There are a number of large water reservoirs.

### Sampling

Specimens of *Tarebia granifera* were collected throughout Thailand. For reference, we compare with samples available to MG from Timor Leste through the courtesy of Vince Kessner, who collected there recently. All samples were preserved in 95 % ethanol. Voucher specimens are kept in the collection of the Center of Natural History (CeNak), Zoological Museum, Universität Hamburg, Germany (ZMH) and the collection of the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Thailand (SUT).

### Geographic data and maps

To reconstruct in detail the distributional range, in addition to own collecting activities in most parts of the region, material was analysed in several major museum collections, as well as literature records which were sufficiently verifiable as to the species identity (in general documented by descriptions and, even better, figures of shells collected).

Geographic coordinates of newly collected material were taken with a GPS device at the sampling site (WGS84 datum). Where GPS data for sampling sites were unavailable, coordinates were determined as accurately as possible from a map. Localities of the samples were mapped on a dot-by-dot basis on a public domain map (NaturalEarth, www.naturalearthdata.com) with ArcMap 10.4.1 (Esri Inc., Redlands, CA, USA). Final maps were compiled using Photoshop CS6 (Adobe Systems Inc., San José, CA, USA). The spelling of localities (whenever possible) follows GeoNames (http://www.geonames.org).

For climatic data, we used information from the climate of the world database (https://www.weatheronline.co.uk/reports/climate/Thailand).

### Shell morphology and biometry

The snails identified as belonging to *Tarebia* were grouped according to their morphological characteristics and geographic origin in four preliminary classes or morphs (see Fig. 2): 1.) specimens from Timor corresponding morphologically to *T. granifera*, i.e. without spiral pattern of narrow brown bands (Timor), 2.) specimens from Thailand corresponding to *T. granifera* (morph A), 3.) specimens from Thailand corresponding to *T. lineata*, i.e. specimens with a pattern of narrow brown spiral ridges (morph B), and 4.) *Tarebia* specimens from Thailand with broad brown spiral bands (morph C). In a second approach, specimens were grouped according to mitochondrial clades for morphological comparisons and analyses (see below).

The following biometrical parameters of the adult shells were taken with a digital calliper (accuracy: 0.1 mm): height of shell (h), width of shell (w), length of aperture (la), width of aperture (wa), height of body whorl (hbw), height of the last three whorls (l3w) and number of whorls (nw) (Fig. 3a). We were able to obtain these measurements from a total of 1,154 specimens. Analyses of shell parameters were performed using RStudio (RStudio Team 2016), with packages "ade4" (Chessel et al. 2004), "lawstat" (Hui et al. 2008), "agricolae" (Mendiburu 2010) "dunn.test" (Dinno 2017), and "car" (Fox and Weisberg 2011). For further testing the data set was partitioned in different predefined groups. These were: (a) four different morphs (see above) and (b) two different mitochondrial clades based on our molecular genetic analyses (see below). These different subdivisions of the data required slightly different approaches with regard to statistical testing. For the four different morphs, we first tested for normal distribution. Hence, we conducted the Shapiro-Wilk test for each subgroup individually. In case all measured variables for each group were normally distributed (Shapiro-Wilk-test $p > 0.05$), we performed an analysis of variance (ANOVA). If significant, it was followed by a Bonferroni-corrected LSD-Test. If at least one test for normal

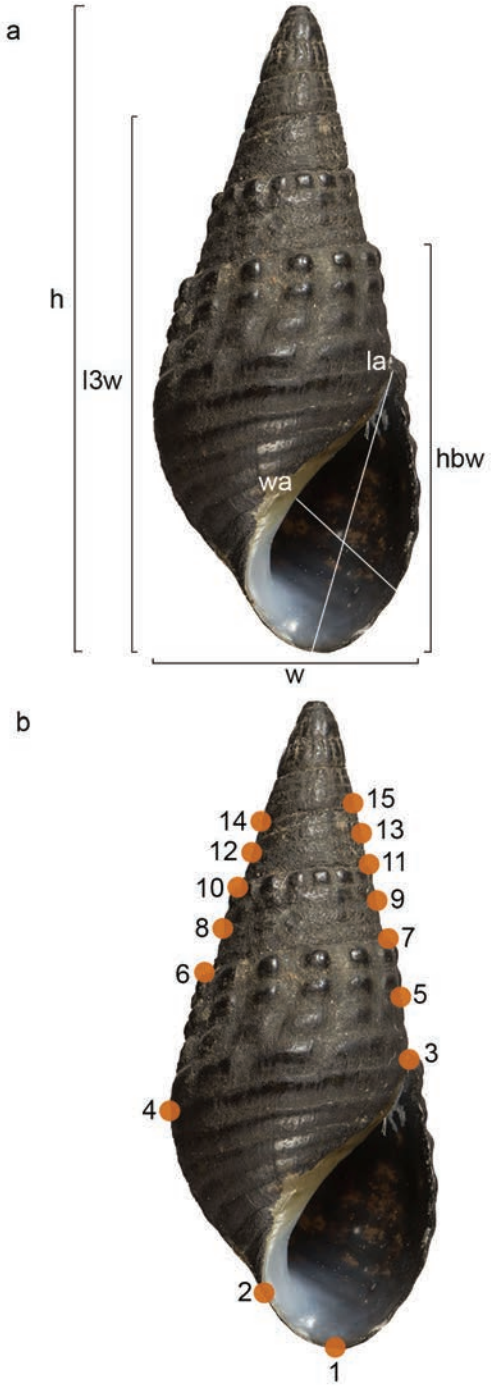


**Figure 3.** Biometrical parameters (**a**) and position of landmarks (**b**). Abbreviations: height of shell (h), width of shell (w), length of aperture (la), width of aperture (wa), height of body whorl (hbw) and height of last three whorls (l3w).

distribution (Shapiro-Wilk-test $p < 0.05$) was not significant, we instead deployed a Kruskal-Wallis-rank sum test. If the latter was found to be significant, a Bonferroni-corrected Dunn-test was conducted subsequently.

For the two different mitochondrial clades, the Shapiro-Wilk-test was performed on all measured variables for each group individually to test for normal distribution. If at least one group was not normally distributed, we conducted a Wilcoxon signed rank test with continuity correction, to test for significant differences between clades. If the data for both groups were normally distributed, a Levene-test based on absolute deviations from the mean was performed to check for homoscedasticity. In case homoscedasticity was detected, we tested different groups using a two-sample t-test. Otherwise, we performed Welch's heteroscedastic t-test.

### Geometric morphometrics

All available type specimens and the other examined material was photographed by remote shooting with EOS Utility 2.12.2.1 for Windows (Canon Inc., Tokyo, Japan) and Digital Photo Professional 3.12.51.2 for Windows (Canon Inc.) using a digital camera (EOS 5D MKII with Canon macro photograph lens MP-E 65 mm and compact macro lens EF 50 mm, Canon Inc.). Shell orientation was adjusted so that the apertural plane of the shell was perpendicular in relation to the optical axis of the camera and the shell's columella parallel to the background. Photo stacks were assembled in Helicon Focus 5.3.14.2 for Windows (Helicon Soft Ltd., Kharkiv, Ukraine). The images were then edited with Photoshop CS6 (Adobe Systems Inc.).

A total of 1,169 standardized images of adult, unbroken shells could be included in our geometric morphometrics data set. Using tpsUtil version 1.74 (Rohlf 2017a), a tps-file including all specimens was assembled. We placed 15 landmarks (see Fig 3b for landmark positions) with tpsDig2 version 2.30 (Rohlf 2017b). Data were analysed in RStudio, with "geomorph" (Adams and Otárola-Castillo 2013) and all packages listed for our biometry data analysis. After performing a Procrustes superimposition, a principal component analysis (PCA) was conducted to identify major axes of variance and to reduce dimensionality. Only axes with a relevant proportion of variance (> 0.05) were included. Following this procedure, we analysed the data set with partitions and tests as described above for the analyses biometric measurements (for each partition and principal component).

### Reproductive biology – brood pouch content

The content of the brood pouch was counted as best proxy for differences in the thiarid reproductive strategy following the method described in Glaubrecht et al. (2009) and Maaß and Glaubrecht (2012). The shells were cracked with a small vice, the operculum cut off from the posterior part of the foot using a scalpel and the soft body opened under a stereo microscope. After opening the brood pouch, which is located in the neck region of the female, care was taken to count all embryos and shelled juveniles contained within the marsupium, according to the nine standard size classes established for Thiaridae before by Glaubrecht et al. (2009): 1.) early embryos, 2.) late embryos, 3.) juveniles up to 0.5 mm, 4.) juveniles between 0.6 mm and 1.0 mm, 5.) juveniles between 1.1 mm and 1.5 mm, 6.) juveniles between 1.6 mm and 2.0 mm, 7.) juveniles between 2.1 mm and 2.5 mm, 8.) ju-

veniles between 2.6 mm and 3.0 mm, and 9.) juveniles > 3.0 mm. We compared brood pouch contents for the different predefined morphological/geographic groups as described above, the main mitochondrial clades and for the different river systems in Thailand.

### Molecular Phylogeny

Sequences from 131 specimens of *T. granifera* from 95 populations in Thailand and 12 specimens from 11 populations in Timor Leste were generated (see Table 1). Two specimens of *Thiara amarula* (Linnaeus, 1748) were selected as outgroup. Total genomic DNA was extracted from ethanol-preserved foot tissue using a CTAB protocol (Winnepenninckx et al. 1993). For phylogenetic analyses fragments of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*; 658 bp) gene using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3', Folmer et al. 1994) and HCO2198var (5'-TAW ACT TCT GGG TGK CCA AAR AAT-3', Rintelen et al. 2004) and the 16 S rRNA (16S; c. 780 bp aligned) gene using the primers 16S_F_Thia2 (5'-CTT YCG CAC TGA TGA TAG CTA G-3', Rintelen, unpublished data, see Gimnich 2015) and H3059 (5'-CCG GTY TGA ACT CAG ATC ATG T-3', Wilson et al. 2004) were amplified by PCR. Amplifications were conducted in 25 µl volumes containing, 2.5 µl 10× DreamTaq Green Buffer (Thermo Fisher Scientific, Waltham, MA, USA), 1.0 µl dNTP mix (5 mM each), 1.0 µl of each primer (10 µM), 0.2 µl of DreamTaq DNA polymerase (Thermo Fisher Scientific), 1.0 µl DNA template and 18.3 $ddH_2O$. After an initial denaturation step of 3 min at 94 °C, 35 cycles of 30 s at 94 °C, 60 s at 45–62 °C and 60–120 s at 72 °C were performed, followed by a final extension step of 5 min at 72 °C. Prior to sequencing, PCR products were enzymatically cleaned by adding 0.65 µl thermosensitive alkaline phosphatase (Thermo Fisher Scientific) and 0.35 µl exonuclease I (Thermo Fisher Scientific) to a 5 µl aliquot of the PCR reaction followed by an incubation step at 37°C for 15 min and enzyme inactivation at 85 °C for 15 min. Both strands of the amplified products were sequenced at Macrogen Europe Laboratory (Amsterdam, The Netherlands).

Forward and reverse strands were assembled using the program Geneious (Biomatters Limited, Auckland, New Zealand) and corrected by eye. The protein coding *cox1* sequences were aligned with MUSCLE (Edgar 2004) as implemented in MEGA7 (Kumar et al. 2016) under default settings. The 16S sequences were aligned with MAFFT (Katoh and Standley 2013) using the Q-INS-i iterative refinement algorithm and otherwise default settings, because this algorithm has been described to perform better for the alignment of sequence data sets that may contain deletions and insertions than alternative multiple sequence alignment methods (Golubchik et al. 2007).

For information on vouchers and GenBank accession numbers, see Table 1. Pairwise genetic p-distances for the *cox1* and 16S data sets were calculated with MEGA7.

### Phylogenetic analyses

Bayesian Inference (BI), Maximum likelihood (ML) and maximum parsimony (MP) approaches were used to reconstruct the phylogenetic relationships. The sequence data set was initially divided into four partitions for the nucleotide model-based ML and BI approaches: 1.) 1st codon positions of *cox1*, 2.) 2nd codon positions of *cox1*, 3.) 3rd codon positions of *cox1*, and 4.) 16S. To select an appropriate partitioning scheme and/or evolutionary models for the mitochondrial sequences, the data set was analysed with PartitionFinder 2.1.1 (Lanfear et al. 2012) conducting an exhaustive search and allowing for separate estimation of branch lengths for each partition using the Bayesian information criterion as recommended by Luo et al. (2010) for model selection. Models to choose from were restricted to those available in MrBayes 3.2.6 (Ronquist et al. 2012) as well as in Garli 2.1 (Zwickl 2006). As best-fit partitioning scheme, the PartitionFinder analysis suggested to combine all predefined partitions into a single partition, with the HKY+G model as best-fit model under the Bayesian information criterion.

The BI analysis was performed using MrBayes 3.2.6. Metropolis-coupled Monte Carlo Markov chain ($MC^3$) searches were run with four chains in two separate runs for 50,000,000 generations with default priors, trees and parameters sampled every 1,000 generations under default heating using the best-fit model as suggested by PartitionFinder. Diagnostic tools in MrBayes, including estimated sample size (ESS) values ≥ 200, were used to ensure that the $MC^3$ searches had reached stationarity and convergence. The first 5,000,000 generations were discarded as burn-in.

Heuristic ML analysis was performed with Garli using the best-fit models as suggested by PartitionFinder. Support values were computed by bootstrapping with 1,000 replications.

Heuristic MP searches were carried out with PAUP v4.0b10 (Swofford 2002) using 100 random-addition-sequence replicates and TBR branch swapping. Support values were computed by bootstrapping with 1,000 replications.

Bayesian posterior probabilities (PP) values ≥ 0.95 and bootstrap (BS) values ≥ 70% and were interpreted as significant/meaningful support. BS values from the ML and MP analyses were mapped onto the Bayesian 50% majority-rule consensus tree with SumTrees 3.3.1, which is part of the Dendropy 3.8.0 package (Sukumaran and Holder 2010).

### Molecular species delimitation and dating

We used the General Mixed Yule-coalescent (Pons et al. 2006) in its Bayesian implementation (bGMYC) (Reid and Carstens 2012) and the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) with p-distances for DNA sequence-based species delimitation. The bGMYC method allows for taking phylogenetic uncertainty into account by basing the analysis on several ultrametric trees sampled from the same posterior distribution. We constructed ultrametric trees for the concatenated 16S and *cox1* data set

**Table 1.** Collection voucher numbers, geographic coordinates of sampling sites and GenBank accession numbers for specimens of *Tarebia granifera* (Lamarck, 1816) used in the molecular analyses.

| Voucher Number | Latitude | Longitude | GenBank accession number | |
|---|---|---|---|---|
| | | | *cox1* | 16 S rRNA |
| SUT 0514050 | 18°17’08.5”N | 098°39’16.9”E | MK000303 | MK025577 |
| SUT 0514051 | 18°17’04.4”N | 098°39’15.0”E | MK000304 | – |
| SUT 0514054 (A) | 18°17’23.0”N | 098°39’03.6”E | MK000307 | MK025580 |
| SUT 0514054 (B) | 18°17’23.0”N | 098°39’03.6”E | – | MK025581 |
| SUT 0514052 (B) | 18°16’26.1”N | 098°38’54.0”E | MK000305 | MK025578 |
| SUT 0514052 (C) | 18°16’26.1”N | 098°38’54.0”E | MK000306 | MK025579 |
| SUT 0515081 ($B_1$) | 19°28’33.6”N | 098°07’02.4”E | MK000331 | – |
| SUT 0515081 ($B_9$) | 19°28’33.6”N | 098°07’02.4”E | – | MK025609 |
| SUT 0515077 | 19°25’31.1”N | 097°59’27.2”E | – | MK025606 |
| SUT 0515083 | 19°22’19.6”N | 098°26’35.9”E | MK000332 | MK025610 |
| SUT 0515078 | 19°21’54.8”N | 097°58’10.7”E | MK000329 | MK025607 |
| SUT 0515079 ($C_3$) | 19°15’31.6”N | 097°54’44.6”E | MK000330 | – |
| SUT 0515079 ($C_5$) | 19°15’31.6”N | 097°54’44.6”E | – | MK025608 |
| SUT 0516119 | 18°51’22.2”N | 100°11’09.1”E | MK000350 | MK025628 |
| SUT 0514045 ($B_1$) | 18°56’00.5”N | 099°38’54.6”E | MK000300 | – |
| SUT 0514045 ($B_2$) | 18°56’00.5”N | 099°38’54.6”E | – | MK025574 |
| SUT 0514044 (A) | 18°52’47.5”N | 099°40’01.0”E | MK000298 | MK025572 |
| SUT 0514044 ($B_1$) | 18°52’47.5”N | 099°40’01.0”E | MK000299 | – |
| SUT 0514044 ($B_2$) | 18°52’47.5”N | 099°40’01.0”E | – | MK025573 |
| SUT 0514046 | 18°46’39.8”N | 099°38’38.7”E | MK000301 | MK025575 |
| SUT 0516124 | 18°42’14.8”N | 099°35’31.7”E | MK000353 | MK025631 |
| SUT 0515090 | 19°11’30.4”N | 101°12’13.2”E | MK000336 | MK025614 |
| SUT 0516114 | 18°51’45.1”N | 100°28’37.1”E | MK000348 | MK025625 |
| SUT 0516108 | 18°05’03.1”N | 100°13’00.1”E | – | MK025621 |
| SUT 0516113 (B) | 18°00’50.6”N | 100°08’22.6”E | MK000346 | MK025623 |
| SUT 0516113 ($C_1$) | 18°00’50.6”N | 100°08’22.6”E | – | MK025624 |
| SUT 0516113 ($C_2$) | 18°00’50.6”N | 100°08’22.6”E | MK000347 | – |
| SUT 0516112 ($B_2$) | 17°52’19.5”N | 100°18’02.1”E | MK000345 | – |
| SUT 0516112 ($B_3$) | 17°52’19.5”N | 100°18’02.1”E | – | MK025622 |
| SUT 0513019 (A) | 17°52’29.5”N | 100°18’25.6”E | MK000292 | – |
| SUT 0513019 (B) | 17°52’29.5”N | 100°18’25.6”E | – | MK025563 |
| SUT 0513023 | 17°52’51.3”N | 100°16’14.9”E | – | MK025564 |
| SUT 0516109 | 17°43’42.3”N | 099°58’49.6”E | MK000344 | – |
| SUT 0515075 ($B_1$) | 17°13’23.4”N | 098°13’34.2”E | MK000327 | – |
| SUT 0515075 ($B_2$) | 17°13’23.4”N | 098°13’34.2”E | – | MK025604 |
| SUT 0515076 ($B_1$) | 17°26’04.8”N | 098°03’33.3”E | MK000328 | – |
| SUT 0515076 ($B_2$) | 17°26’04.8”N | 098°03’33.3”E | – | MK025605 |
| SUT 0516126 ($C_1$) | 16°52’29.3”N | 099°07’13.6”E | MK000355 | – |
| SUT 0516126 ($C_2$) | 16°52’29.3”N | 099°07’13.6”E | – | MK025633 |
| SUT 0515073 | 16°42’38.5”N | 098°30’22.2”E | MK000326 | MK025602 |
| SUT 0515072 | 16°41’39.3”N | 098°31’04.4”E | MK000325 | MK025601 |
| SUT 0515074 | 16°40’58.4”N | 098°31’06.9”E | – | MK025603 |
| SUT 0516103 ($B_1$) | 17°33’16.2”N | 099°29’48.2”E | MK000343 | – |
| SUT 0516103 ($B_2$) | 17°33’16.2”N | 099°29’48.2”E | – | MK025620 |
| SUT 0515086 ($A_1$) | 17°01’07.6”N | 100°55’36.0”E | MK000333 | – |
| SUT 0515086 ($A_2$) | 17°01’07.6”N | 100°55’36.0”E | – | MK025611 |
| SUT 0515087 | 16°57’21.3”N | 100°55’31.0”E | MK000334 | MK025612 |
| SUT 0516118 (A) | 16°52’13.1”N | 100°50’17.4”E | MK000349 | MK025626 |
| SUT 0516118 (B) | 16°52’13.1”N | 100°50’17.4”E | – | MK025627 |
| SUT 0515067 | 16°50’36.3”N | 100°45’16.1”E | MK000319 | MK025595 |
| SUT 0516130 | 16°39’46.3”N | 101°08’09.8”E | – | MK025637 |
| SUT 0516121 | 16°37’23.8”N | 100°54’00.5”E | – | MK025629 |
| SUT 0516120 | 16°36’01.3”N | 100°54’29.9”E | MK000351 | – |
| SUT 0516123 | 16°34’24.1”N | 100°59’23.6”E | MK000352 | MK025630 |
| SUT 0515088 ($A_1$) | 16°32’51.7”N | 100°54’03.2”E | MK000335 | – |
| SUT 0515088 ($A_2$) | 16°32’51.7”N | 100°54’03.2”E | – | MK025613 |
| SUT 0516129 ($B_2$) | 16°32’25.6”N | 101°04’58.4”E | MK000358 | – |
| SUT 0516129 ($B_3$) | 16°32’25.6”N | 101°04’58.4”E | – | MK025636 |
| SUT 0514041 | 15°47’54.2”N | 101°14’08.1”E | – | MK025570 |
| SUT 0514042 | 15°47’52.2”N | 101°13’54.4”E | MK000296 | – |
| SUT 0514040 (B) | 15°47’29,7”N | 101°13’30,7”E | – | MK025568 |
| SUT 0514040 (C) | 15°47’29,7”N | 101°13’30,7”E | – | MK025569 |
| SUT 0514043 ($B_1$) | 15°47’19.3”N | 101°15’07.4”E | MK000297 | – |
| SUT 0514043 ($B_2$) | 15°47’19.3”N | 101°15’07.4”E | – | MK025571 |

| Voucher Number | Latitude | Longitude | GenBank accession number | |
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| | | | *cox1* | 16 S rRNA |
| SUT 0515068 | 17°23'24.7"N | 101°22'27.3"E | MK000320 | MK025596 |
| SUT 0516125 | 17°04'38.0"N | 101°29'20.6"E | MK000354 | MK025632 |
| SUT 0516128 ($B_4$) | 17°03'03.9"N | 101°31'38.7"E | MK000357 | – |
| SUT 0516128 ($B_5$) | 17°03'03.9"N | 101°31'38.7"E | – | MK025635 |
| SUT 0515064 ($B_4$) | 16°34'45.6"N | 102°50'22.5"E | MK000317 | – |
| SUT 0515064 ($B_5$) | 16°34'45.6"N | 102°50'22.5"E | – | MK025593 |
| SUT 0516131 (B) | 14°35'32.3"N | 101°50'30.1"E | MK000359 | MK025638 |
| SUT 0516131 (C) | 14°35'32.3"N | 101°50'30.1"E | MK000360 | MK025639 |
| SUT 0516135 | 12°37'50.0"N | 101°20'35.0"E | MK000362 | MK025642 |
| SUT 0516127 ($B_1$) | 15°40'59.6"N | 100°14'59.3"E | MK000356 | – |
| SUT 0516127 ($B_2$) | 15°40'59.6"N | 100°14'59.3"E | – | MK025634 |
| SUT 0516132 | 14°55'12.3"N | 101°13'10.9"E | MK000361 | MK025640 |
| SUT 0516133 | 14°44'06.4"N | 101°11'31.4"E | – | MK025641 |
| SUT 0515055 ($C_1$) | 13°49'01.2"N | 100°02'27.9"E | MK000308 | – |
| SUT 0515055 ($C_2$) | 13°49'01.2"N | 100°02'27.9"E | – | MK025582 |
| SUT 0515091 ($C_1$) | 14°37'25.9"N | 098°43'40.5"E | MK000337 | – |
| SUT 0515091 ($C_2$) | 14°37'25.9"N | 098°43'40.5"E | – | MK025615 |
| SUT 0515092 ($B_1$) | 14°26'03.0"N | 098°51'14.7"E | MK000338 | – |
| SUT 0515092 ($B_2$) | 14°26'03.0"N | 098°51'14.7"E | – | MK025616 |
| SUT 0515093 | 14°14'27.6"N | 099°03'55.9"E | MK000339 | – |
| SUT 0515061 (B) | 13°54'18.1"N | 099°23'07.8"E | – | MK025591 |
| SUT 0515061 (C) | 13°54'18.1"N | 099°23'07.8"E | MK000316 | MK025592 |
| SUT 0515060 ($B_1$) | 13°51'17.7"N | 099°22'58.9"E | MK000315 | – |
| SUT 0515060 ($B_2$) | 13°51'17.7"N | 099°22'58.9"E | – | MK025590 |
| SUT 0515059 ($A_1$) | 13°46'44.8"N | 099°25'26.7"E | MK000313 | – |
| SUT 0515059 ($A_2$) | 13°46'44.8"N | 099°25'26.7"E | – | MK025588 |
| SUT 0515059 (B) | 13°46'44.8"N | 099°25'26.7"E | MK000314 | MK025589 |
| SUT 0515058 | 13°45'00.5"N | 099°26'27.4"E | MK000312 | MK025587 |
| SUT 0515057 ($B_1$) | 13°41'28.1"N | 099°29'08.1"E | MK000311 | – |
| SUT 0515057 ($B_2$) | 13°41'28.1"N | 099°29'08.1"E | – | MK025586 |
| SUT 0515056 (A) | 13°37'00.15"N | 099°24'36.9"E | – | MK025583 |
| SUT 0515056 (B) | 13°37'00.15"N | 099°24'36.9"E | MK000309 | MK025584 |
| SUT 0515056 (C) | 13°37'00.15"N | 099°24'36.9"E | MK000310 | MK025585 |
| SUT 0515070 ($B_1$) | 13°32'54.2"N | 099°21'42.3"E | MK000322 | – |
| SUT 0515070 ($B_2$) | 13°32'54.2"N | 099°21'42.3"E | – | MK025598 |
| SUT 0515070 (C) | 13°32'54.2"N | 099°21'42.3"E | MK000323 | MK025599 |
| SUT 0515069 | 13°32'52.2"N | 099°17'33.7"E | MK000321 | MK025597 |
| SUT 0515071 | 13°32'07.4"N | 099°20'31.8"E | MK000324 | MK025600 |
| SUT 0515066 | 13°19'29.2"N | 099°14'22.0"E | MK000318 | MK025594 |
| SUT 0513032 | 12°48'02.7"N | 099°58'53,2"E | MK000293 | MK025565 |
| SUT 0516146 ($B_3$) | 11°55'29.1"N | 099°42'40.9"E | MK000372 | – |
| SUT 0516146 ($B_7$) | 11°55'29.1"N | 099°42'40.9"E | – | MK025652 |
| SUT 0516146 (C) | 11°55'29.1"N | 099°42'40.9"E | MK000373 | MK025653 |
| SUT 0514037 ($A_1$) | 11°36'50.0"N | 099°40'07.9"E | – | MK025566 |
| SUT 0514037 ($A_7$) | 11°36'50.0"N | 099°40'07.9"E | MK000294 | – |
| SUT 0514038 | 11°26'14.4"N | 099°26'33.0"E | MK000295 | MK025567 |
| SUT 0511149 | 10°44'28,8"N | 099°12'54.9"E | MK000291 | MK025562 |
| SUT 0516137 ($B_1$) | 08°48'06.9"N | 099°26'45.1"E | MK000363 | – |
| SUT 0516137 ($B_2$) | 08°48'06.9"N | 099°26'45.1"E | – | MK025643 |
| SUT 0514048 | 08°52'18.8"N | 099°25'59.1"E | MK000302 | MK025576 |
| SUT 0516147 | 09°12'39.8"N | 099°11'55.7"E | MK000374 | MK025654 |
| SUT 0516148 | 09°12'25.7"N | 099°12'25.7"E | MK000375 | MK025655 |
| SUT 0516142 (B) | 09°08'07.2"N | 099°40'31.6"E | MK000367 | MK025647 |
| SUT 0516142 (C) | 09°08'07.2"N | 099°40'31.6"E | MK000368 | MK025648 |
| SUT 0516139 | 08°47'23.0"N | 099°38'13.2"E | MK000365 | MK025645 |
| SUT 0516145 ($B_1$) | 08°43'17.3"N | 099°40'14.8"E | MK000371 | – |
| SUT 0516145 ($B_2$) | 08°43'17.3"N | 099°40'14.8"E | – | MK025651 |
| SUT 0515097 ($A_1$) | 08°10'20.8"N | 098°47'37.6"E | MK000341 | – |
| SUT 0515097 ($A_2$) | 08°10'20.8"N | 098°47'37.6"E | – | MK025618 |
| SUT 0515098 | 08°09'49.2"N | 098°47'50.9"E | MK000342 | MK025619 |
| SUT 0515095 | 07°22'11.0"N | 099°40'47.9"E | MK000340 | MK025617 |
| SUT 0516138 | 07°42'48.3"N | 099°51'33.6"E | MK000364 | MK025644 |
| SUT 0516144 ($A_1$) | 07°13'36.6"N | 100°31'41.8"E | – | MK025650 |
| SUT 0516144 ($A_2$) | 07°13'36.6"N | 100°31'41.8"E | MK000370 | – |
| SUT 0516141 ($B_1$) | 06°52'29.3"N | 100°19'48.4"E | MK000366 | – |
| SUT 0516141 ($B_2$) | 06°52'29.3"N | 100°19'48.4"E | – | MK025646 |

| Voucher Number | Latitude | Longitude | GenBank accession number | |
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| | | | *cox1* | 16 S rRNA |
| SUT 0516143 | 06°49'29.5"N | 100°19'49.7"E | MK000369 | MK025649 |
| ZMH 119364 | 08°31'32.3"S | 125°58'50.0"E | – | MK025664 |
| ZMH 119359 | 09°00'30.6"S | 126°03'45.0"E | MK000382 | MK025661 |
| ZMH 119358 | 09°00'44.8"S | 126°03'49.2"E | MK000381 | MK025660 |
| ZMH 119354 | 09°01'11.4"S | 126°03'58.3"E | MK000377 | MK025656 |
| ZMH 119357 | 08°26'36.3"S | 126°28'11.4"E | MK000380 | MK025659 |
| ZMH 119356 | 08°20'32.1"S | 127°01'07.9"E | MK000379 | MK025658 |
| ZMH 119353 | 08°25'34.6"S | 126°41'42.5"E | MK000376 | – |
| ZMH 119362 | 08°56'47.1"S | 124°58'28.4"E | MK000385 | – |
| ZMH 119355 | 08°44'36.4"S | 126°22'49.7"E | MK000378 | MK025657 |
| ZMH 119360 | 08°47'05.0"S | 126°22'32.0"E | MK000383 | MK025662 |
| ZMH 119363 | 08°47'05.0"S | 126°22'32.0"E | MK000386 | – |
| ZMH 119361 | 09°01'59.6"S | 125°59'35.9"E | MK000384 | MK025663 |

Outgroup: *Thiara amarula*: ZMB (Museum für Naturkunde, Berlin, Germany) 107472, Indonesia, Ambon Island (*cox1*: MK000289; 16S: MK025560) and ZMB 191489, Indonesia, Obi Island (*cox1*: MK000290; 16S: MK025561).

with Beast 2.4.1 (Bouckaert et al. 2014) assuming a strict clock and the same evolutionary model as in the Bayesian and ML analyses (root age was set to one using a lognormal prior). Chains were run for 10,000,000 generations discarding the first 50% of the generations as burn-in and sampling every 50,000$^{th}$ tree resulting in a set of 100 ultrametric trees which were used in the bGMYC analyses. For each of the 100 ultrametric trees in the 16S and *cox1* data set, the Markov-chain Monte Carlo sampler implemented in the bGMYC R package (Reid and Carstens 2012) was run for 100,000 generations, discarding the first 90,000 generations as burn-in and sampling every 100 generations.

We dated the divergence times for the main clades of *Tarebia* included in this study using the Bayesian algorithm implemented in Beast 2 based on the concatenated mitochondrial data assuming a strict molecular clock as the test implemented in MEGA 7 (Kumar et al. 2016) rejected a strict molecular clock at $\alpha = 0.05$. The same partitioning scheme and nucleotide substitution models as in the MrBayes analysis described above were used. As tree prior the Yule speciation model was chosen. In the absences of fossil calibration points, a constant substitution rate of 1% per Ma was assumed as has previously been done by Köhler and Glaubrecht (2010) for related freshwater cerithioideans in the Pachychilidae. The Beast 2 analysis was run for 10,000,000 generations with a sampling frequency set to 10,000. Tracer 1.7 (Rambaut et al. 2018) was used to assess convergence of runs and to check whether effective sample sizes for all estimated parameters were above 200. A maximum clade credibility tree with median node heights was calculated with Treeannotator v1.8.2 from the BEAST 2 package discarding 10% of generations as burn-in.

# Results

### *Tarebia granifera* Lamarck, 1816

**Type material.** 4 syntypes (MHNG 1093/72/1-4).

**Type locality.** Originally given as "Timor" by Lamarck (1822). This island, of which the western part is today a province of Indonesia (the eastern part, in contrast, forms the recently independent state of East Timor, or Timor Leste), was an important stop-over for major expeditions of discovery in the Indo-West-Pacific and Australia in particular (see Glaubrecht 2002). However, at that time and the time of collecting, around 1800, all expeditions known to us have anchored at the natural harbor of Kupang. Thus, we here restrict the type locality on this island to the vicinity of its western part (see Fig. 1). Nevertheless, we regard material collected recently by Vince Kessner elsewhere on this island of Timor and used in the present study as reference and for comparison as to qualify as topotypical material.

**Taxonomy.** Lamarck (1816) depicted for the first time shells of this thiarid, creating the name *Melania granifera*, however without any further description. Later, Lamarck (1822) described this new species and its shell morphology in more detail; see also Mermod (1952: 75, fig. 137). Adams and Adams (1854) transferred *Melania granifera* to its own genus *Tarebia*. Many subsequent authors, though, referring to Lamarck (1822) continue to use the generic allocation as "*Melania*" *granifera*; see e.g. Brot (1874–1879) in his widely used monography that was followed by most authors for nearly a century. However, the generic allocation remains vage, as e.g. Benthem-Jutting (1937) either used *Thiara* while she later employed *Melanoides* (see Benthem-Jutting 1956). Starmühlner (1976), in his thorough faunistic revision, provided an extensive list of synonyms for this taxon.

In addition, in the past some authors employed "*Melania" lineata* for shells found to exhibit spiral ridges and/or dark bands on its body whorls. Accordingly, Rensch (1934) divided *Tarebia* into two subspecies, namely "*Melania*" *granifera granifera* and "*Melania*" *granifera lineata*. In contrast, for Thailand, Brandt (1974) considered and employed *Tarebia granifera* as the only congeneric species to exist there; as was also done by Glaubrecht (1996).

### Biogeography

The distributional range of *Tarebia granifera* (Fig. 1) extends from mainland Southeast Asia, with Thailand and

Vietnam at its northern most margin, to the island of Taiwan and the Philippines. It also comprises, from the Malay Peninsula south and east, the region of the entire Sunda shelf area, with occurrences on the larger Sunda Islands Sumatra, Java and Borneo, as well as the islands of Nusa Tenggara (or Lesser Sunda islands), i.e. from Bali east to Timor. The species is also abundant in Wallacea, i.e. on Sulawesi and on several islands of the Moluccas (e.g. Halmahera, Ceram, Ambon). From there, it extends east into the Indo-West Pacific, with occurrences in western and eastern New Guinea and the Bismarck Archipelago.

In Thailand, this species occurs in most lentic and lotic water bodies ranging throughout the various regions, provinces and river systems. There, *T. granifera* was found in both natural and artificial water bodies on a variety of substrata, such as e.g. sand, mud, rock (and, alternatively, concrete bridge foundations, concrete walls), on bottoms of reservoirs, irrigation canals and ornamental ponds. This species is usually found together with other thiarids, most often with *M. tuberculata* and *Mieniplotia scabra*. We were not able to correlate any consistent ecological features that clearly distinguish either at particular locations or specific habitat and/or populations where *T. granifera* was found to occur. Thus, the ecological requirements of this taxon, in particular contrasting those to that of other thiarids, remain insufficiently known.

**Material examined**

In the following we document here in detail the geographical origin of material studied from Thailand, in comparison with the syntypes as well as topotypical material from Timor as reference (see above). Data on other localities indicated in Fig. 2 to depict the extension of the entire distribution range of the species, will be provided and analysed elsewhere (Glaubrecht et al., in prep.).

**Thailand:1**

**Pai drainage (Salween river system):** Mae Hong Son province: Pang Mapha district, Huai Pa Hung, 19°22’20”N, 098°26’36”E, 435 m (SUT 0515083, 03. V. 2015); Mueang Mae Hong Son district, Huay Nam Kong, 19°28’34”N, 098°07’02”E, 425 m (SUT 0515081, 03. V. 2015); Tham Pla, 19°25’31”N, 097°59’27”E, 300 m (SUT 0515077, 02. V. 2015); Pai River, 19°21’55”N, 097°58’11”E, 215 m (SUT 0515078, 02. V. 2015); Huay Sua Tao, 19°15’32”N, 097°54’45”E, 235 m (SUT 0515079, 02. V. 2015).

**Moei drainage (Salween river system):** Tak province: Tha Song Yang district, check point near Moei River, 17°13’23”N, 098°13’34”E, 130 m (SUT 0515075, 02. V. 2015); Mae Salit Luang harbour, 17°26’05”N, 098°03’33”E, 110 m (SUT 0515076, 01. V. 2015); Mae Sot district, Ban Wang Takhian, 16°42’39”N, 098°30’22”E, 195 m (SUT 0515073, 30. IV. 2015); Thong Dee harbour, 16°41’39”N, 098°31’04”E, 205 m (SUT 0515072, 30. IV. 2015); Ban Huay Muang, 16°40’58”N, 098°31’07”E, 200 m (SUT 0515074, 30. IV. 2015).

**Ping drainage (Chao Phraya river system):** Chiang Mai province: Chom Thong district, Mae Soy bridge, 18°17’23”N, 098°39’04”E, 270 m (SUT 0514054, 24. VI. 2014); Ban Huay Phang, 18°17’09”N, 098°39’17” E, 260 m, SUT 0514050, 25. VI. 2014; Ban Mae Suai Luang, 18°17’04”N, 098°39’15”E, 270 m (SUT 0514051, 25. VI. 2014); Ban Mai Saraphi, 18°16’26”N, 098°38’54”E, 275 m (SUT 0514052, 25. VI. 2014); Tak province: Mueang Tak district, Ban Pak Huay Mae Tho, 16°52’29”N, 099°07’14”E, 105 m (SUT 0516126, 10. III. 2016).

**Wang drainage (Chao Phraya river system):** Lampang province: Chae Hom district, Wang river, 18°56’01”N, 099°38’55”E, 375 m (SUT 0514045, 23. IV. 2014); Ban Thung Hang stream, 18°52’48”N, 099°40’01”E, 375 m (SUT 0514044, 23. IV. 2014); Huay MaeYuak, 18°46’40”N, 099°38’39”E, 350 m (SUT 0514046, 22. IV. 2014); km. 40 + 075 bridge, 18°42’15”N, 99°35’32”E, 330 m (SUT 0516124, 09. III. 2016).

**Yom drainage (Chao Phraya river system):** Phayao province: Chiang Muan district, Thansawan waterfall, 18°51’22”N, 100°11’09”E, 230 m (SUT 0516119, 08. III. 2016); Phrae province: Mueang Phrae district, Mae Nam Saai km 9/457 bridge, 18°05’03”N, 100°13’00”E, 170 m (SUT 0516108, 07. III 2016); Sung Men district, Mae Marn reservoir, 18°00’51”N, 100°08’23”E, 205 m (SUT 0516113, 07. III 2016); Sukhothai province: Si Satchanalai district, Tat Duen waterfall, 17°33’16”N, 099°29’48”E, 135 m (SUT 0516103, 06. III. 2016).

**Nan drainage (Chao Phraya river system):** Nan province: Bo Kluea district, Wa river, 19°11’30”N, 101°12’13”E, 715 m (SUT 0515090, 11. VI. 2015); Ban Luang district, Huay Si Pun reservoir, 18°51’45”N, 100°28’37”E, 430 m (SUT 0516114, 08. III. 2016); Uttaradit province: Tha Pla district, Kaeng Sai Ngam, 17°52’20”N, 100°18’02”E, 255 m (SUT 0516112, 07. III 2016); Kaeng Wang Wua, 17°52’30”N, 100°18’26”E, 230 m (SUT 0513019, 28. VI. 2013); Huai Nam Re Noi, 17°52’51”N, 100°16’15”E, 270 m (SUT 0513023, 28. VI. 2013); Laplae district, Mae pool waterfall, 17°43’42”N, 099°58’50”E, 125 m (SUT 0516109, 07. III. 2016).

**Khek drainage (Chao Phraya river system):** Phitsanulok province: Nakhon Thai district, Huai Nam Sai, 17°01’08”N, 100°55’36”E, 215 m (SUT 0515086, 20. V. 2015); Ban Kaeng Lat, 16°57’21”N, 100°55’31”E, 325 m (SUT 0515087, 20. V. 2015); Wang Thong district, Kaeng Sopha, 16°52’13”N, 100°50’17”E, 415 m (SUT 0516118, 08. III. 2016); Poi waterfall, 16°50’36”N, 100°45’16”E, 200 m (SUT 0515067, 08. II. 2015); Khao Kho district, Kaeng Wang Nam Yen, 16°37’24”N, 100°54’01”E, 710 m (SUT 0516121, 09. III. 2016); Rajapruek resort, 16°36’01”N, 100°54’30”E, 705 m (SUT 0516120, 09. III. 2016); Phetchabun province: Khao Kho district, Huai Sa Dao Pong, 16°34’24”N, 100°59’24”E, 320 m (SUT 0516123, 10. III. 2016); Kaeng Bang Ra Chan, 16°32’52”N, 100°54’03”E, 600 m (SUT 0515088, 21. V. 2015).

**Pa Sak drainage (Chao Phraya river system):** Phetchabun province: Lom Sak district, Than Thip waterfall, 16°39’46”N, 101°08’10”E, 375 m (SUT 0516130, 11. III.

2016); Khao Kho district, Samsipkhot waterfall, 16°32'26"N, 101°04'58"E, 385 m (SUT 0516129, 11. III. 2016); Wichian Buri district, Ban Wang Ta Pak Moo 13, 15°47'54"N, 101°14'08"E, 120 m (SUT 0514041, 27. VI. 2014); Huai Leng, 15°47'52"N, 101°13'54"E, 115 m (SUT 0514042, 27. VI. 2014); Ban Wang Tian, 15°47'30"N, 101°13'31"E, 120 m (SUT 0514040, 27. VI. 2014); Huay Range reservoir at Ban Wang Ta Pak, 15°47'19"N, 101°15'07"E, 140 m (SUT 0514043, 27. VI. 2014); Lop Buri province: Phatthan Nikhom district, Suanmaduea waterfall, 14°55'12"N, 101°13'11"E, 135 m (SUT 0516132, 26. IV. 2016); Sara Buri province: Muak Lek district, Dong Phaya Yen waterfall, 14°44'06"N, 101°11'31"E, 155 m (SUT 0516133, 26. IV. 2016). Nakhon Sawan province: Mueand Nakhon Sawan district, Bungboraped, 15°41'00"N, 100°14'59"E, 30 m (SUT 0516127, 10. III. 2016).

**Loei drainage (Mekong river system):** Loei province: Phu Ruea district, Pla Ba waterfall, 17°23'25"N, 101°22'27"E, 665 m (SUT 0515068, 07. II. 2015); Phu Luang district, km. 50/350 at Loei River, 17°04'38"N, 101°29'21"E, 675 m (SUT 0516125, 10. III. 2016); Tatkoktup waterfall, 17°03'04"N, 101°31'39"E, 690 m (SUT 0516128, 10. III. 2016).

**Chee drainage (Mekong river system):** Khon Kaen province: Mueang Khon Kaen district, Bueng Thung Sang, 16°34'46"N, 102°50'23"E, 170 m (SUT 0515064, 05. II. 2015).

**Moon drainage (Mekong river system):** Nakhon Ratchasima province: Pak Thong Chai district, Lamphraphloeng reservoir, 14°35'32"N, 101°50'30"E, 260 m (SUT 0516131, 22. III. 2016).

**Khwae drainage (Mae Klong river system):** Kanchanaburi province: Thong Pha Phum district, Hindad hot spring, 14°37'26"N, 098°43'41"E, 160 m (SUT 0515091, 27. VI. 2015); Sai Yok district, Sai Yok Yai waterfall, 14°26'03"N, 098°51'15"E, 105 m (SUT 0515092, 27. VI. 2015); Sai Yok Noi waterfall, 14°14'28"N, 099°03'56"E, 115 m (SUT 0515093, 27. VI. 2015).

**Phachi drainage (Mae Klong river system):** Kanchanaburi province: Dan Makham Tia district, Ban Thung Makham Tia, 13°54'18"N, 099°23'08"E, 45 m (SUT 0515061, 17. III. 2015); Ban Ta Pu, 13°51'18"N, 099°22'59"E, 55 m (SUT 0515060, 17. III. 2015); Ban Nong Phai, 13°46'45"N, 099°25'27"E, 70 m (SUT 0515059, 17. III. 2015); Ratchaburi province: Chom Bueng district, Phachi River bridge, 13°45'01"N, 099°26'27"E, 65 m (SUT 0515058, 17. III. 2015); Ban Dan Thap Tako, 13°41'28"N, 099°29'08"E, 80 m (SUT 0515057, 17. III. 2015); Ban Pa Wai, 13°37'00"N, 099°24'37"E, 75 m (SUT 0515056, 17. III. 2015); Suan Phueng district, Lum Nam Phachi, 13°32'54"N, 099°21'42"E, 110 m (SUT 0515070, 23. I. 2015); Huai Ban Bor, 13°32'07"N, 099°20'32"E, 135 m (SUT 0515071, 23. I. 2015); Huay Nueng, 13°32'52"N, 099°17'34"E, 155 m (SUT 0515069, 23. I. 2015); Suan Phueng district, Ban Purakom, 13°19'29"N, 099°14'22"E, 275 m (SUT 0515066, 23. I. 2015).

**Mae Klong river system:** Nakhon Pathom province: Mueang Nakhon Pathom district, pond on campus of Silpakorn University, 13°49'01"N, 100°02'28"E, 80 m (SUT 0515055, 13. I. 2015).

**Gulf of Thailand:** Rayong province: Mueang Rayong district, Mae Rumphueng beach (Mae Rumphueng canal), 12°37'50"N, 101°20'35"E, 10 m (SUT 0516135, 28. IV. 2016); Phetchaburi province: Cha-am district, Khlong Cha-am (Cha-am canal), 12°48'03"N, 099°58'53"E, 20 m (SUT 0513032, 16. X. 2013); Prachuap Khiri Khan province: Mueang Prachuap Khiri Khan district, Khlong Bueng reservoir, 11°55'29"N, 099°42'40.9" E, 70 m (SUT 0516146, 11. V. 2016); Huai Yang district, Khlong Huai Yang (Yang canal), 11°36'50"N, 099°40'08"E, 55 m (SUT 0514037, 23. XI. 2014); Bang Saphan district, Kar on waterfall, 11°26'14"N, 099°26'33"E, 55 m (SUT 0514038, 23. XI. 2014); Chumphon province: Tha Sae district, Krapo waterfall, 10°44'29"N, 099°12'55"E, 75 m (SUT 0511149, 2. VII. 2011); Surat Thani province: Tha Chang district, Khlong Tha Sai (Takhoei canal), 09°12'40"N, 099°11'56"E, 10 m (SUT 0516147, 04. VI. 2016); Phunphin district, Ban Tung Ao (Ta Khoei canal), 09°12'26"N, 099°12'26"E, 5 m (SUT 0516148, 04. VI. 2016); Don Sak district, Vibhavadi waterfall (Tha Thong canal), 09°08'07"N, 099°40'32"E, 25 m (SUT 0516142, 09. V. 2016); Ban Na San district, Dat Fa waterfall, 08°52'19"N, 099°25'59"E, 80 m (SUT 0514048, 22. XI. 2014); Khlong Klai (Nong Noi canal), 08°48'07"N, 099°26'45"E, 110 m (SUT 0516137, 9. V. 2016); Nakhon Si Thammarat province: Nopphitam district, Khlong Prong (Klai canal), 08°47'23"N, 099°38'13"E, 100 m (SUT 0516139, 09. V. 2016); Krung Ching waterfall, 08°43'17"N, 099°40'15"E, 195 m (SUT 0516145, 09. V. 2016); Phatthalung province: Si Banphot district, Khlong Tha Leung (Tha Nae canal), 07°42'48"N, 099°51'34"E, 70 m (SUT 0516138, 08. V. 2016); Songkhla province: Singhanakhon district, Khlong Sathing Mo (Songkhla lake), 07°13'37"N, 100°31'42"E, 10 m (SUT 0516144, 08. V. 2016); Khlong Hoi Khong district, Khlong La reservoir, 06°52'29"N, 100°19'48"E, 60 m (SUT 0516141, 07. V. 2016); Khlong Cham Rai reservoir, 06°49'30"N, 100°19'50"E, 55 m (SUT 0516143, 07. V. 2016).

**Andaman Sea:** Krabi province: Mueang Krabi district, Khlong Sai (Khlong Sai canal), 08°10'20.8" N, 098°47'38" E, 25 m (SUT 0515097, 30. X. 2015); Wang Than Thip (Wang Than Thip canal), 08°09'49"N, 098°47'51"E, 20 m (SUT 0515098, 30. X. 2015); Trang province: Yan Ta Khao district, Khlong Palian (Palian canal), 07°22'11"N, 099°40'48"E, 20 m (SUT 0515095, 29. X. 2015).

**Timor Leste:** Manatuto district, W bank of Laclo river near Condae, ca. 4 km WSW of Manatuto, 08°31'32"S, 125°58'50"E, 35 m (ZMH 119364, 21 VI. 2012); south coast, 3.8 km N of Nancuro beach, 4.7 km SE of Natarbora, 09°00'31"S, 126°03'45"E, 20 m (ZMH 119359, 13. XI. 2011); 3.4 km N of Nancuro beach, 5 km SE of Natarbora, 09°00'45"S, 126°03'49"E, 20 m (ZMH 119358, 13. XI. 2011); 2.5 km N of Nancuro beach, 5.7 km SE of Natarbora, 09°01'11"S, 126°03'58"E, 15 m (ZMH 119354, 13. XI. 2011); Baucau district, NE of Baucau, Watabo beach, 08°26'36"S, 126°28'11"E, 20 m (ZMH 119357,

9. XI. 2011); Lautem district, Ira-Ara village, Lutu-Ira, 08°20'32"S, 127°01'08"E, 100 m (ZMH 119356, 23. V. 2011); near the Baucau/Lautem district border marker, 11.8 km NE of Laga, 08°25'35"S, 126°41'43"E, 5 m (ZMH 119353, 10. XI. 2011); Bobonaro district, north coast, 0.5 km from the mouth, Large seasonal stream in Batugade, 08°56'47"S, 124°58'28"E, 10 m (ZMH 119362, 20. V. 2012); Viqueque district, Ossu subdistrict, near village Usu Decima, Wai-eu-Lau, 08°44'36"S, 126°22'50"E, 670 m (ZMH 119355, 13. V. 2011); spring in the village, Loihuno, 08°47'05"S, 126°22'32"E, 255 m (ZMH 119360, 11. XI. 2011); spring in the village, Loihuno, 08°47'05"S, 126°22'32"E, 255 m (ZMH 119363, 17.V. 2012); Manufahi district, south coast, Fatuhcahi village, Wetetefuik creek, 09°02'00"S, 125°59'36"E, 30 m (ZMH 119361, 12 XI. 2011).

### Phylogenetic analyses

The final alignment of the *cox1* sequences had a length of 658 base pairs (bp) and that of the 16S sequences 781 bp. Genetic p-distances for *cox1* sequences of specimens determined as *T. granifera* from Thailand ranged from 0% to 14.7%, whereas all *cox1* sequences obtained from specimens from Timor Leste were identical.

For 16S sequences, p-distances among specimens from Thailand ranged from 0% to 10.4% and for Timor Leste, pairwise p-distance between specimens were very low, ranging from 0% to 0.1%.

All three phylogenetic analyses recovered two deeply divergent clades of specimens assigned to *T. granifera* (clades A and B, Fig. 4), with high to very high support (clade A, PP: 1.00, BS (ML): 95, BS (MP): 100; clade B, PP: 1.00, BS (ML): 90, BS (MP): 100). Genetic p-distances between these two clades were distinctly higher than p-distances within either clade A or clade B, 13.8% for *cox1* and 10% for 16S sequences. Genetic p-distances within clade A were with 0% to 3.34% for *cox1* and 0% to 1.44% for 16S sequences rather low.

All specimens from Timor Leste were included in clade A together with specimens mostly from the southern to southern-central parts of Thailand (Fig. 4), viz. those from the provinces Songkhla, Trang, Krabi, Nakhon Si Thammarat, Surat Thani, Chumphon, Prachuap Khiri Khan, Phetchaburi, Ratchaburi, Kanchanaburi, Nakhon Pathom, Sara Buri and Nakhon Sawan. But this clade included also specimens from the northern part of the country, viz. Chang Mai, Lampang, Phrae and Phitsanulok, and specimens from Nakhon Ratchasima and Rayong in northeast to eastern Thailand. Within clade A, relationships among specimens were generally not well-supported (Fig. 4). However, there is a general pattern that Thai specimens of *T. granifera* assigned to clade A were more frequent in the southern part of the country.

In contrast, specimens of *T. granifera* assigned to clade B were more frequent in the northern part of Thailand, i.e. the majority of specimens in this clade originate from the northern to north east Thai provinces, such as Chang Mai, Mueang Mae Hong Son, Phayao, Lampang, Nan, Uttaradit, Tak, Sukhothai, Phitsanulok, Phetchabun and Loei, while only few specimens in this clade are from the southern-central Thai provinces Phatthalung, Nakhon Si Thammarat, Surat Thani, Ratchaburi, Kanchanaburi and Lop Buri. Almost all specimens assigned to clade B were placed in a polytomy in the tree shown in Fig. 4. Corresponding to the results of the phylogenetic analyses, genetic p-distances within clade B were very low, with 0% to 0.46% for *cox1* and 0% to 0.52% for 16S sequences.

When analysed by drainage systems, we found that all specimens from the north-western part of Thailand, which is drained through the Salween river system into the Andaman Sea, were included in clade B. Likewise, specimens from the headwaters of the Ping, Wang, Yom and Nan rivers belonging to the Chao Phraya system, with few exceptions, were assigned to clade B in the phylogenetic analyses. In the lower courses of northern to northern-central Thai drainages, such as e.g. the Chao Phraya and Mae Klong drainages that run into the Gulf of Thailand, specimens assigned to both clades are present.

Similarly, specimens belonging to both mitochondrial clades are present in the Mekong drainage, whereas specimens assigned to clade A predominate in the smaller rivers in the Thai parts of the Malay Peninsula to the north and south of the Isthmus of Kra that either drain into the Gulf of Thailand or the Andaman Sea (Fig. 4). Noteworthy are a few populations from the somewhat more elevated parts of the provinces Surat Thani (SUT 0516137), Nakhon Si Thammarat (SUT 0516139) and Phatthalung (SUT 0516138) on the Malay Peninsula that were assigned to clade B (Fig. 4).

In contrast to this geographical pattern in *Tarebia granifera*, with broadly speaking an essentially southern clade A and an essentially northern clade B, we found no correspondence of specimens from the three morphotypes with the two genetically differentiated clades as outlined above as all morphs were present in both clades (data not shown).

### Haplotype networks, molecular species delimitation and dating

Evolutionary relationships among haplotypes were inferred applying a median-joining network approach that showed the two mitochondrial clades A and B to be separated by > 60 steps (*cox1* and 16S; Fig. 5a, b), while within these clades haplotypes were separated by usually only a few steps (Fig. 5a, b).

The ABGD approach suggested that the *T. granifera* clades A and B could be classified as two species for prior intraspecific divergences ($d$) of the combined *cox1* and 16S data set of $d \geq 0.0077$. The bGMYC analysis (Fig. 5c) recovered a probability of conspecifity of less than 0.05 for specimen pairs belonging to both, the mitochondrial clades A and B. For specimen pairs assigned to clade A in the phylogenetic analyses a probability of conspecifity of more than 0.7 was recovered, with most pairs having a probability of conspecifity of more than 0.95. All specimen pairs assigned to clade B in the phylogenet-

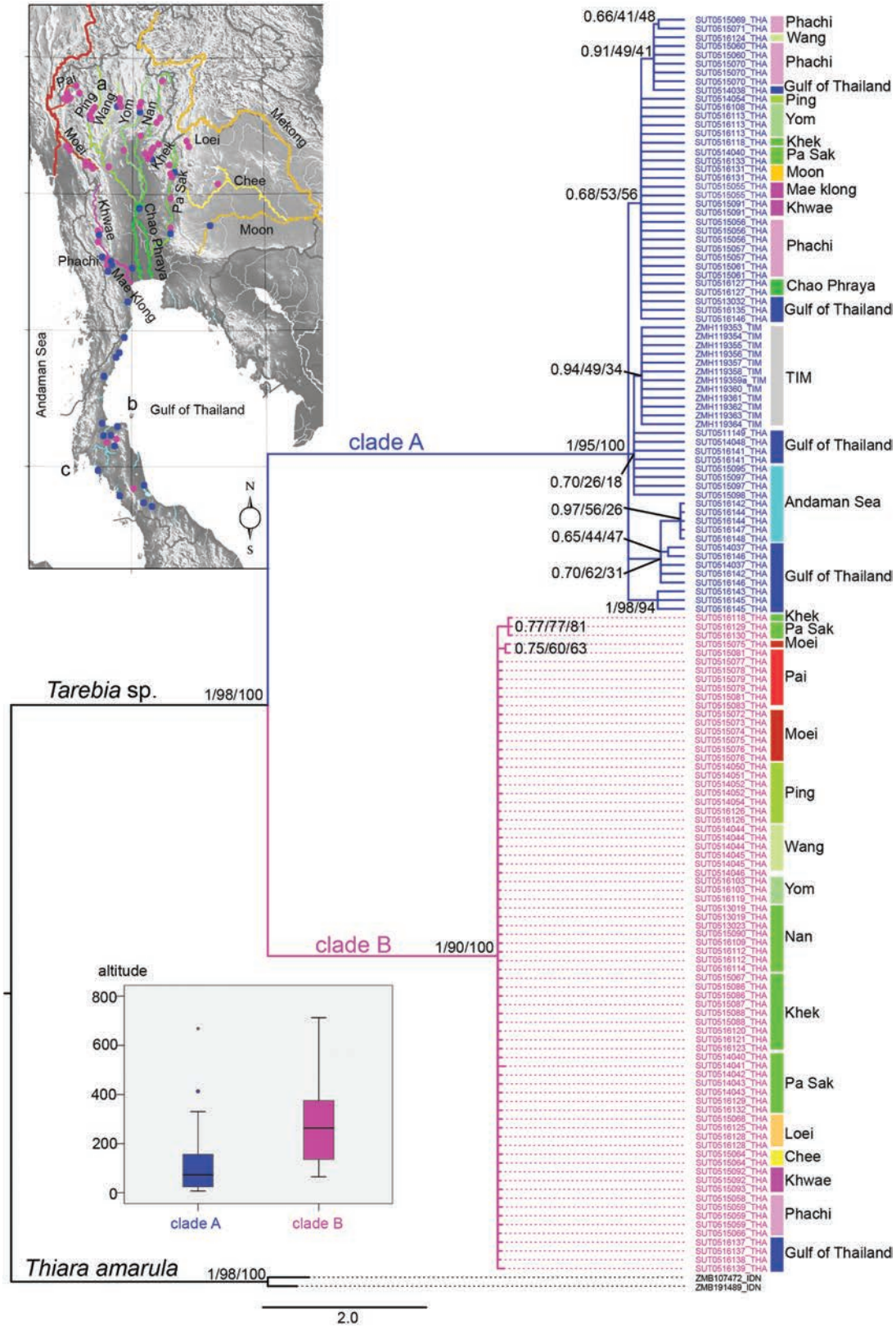


**Figure 4.** Bayesiam 50% majority-rule consensus tree showing two major mitochondrial clades in *Tarebia granifera* (Lamarck, 1816). Numbers at the nodes correspond to posterior probabilities (left), maximum likelihood (middle) and maximum parsimony (right) bootstrap values. At the tips of the tree voucher numbers (see material list in the main part of the text), country codes (THA: Thailand; TIM: Timor Leste; IDN: Indonesia) and the river where specimens were collected are indicated. The inset map shows the distribution of mitochondrial clades in Thailand (clade A: blue dots; clade B: magenta dots) and major river systems. The letters a–c in the map refer to localities, for which climatic data were available (see also Fig. 12). The inset with box plots shows the altitudinal distribution of mitochondrial caldes A and B, respectively.

ic analyses were assigned a probability of conspecificity of more than 0.95 in the bGMYC analysis.

The results of the BEAST analysis assuming a strict molecular clock and a divergence rate of 1% per million years (Fig. 5d) suggests, following the split of *Tarebia* (*granifera*) from *Thiara* (*amarula*) at about 7.1 million years ago (Mya), a separation of the mitochondrial clades A and B at about 5.3 Ma BP (95% highest posterior density interval (HPD): c. 6.5–4.0 Mya). The diverification within clade A is suggested to have started c. 0.65 Mya (95% HPD: 0.95–0.45 Ma BP), while the slitting within clade B occurred presumably c. 0.33 Mya (95% HPD: 0.50–0.25 Mya).

### Shell morphology

The shells of *Tarebia granifera* (Fig. 2), which are often of greenish or brownish colour, are medium-sized, with 12 to 44 mm, of elongately ovate-conoidal or turreted shape, much shorter than *Melanoides* and rather thick, the body whorl being greater in length than half the entire length of the shell. The spire is usually sharp, the whorls are not much convex, almost flat in the spire. The sculpture consists of spiral grooves and tubercles on the whorl. The shape of the aperture is oval with sharp peristome and curved columella; the umbilicus is closed.

As shown in Fig. 2 *Tarebia granifera* exhibits a wide phenotypical spectrum of shell morphology, which varies with respect to size and shape and in particular in sculpture and colouration including banding patterns. We separated, based on superficial "Gestaltwahrnehmung" of morphologically distinct shells, three groups called morphs A, B and C here, without implying morphotypes in the sense of species under a respective species concept, but for convenience only and to fasciliate further research into the potential correlation of phenotypical and genetic proprinquity.

Starting off from the type series of *T. granifera* from Timor (Fig. 2a) and comparing to topotypical material collected in Timor Leste (Fig. 2t–y) we distinguished based on phenotype only three major morphologies, comprising a combination of several distinct features, which taken together allows to differentiate the three morphs. The first (morph A) is similar to and characteristic by shell features also visible in the Timor types (Fig. 2b–g), with shell shape ovate-conoidal to moderately turreted and rather thick; the apex is pointed and often eroded; the colour is highly variable, ranging from yellowish-brown to dark brown and even nearly black. The number of whorls is mostly between 3 and 7, with a high spire and regularly increasing size. The body whorl is large and measures about half the length of the shell. The sculpture consists of spiral grooves and tubercles on the whorl, the suture is shallow. Next we separated those shells as morph B which agree to features similar to the description of *T. lineata* (Gray, 1828), as shown in Fig. 2(h-m), with the shell being moderately thick and elongately or ovate-conoidal, with 3–9 whorls and the body whorl being two-thirds of the shell. The colour is mostly yellowish-brown to dark brown. The sculpture of these shells were found to have small brown spiral ridges on the whorl, sometimes built as rows of tubercles. Morph C is represented by shells which combine features from both of the former morphs, but were differentiated here primarily due to the pronounced banding pattern (Fig. 2n–s).

We were not able to find any correlation of shell morphology with molecular genetic clusters as described above, or any other geographical or ecological factor matching these distinct phenotypes in *Tarebia granifera*.

### Biometry

For ranges and mean values of measured shell parameters for the different predefined groups, i.e. shell morphs/geographic groups or genetic clades, see Table 2. For all but one of the shell parameters tested, at least one group was present that was not normally distributed (Shapiro-Wilk-test, $p < 0.05$). The exception was the length of the last three whorls (l3w). Here, normally distributed data was found in every tested shell morph/geographic group (Shapiro-Wilk-test, $p > 0.05$). Hence, we conducted an ANOVA, scoring significant ($p < 0.05$) followed by a Bonferroni-corrected LSD-test. The latter found significant differences ($p < 0.025$) between the means of morph B and C. For all other parameters we performed a Kruskal-Wallis-rank sum test, significant ($p < 0.05$) for shell height and width, but not for the index of lw3/w ($p > 0.05$). Hence, the latter was found to contain no differences between groups. For shell height, a subsequent Bonferroni-corrected Dunn-test identified significant differences between the means of morph A and B ($p < 0.025$). The same test identified significant differences of means in shell width between morph B and C ($p < 0.025$). It has to be noted, however, that the ranges of all measured shell parameters widely overlap and, therefore, do not qualify as diagnostic characteristics (see boxplots in Fig. 6a–d).

Between genetic clades at least one of the groups was found to be not normally distributed (Shapiro-Wilk-test, $p < 0.05$) for shell width and l3w/w. By contrast normal distribution was found for lw3 and shell height. Subsequent Levene-testing identified the height and l3w data sets as homoscedastic ($p > 0.05$), hence a two-sample t-test was performed, identifying significant differences ($p < 0.025$) between the means for the two clades for lw3 and no significant differences for shell height. For shell width and l3w/w a Wilcoxon signed rank test was performed, revealing significant differences ($p < 0.025$) for the mean of both shell parameters. However, similar to the situation when comparing the different shell morphs/geographical groups, it has to be noted that the ranges of all measured shell parameters widely overlap and, therefore, do not allow to derive diagnostic characteristics for the two main clades found in the phylogenetic analyses (see boxplots in Fig. 7a–d).

### Geometric morphometrics

A principal component analysis (PCA) identified the first six major axes to account for a relevant proportion of

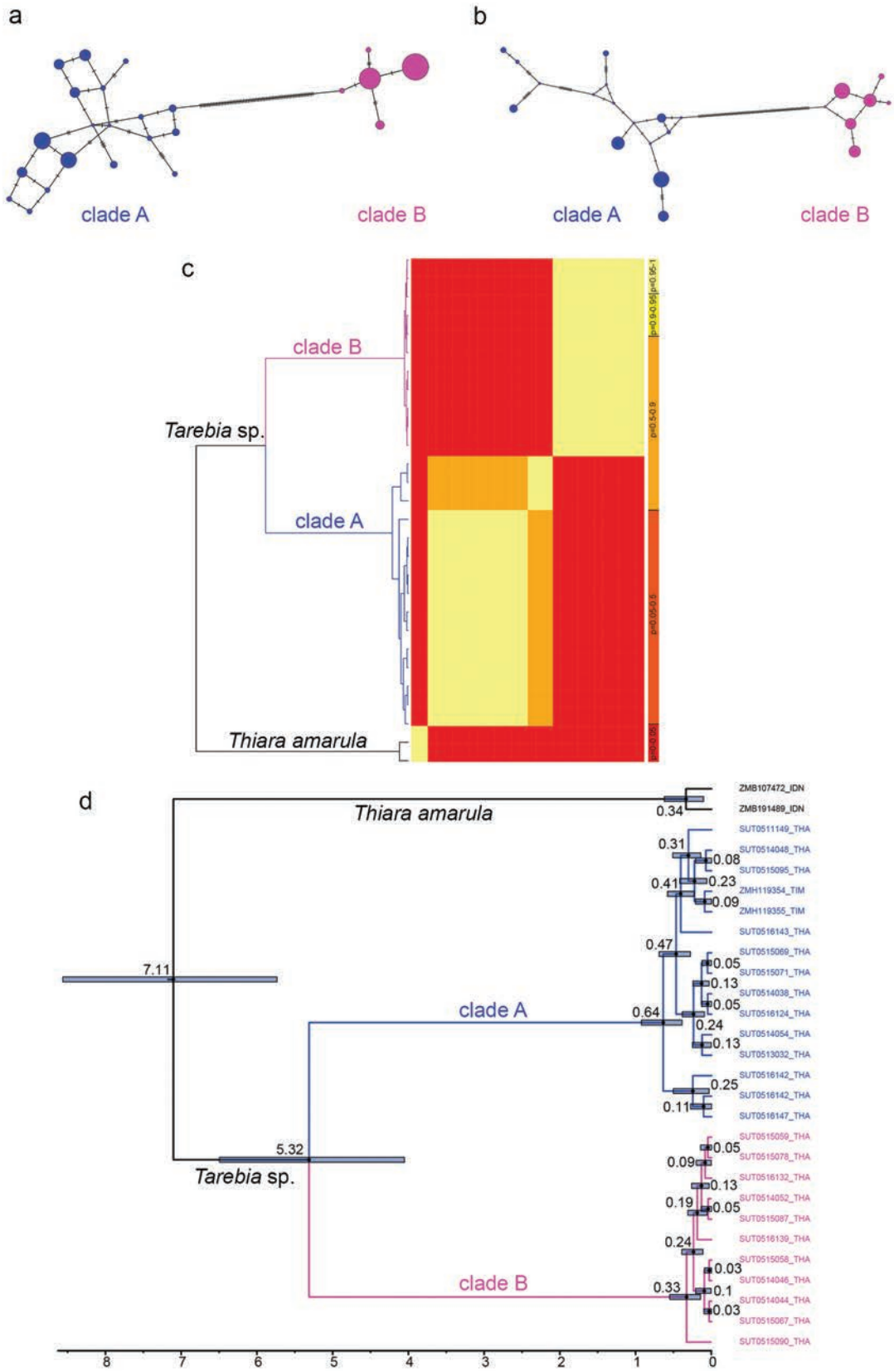


**Figure 5.** Molecular analysis of *Tarebia*. **a–b**. Median-joining haplotype networks based on 16S (**a**) and *cox1* (**b**) sequence data of *Tarebia granifera* (Lamarck, 1816). The size of each circle represents the frequency of a haplotype and the colour refers to main mitochondrial clades obtained from the phylogenetic analyses (Fig. 4; blue: clade A, magenta: clade B). Tick marks between circles represent evolutionary steps. **c**. Results of the bGMYC analysis. Colouration of the matrix cells represents pairwise probabilities of conspecificity. **d**. Dated molecular tree (only unique haplotypes were included). Numbers at the nodes are node ages in Ma, bars represent 95% highest posterior probabilitiy intervals.

variance ($p > 0.05$) (PC1: 0.303; PC2: 0.181; PC3: 0.117; PC4: 0.090; PC5: 0.058; PC6: 0.052), explaining a cumulative proportion of 0.801 of variance.

Principal components (PC) 1–6 had all at least one group that proved to be not normally distributed (Shapiro-Wilk-test, $p < 0.05$). Subsequent Kruskal-Wallis-testing was significant ($p < 0.05$) in PC1–5 and not significant in PC6. Hence, no further testing was done for PC6. The Bonferroni-corrected Dunn-test identified the mean value for specimens from Timor to be significantly different ($p > 0.025$) from all other morphs on PC1. By contrast, examining PC2 and PC4 with the same test, proved morph A and B to be the only groups not significantly different (with regard to mean values) from one another. Finally, on PC3 and PC5 the Bonferroni-corrected Dunn-Test revealed the mean value of morph C not to be significantly different from all other groups, but the means of morph A and B to be significantly different to that of the specimens from Timor.

Finally, when morph C was integrated into morph B (since these were only differentiated on the basis of slight differences in banding pattern), PC1–5 supported only the group consisting of specimens from Timor to have significantly different means from all other specimens (data not shown). The scatter plot in Fig. 6e shows the distribution of PC1 vs. PC2, illustrating that all predefined groups widely overlap, which indicates that a clear separation is not possible on the basis of shell shape.

For PC1 and PC3–6 at least one of the groups (clade A/clade B) was not normally distributed (Shapiro-Wilk-test, $p > 0.05$). Hence, we conducted Wilcoxon singed rank tests for all these PC, with none showing significant differences between groups ($p > 0.05$). By contrast, in PC2 both groups showed normally distributed data. Therefore, Levene-testing based on deviations from the mean followed and was found significant ($p < 0.05$). Accordingly, we conducted Welch's two sample t-test, revealing significant differences between the means of the two clades on PC2. The scatter plot in Fig. 7e shows the distribution of PC1 vs. PC2, illustrating that the clusters of specimens assigned either to clade A or clade B widely overlap, which indicates that a clear separation is not possible on the basis of shell shape.

## Brood pouch content

Females of *Tarebia granifera* were found to contain embryos and shelled juveniles in their "marsupium", or subhemocoelic brood pouch, situated in the neck region as in other thiarids studied so far. They usually release crawling juveniles with shells comprising several whorls that are built before hatching from the brood pouch. In this study, we found the snails to possess brood pouches filled with all ontogenetic stages, ranging from early to late embryos and six additional size classes of juveniles, with shells measuring between less than 0.5 to more than 3 mm (see Figs 8–10a).

The frequency of these different size classes in the subhemocoelic brood pouch of the total of n = 1,007 dissected

**Table 2.** Biometric data for different shell morphs/geographic groups (see also Figs 2, 8, 9) and mitochondrial clades (see Figs 4, 8, 9) of *Tarebia granifera* (Lamarck, 1816).

| | Min | Max | Mean | Median | Standard deviation |
|---|---|---|---|---|---|
| **Height** | | | | | |
| Morph A | 9.29 | 29.83 | 18.93 | 18.90 | 3.69 |
| Morph B | 8.56 | 32.38 | 19.73 | 20.06 | 3.87 |
| Morph C | 10.53 | 26.88 | 19.03 | 18.94 | 3.02 |
| Morph B+C | 8.56 | 32.38 | 19.62 | 19.81 | 3.76 |
| Timor | 11.67 | 28.53 | 19.68 | 19.69 | 3.75 |
| Clade A | 8.56 | 32.38 | 19.24 | 19.52 | 3.86 |
| Clade B | 9.45 | 30.67 | 19.66 | 19.68 | 3.64 |
| **Width** | | | | | |
| Morph A | 3.73 | 13.28 | 8.26 | 8.44 | 1.71 |
| Morph B | 3.49 | 14.46 | 8.35 | 8.55 | 1.61 |
| Morph C | 4.39 | 11.58 | 7.94 | 7.98 | 1.32 |
| Morph B+C | 3.49 | 14.46 | 8.28 | 8.39 | 1.58 |
| Timor | 5.04 | 12.18 | 8.15 | 8.20 | 1.42 |
| Clade A | 3.73 | 13.28 | 8.05 | 8.13 | 1.51 |
| Clade B | 3.49 | 14.46 | 8.46 | 8.61 | 1.64 |
| **Aperture height** | | | | | |
| Morph A | 4.38 | 14.39 | 9.23 | 9.31 | 1.84 |
| Morph B | 4.23 | 15.35 | 9.31 | 9.46 | 1.75 |
| Morph C | 4.94 | 18.96 | 9.06 | 8.98 | 1.72 |
| Morph B+C | 4.23 | 18.96 | 9.27 | 9.38 | 1.74 |
| Timor | 5.16 | 13.6 | 9.13 | 9.12 | 1.62 |
| Clade A | 4.38 | 14.39 | 9.06 | 9.10 | 1.72 |
| Clade B | 4.23 | 18.96 | 9.41 | 9.50 | 1.77 |
| **Aperture width** | | | | | |
| Morph A | 1.63 | 8.92 | 4.30 | 4.29 | 0.87 |
| Morph B | 1.68 | 8.91 | 4.25 | 4.25 | 0.90 |
| Morph C | 2.42 | 8.41 | 4.31 | 4.23 | 1.01 |
| Morph B+C | 1.68 | 8.91 | 4.26 | 4.25 | 0.92 |
| Timor | 2.40 | 6.07 | 4.09 | 4.14 | 0.71 |
| Clade A | 1.63 | 8.92 | 4.18 | 4.21 | 0.87 |
| Clade B | 1.68 | 8.91 | 4.32 | 4.29 | 0.91 |
| **Last whorl height** | | | | | |
| Morph A | 5.91 | 19.55 | 12.48 | 12.47 | 2.44 |
| Morph B | 5.77 | 20.37 | 12.60 | 12.78 | 2.35 |
| Morph C | 6.81 | 15.83 | 11.99 | 11.92 | 1.87 |
| Morph B+C | 5.77 | 20.37 | 12.50 | 12.65 | 2.29 |
| Timor | 6.53 | 17.78 | 12.35 | 12.48 | 2.22 |
| Clade A | 5.91 | 17.81 | 12.25 | 12.38 | 2.29 |
| Clade B | 5.77 | 20.37 | 12.69 | 12.81 | 2.33 |
| **Last three whorls height** | | | | | |
| Morph A | 7.93 | 26.43 | 16.84 | 17.04 | 3.29 |
| Morph B | 7.73 | 28.74 | 16.93 | 17.13 | 3.28 |
| Morph C | 9.20 | 21.34 | 15.97 | 15.95 | 2.49 |
| Morph B+C | 7.73 | 28.74 | 16.77 | 16.85 | 3.19 |
| Timor | 9.46 | 23.89 | 16.56 | 16.40 | 3.12 |
| Clade A | 7.93 | 26.22 | 16.49 | 16.54 | 3.22 |
| Clade B | 7.73 | 28.74 | 17.01 | 17.13 | 3.17 |
| **H/W** | | | | | |
| Morph A | 1.77 | 2.95 | 2.31 | 2.29 | 0.25 |
| Morph B | 1.22 | 3.13 | 2.37 | 2.38 | 0.22 |
| Morph C | 1.50 | 3.05 | 2.41 | 2.41 | 0.24 |
| Morph B+C | 1.22 | 3.13 | 2.38 | 2.39 | 0.22 |
| Timor | 1.92 | 2.87 | 2.41 | 2.41 | 0.18 |
| Clade A | 1.50 | 3.13 | 2.39 | 2.41 | 0.22 |
| Clade B | 1.22 | 2.93 | 2.34 | 2.35 | 0.23 |
| **Last three whorls/width** | | | | | |
| Morph A | 1.27 | 2.54 | 2.05 | 2.04 | 0.13 |
| Morph B | 1.22 | 2.53 | 2.03 | 2.03 | 0.15 |
| Morph C | 1.39 | 2.65 | 2.02 | 2.03 | 0.16 |
| Morph B+C | 1.22 | 2.65 | 2.03 | 2.03 | 0.15 |
| Timor | 1.66 | 2.28 | 2.03 | 2.04 | 0.13 |
| Clade A | 1.27 | 2.65 | 2.05 | 2.06 | 0.16 |
| Clade B | 1.22 | 2.38 | 2.02 | 2.01 | 0.13 |

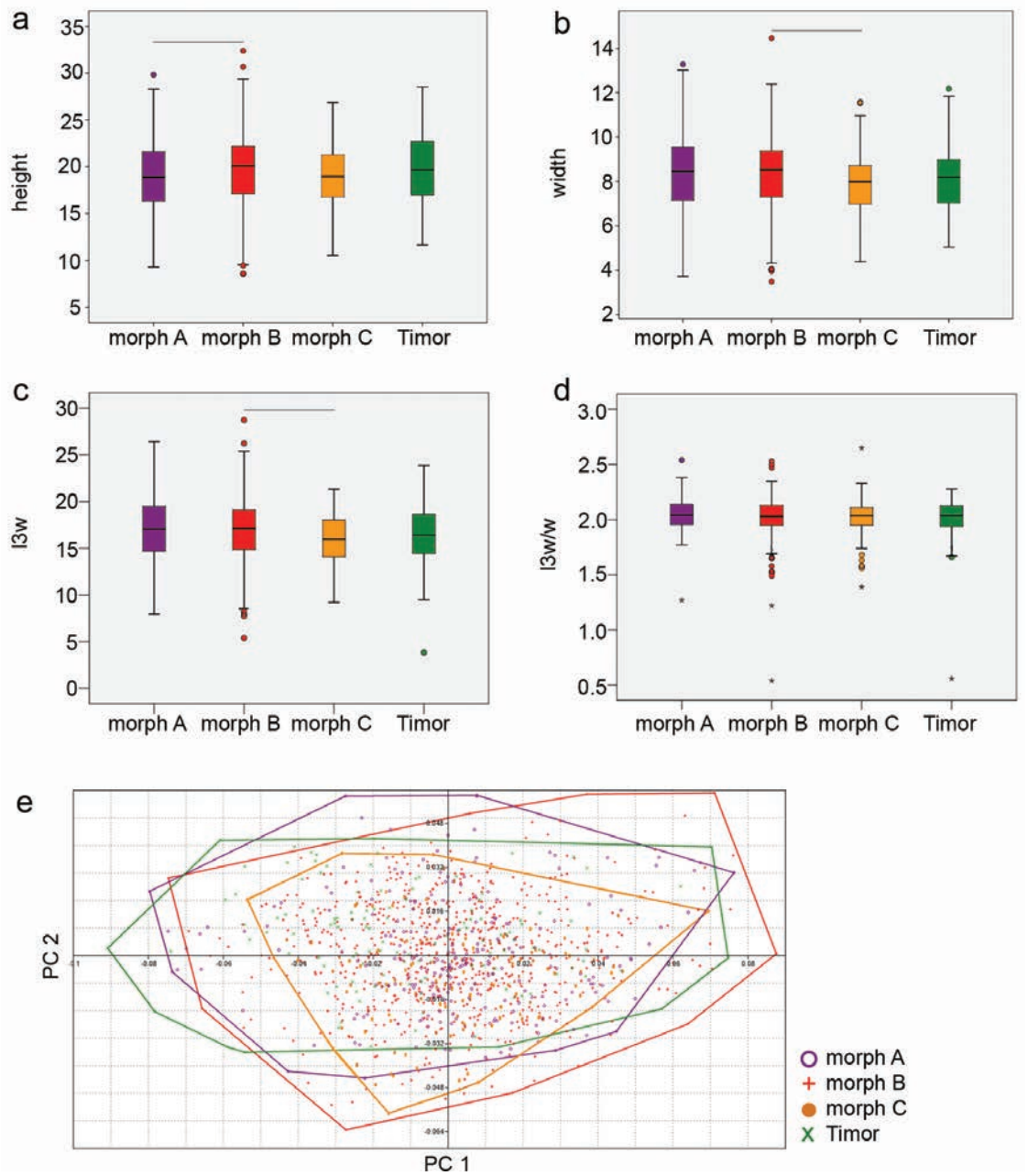


**Figure 6.** Results of biometric (**a–d**) and geometric morphometrics study (**e**), for four different morphs (A,B,C,Timor) of *Tarebia granifera* (Lamarck, 1816). Boxplots of (**a**) shell height, (**b**) shell width, (**c**) height of the last three whorls and (**d**) index of height of last three whorls agaianst shell width. Significant differences between groups are indicated by bars above the boxplots (**e**) Relative variance in shell shape along PC1 and PC2. Colour corresponding planes indicate the spread of each morph in the data set.

females of *Tarebia granifera* from a total of 107 populations from Thailand (n = 95) and Timor Leste (n = 12) is shown as to their geographic occurrence for the two mitochondrial clades A (n = 42) and B (n = 53) as well as the predefined morphs A, B and C in Figs. 8 and 9 a–c. Although the content of the brood pouch varied considerably among individuals and populations, no geographic pattern could be observed, neither for the populations within Thailand nor for those from Timor Leste. We were also unable to find any specific pattern in the distribution of the eight ontogenetic stages in correlation with the two genetic clades A and B or for the different predefined shell morphs (Figs 8, 9).

In all examined populations, the number of early and late embryonic stages was above 50%, in most cases even above 75%; see Fig. 10a for the composition of the brood pouch contents according to the three morphs A-C, and see Fig. 10c for those of the two mitochondrial clades. Nevertheless, in nearly all populations shelled juveniles of the size between less than 0.5 to more than 3.0 mm were present in the female's brood pouches; with the only exception for females (n = 1 and 9) from two populations of morph A and C, both in locations in the south in streams draining to the Gulf of Thailand (see Fig. 9a, b).

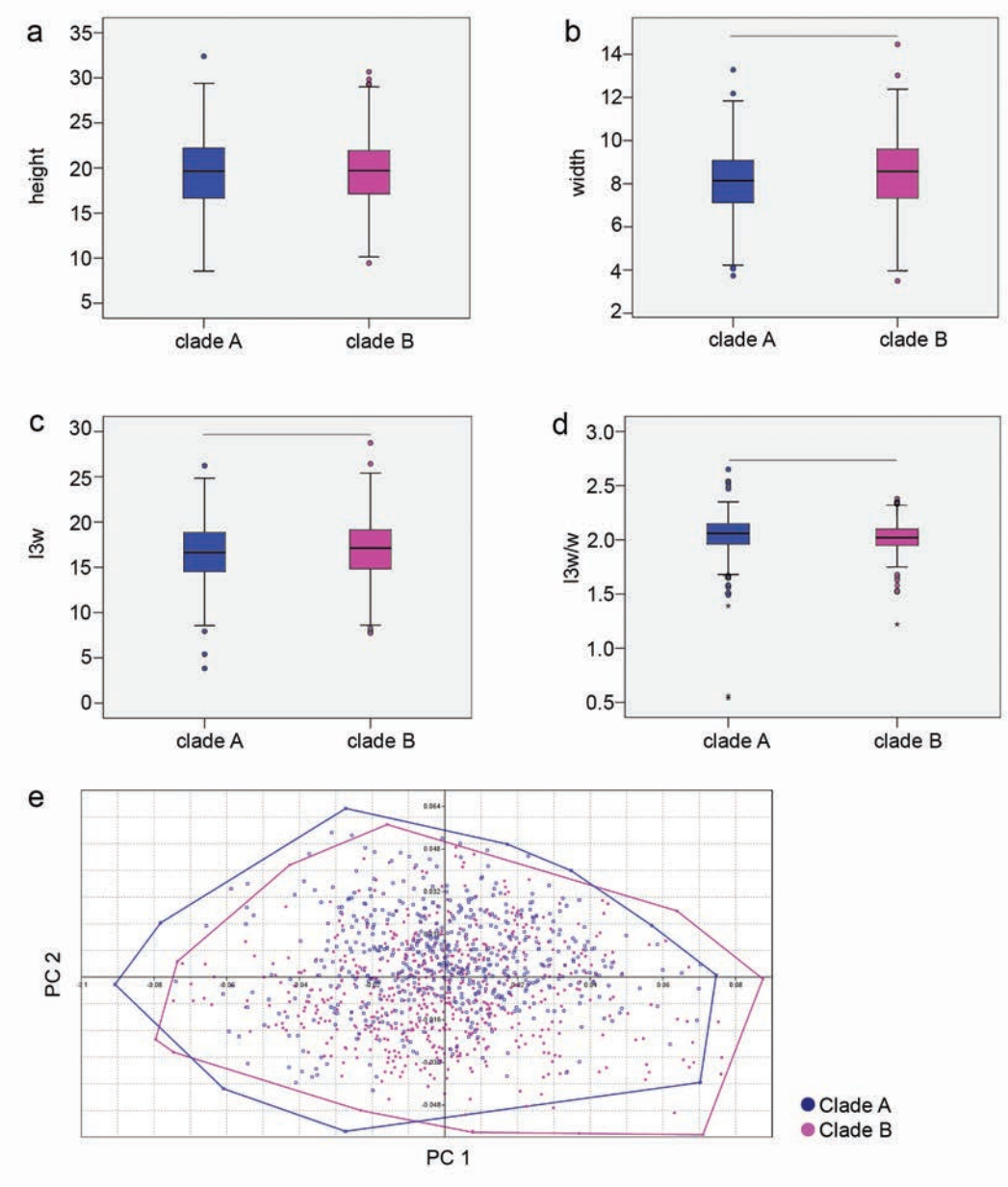


**Figure 7.** Results of biometric (**a–d**) and geometric morphometrics study (**e**), for the two mitochondiral clades of *Tarebia granifera* (Lamarck, 1816) found in this study. Boxplots of (**a**) shell height, (**b**) shell width, (**c**) height of the last three whorls and (**d**) index of height of last three whorls agaianst shell width. Significant differences between groups are indicated by bars above the boxplots (**e**). Relative variance in shell shape along PC1 and PC2. Colour corresponding planes indicate the spread of each morph in the data set.

When considering the overall distribution of different size classes in the different morphs/geographic clusters or mitochondrial clades, the resulting histograms (Fig. 10a, c) all show essentially the same composition of ontogenetic stages, which suggests the presence of the same reproductive strategy in all investigated groupings. The overall ratio of non-gravid vs. gravid specimens was 164:943 (= 17.4%). Among the 255 dissected specimens assigned to morph A, 21 snails were found to be non-gravid (= 8.2%), while among the 652 dissected snails assigned to morph B, in 123 of these no offspring was observed (= 18.9%). For morph C, the ratio of non-gravid vs. gravid specimens was 11:128 (= 8.6%) and that ratio for specimens from Timor Leste was 9:72 (= 12.5%) (Fig. 10b). Considering the two main mitochondrial clades, similar values were observed (Fig. 10d), with the proportion of gravid females well above 85%.

We also compared the size class composition of offspring in the subhemocoelic brood pouches of *Tarebia* populations from different drainage systems. Although considerable variation was present among the rivers and streams of the 17 drainage systems in Thailand (Fig. 11a), clear differences could not be observed. There is, however, one possible exception, i.e. females of *T. granifera* from the Moei River in the Northwest of Thailand, where

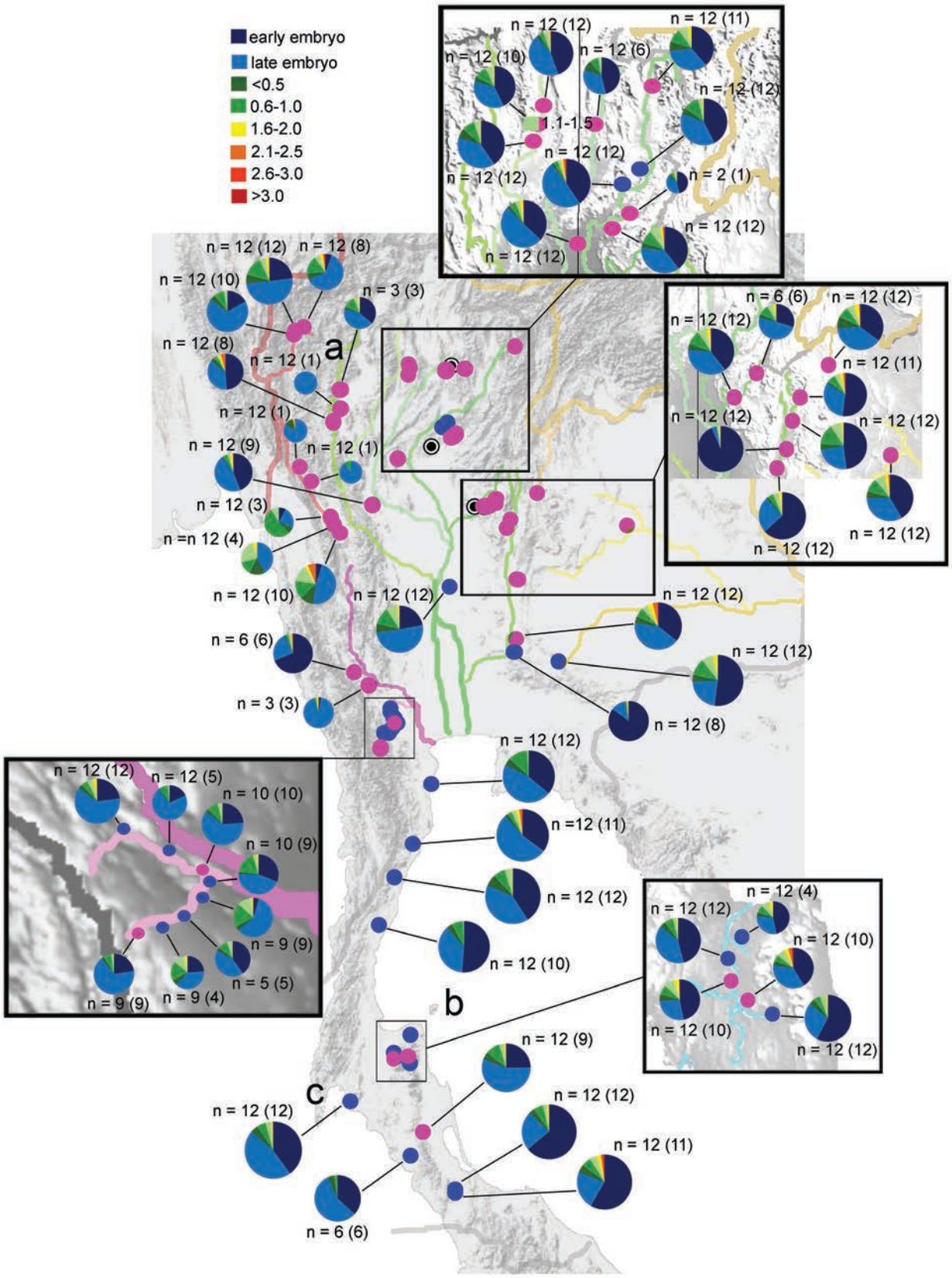


**Figure 8.** Frequency of ontogenetic stages in the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (morph B) depending on occurrence in Thailand. Blue dots: mitochondrial clade A; pink dots: mitochondrial clade B. Size classes are assigned different colours in the pie charts (see legend) and rivers are coloured according to drainage systems; numbers at the pie charts refer to the total number of dissected specimens and the number of gravid females (in parentheses). The small letters refer to the stations Chiang Mai (**a**), Ko Samui (**b**) and Phuket (**c**) for which meteorological data representing the different climatic regions of Thailand were analysed (see Fig. 12).

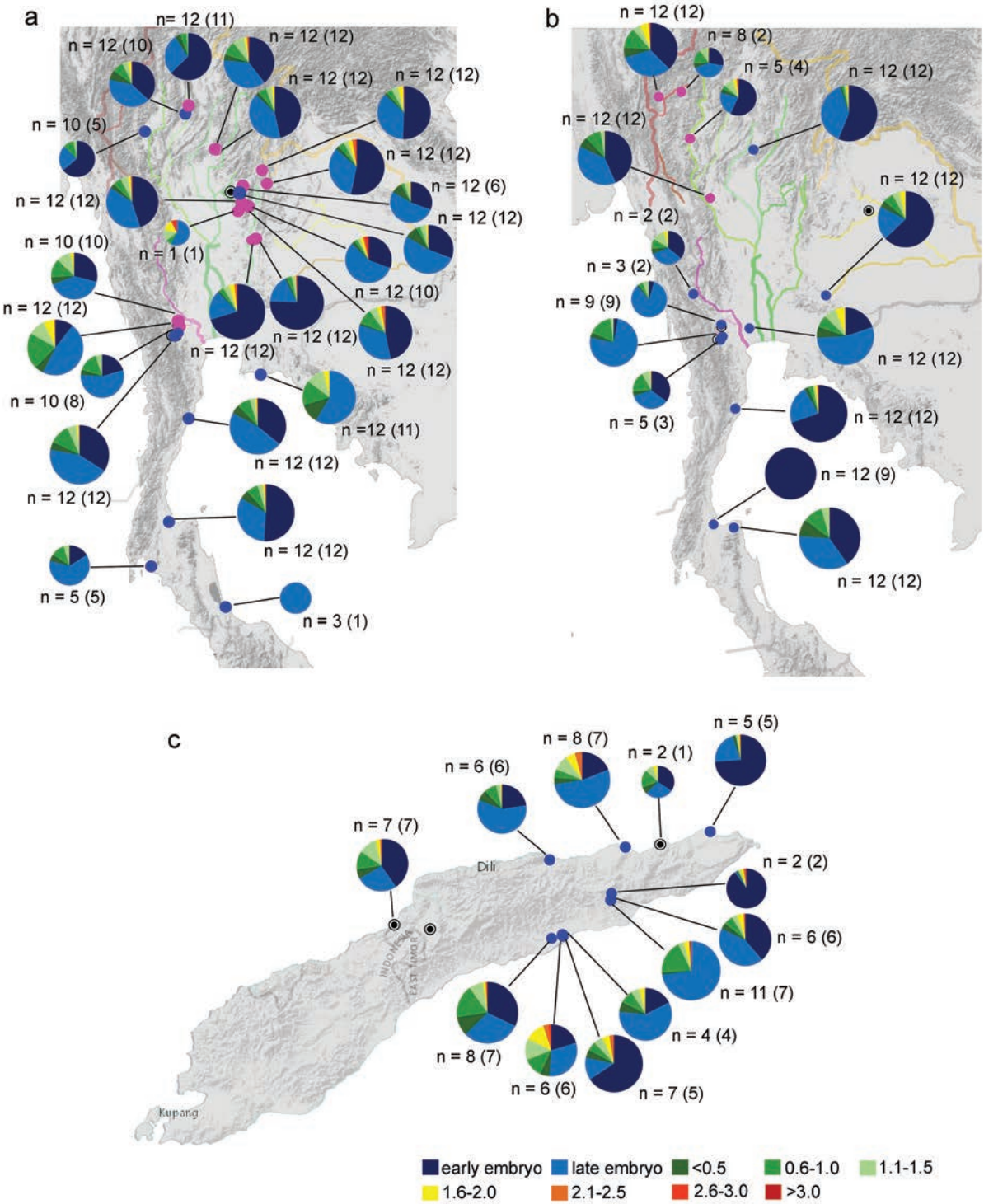


**Figure 9.** Frequency of ontogenetic stages in the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) depending on occurrence in Thailand and Timor Leste. **a**. Morph A in Thailand; **b**. Morph C in Thailand; **c**. Timor Leste. Blue dots: mitochondrial clade A; pink dots: mitochondrial clade B. Size classes are assigned different colours in the pie charts (see legend) and rivers are coloured according to drainage systems; numbers at the pie charts refer to the total number of dissected specimens and the number of gravid females (in parentheses).

a very low amount of early embryonic stages and less later embryonic stages were found, while there was the largest proportion of larger shelled juveniles. Also, there is a slight trend for populations in streams and rivers in the south of Thailand, both draining into the Gulf of Thailand and the Andaman Sea, to exhibit higher proportions of the earliest embryonic stages.

The distribution of gravid vs. non-gravid specimens according to the 17 rivers systems exhibits some variation (Fig. 11b), albeit with usually (far) more gravid

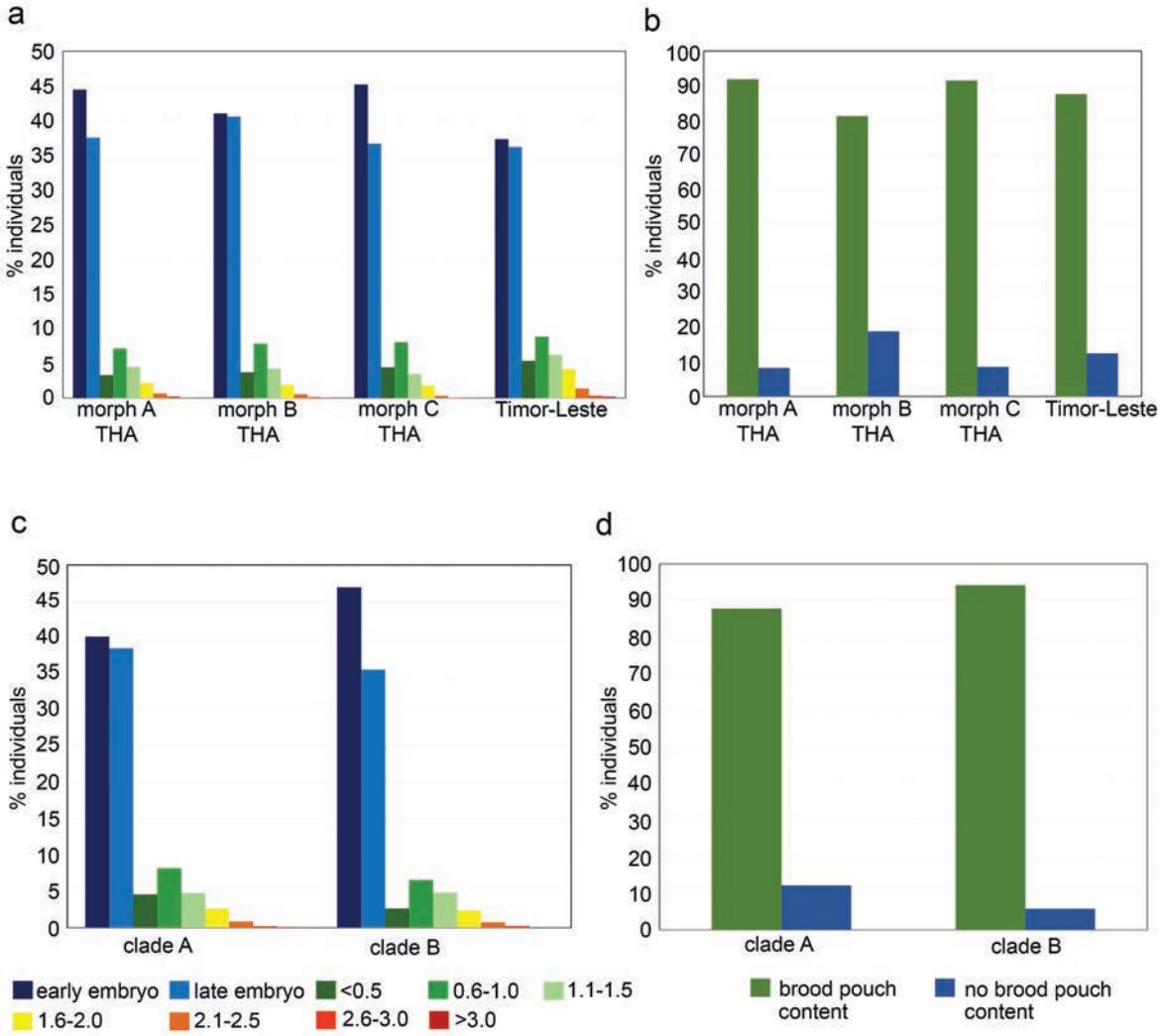


**Figure 10.** Composition of contents of the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (**a, c**) and proportions of gravid animals, i.e. those with filled brood pouch, versus non-gravid specimens (**b, d**) from Thailand and Timor Leste. **a**. Composition of contents of the brood pouches for morph A, B and C from Thailand (THA) and specimens from Timor Leste (see Figs 1, 8 and 9). **b**. Proportion of gravid vs. non-gravid specimens for morph A, B and C from Thailand and specimens from Timor Leste. **c**. Composition of contents of the brood pouches for mitochondrial clades A and B, respectively (see also Figs 4, 8, 9). **d**. Proportion of gravid vs. non-gravid specimens for mitochondrial clades A and B, respectively. For colour coding, see the inset legends.

specimens present in all populations; but again with the exception of females from populations in the Northwest of Thailand, in particular from the rivers Moei, Ping and Pai. The populations in Moei River are in this respect exceptional because only there we found more non-gravid than gravid specimens. Conversely, all females from populations in the rivers Chao Phraya, Loei, Chee, Moon, Khwae, Mae Klong and from streams of the Andaman Sea were found to be gravid, with no non-gravid specimens at all detected in our samples.

Whether reproduction is seasonal, or whether there is any influence of the month of collecting on our data, can currently not be answered with certainty. In an attempt to correlate reproduction (i.e. the frequency of gravid vs. non-gravid females) with climatic effects such as, for example, rainy season resulting in high water levels in rivers and streams, we have used published meteorological data (e.g. minimum/maximum temperature and precipitation) for stations representing the different climatic regions of Thailand, viz. Chiang Mai for northern inland region, Ko Samui for the Gulf of Thailand and Phuket for the Andaman Sea localities (see map in Fig. 8 for these locations). As is evident from Fig. 12, specimens collected in populations from inland places were to a high proportion gravid females at the end of winter (January–February) and into the summer season (March–June). During this first half of the year the proportion of gravid females somehow reflect percipitation in so far, as there is a trend to be high when it is dry (see Fig. 12a); also the proportion of non-gravid females increases towards the rainy season in the North of Thailand (April/May). At localities in the Gulf of Thailand region, high numbers

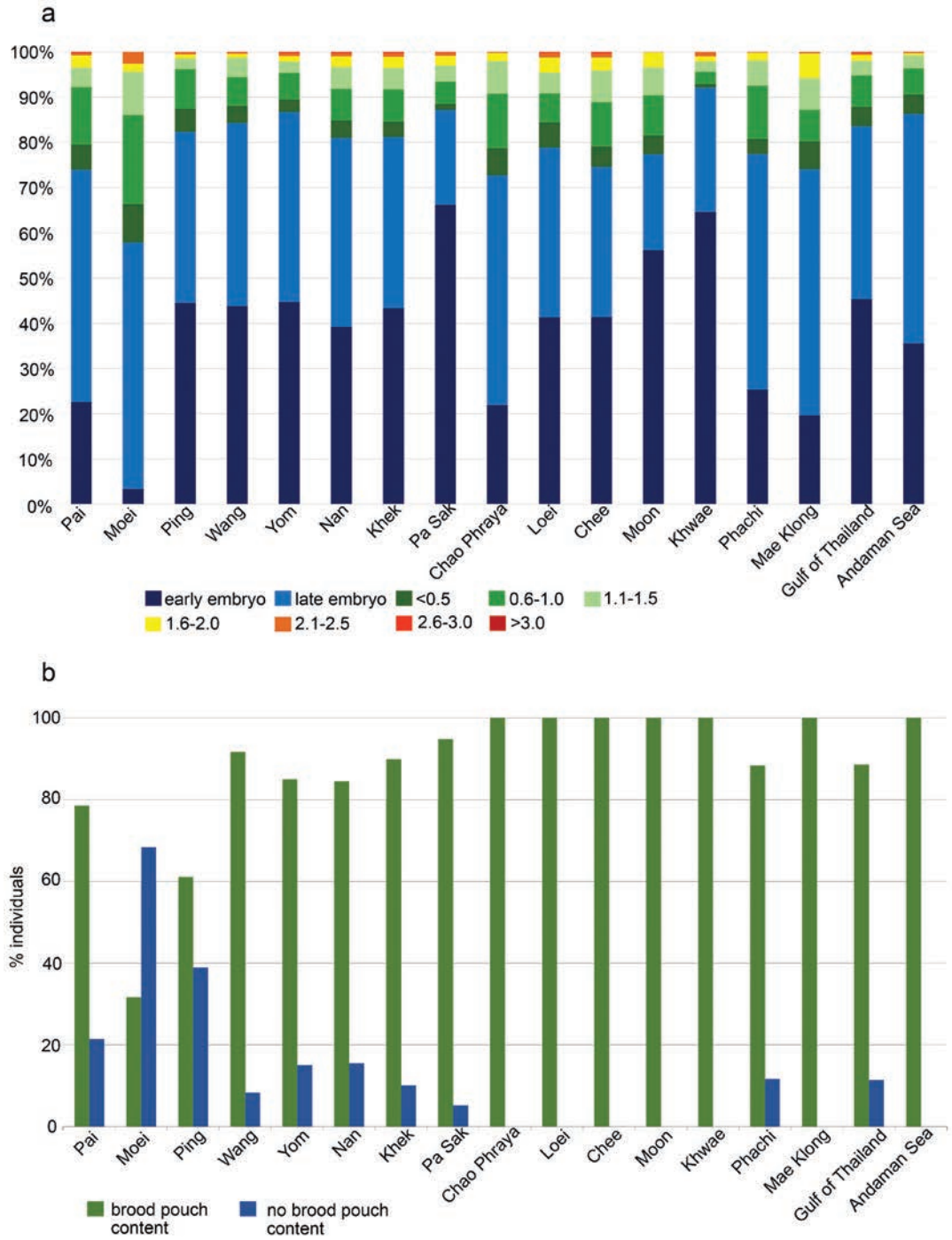


**Figure 11.** Composition of contents of the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (**a**) and proportions of gravid animals, i.e. those with brood pouch containing juveniles or other stages, and non-gravid specimens (**b**) from Thailand grouped according to rivers. For colour coding, see the inset legends.

of specimens with brood pouch content were found both during the little (May–June) and great (Oct–Nov.) rainy season; however, we lack sufficient collecting data for the dry season (Fig. 12b). For the Andaman Sea region, only specimens collected during the rainy season were available, reflecting in general the picture from the Gulf region, though; with ~25% non-gravid specimens at the beginning and only gravid specimens shortly after the peak of the rainy season (Fig. 12c).

## Discussion

As evolutionary biologists working with molluscs, we should aim at testing the universality of known and disputed speciation mechanisms, and it is with a clear focus on these mechanisms we should choose our molluscan models to increase their frequency as a source of data in order to decipher the underlying mechanisms of biodiversity.

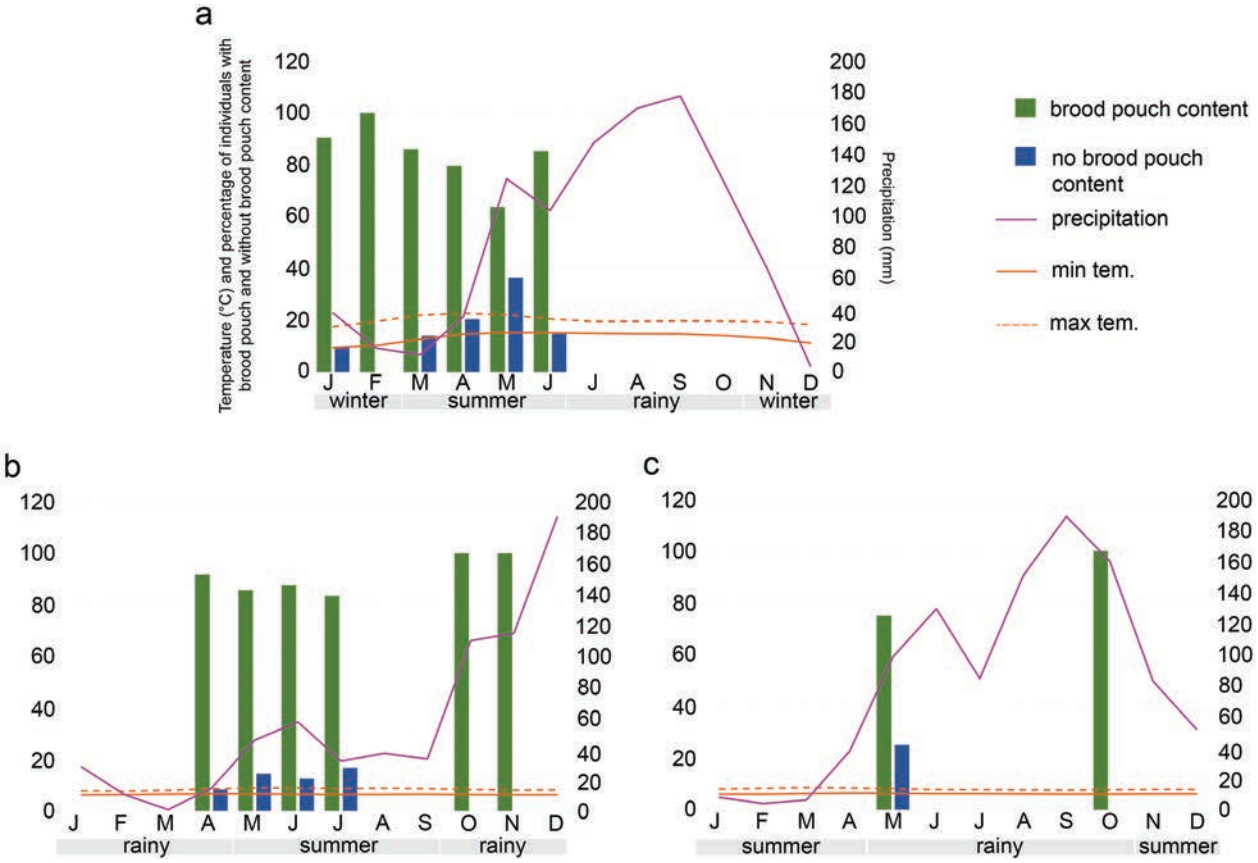


**Figure 12.** Proportions of gravid vs. non-gravid specimens of *Tarebia granifera* (Lamarck, 1816) collected in different months within a given year, plotted on climate charts for localities that are representative for different climatic regimes in Thailand. (**a**) Chiang Mai for inland locations; (**b**) Ko Samui for the Gulf of Thailand; (**c**) Phuket for the Andaman Sea (see also Fig. 8). For colour coding, see the inset legend.

The combination of molecular genetics and phenotypic analyses in concert with information on the geographical occurrence and additional data, e.g. on biological properties such as reproductive strategies, provides a powerful tool for the study of species differentiation, or diversification indicating speciation. It allows truly biological species to be distinguished, not only as perceivable taxonomic or even genetic units, but also as natural entities of evolutionary significance; if we want to make here the careful distinction between a species taxon (with identifying characteristics) and species entity (as a group of coevolving populations); see for the theoretical background of applications of species concepts in freshwater molluscs Glaubrecht (2004, 2009, 2010, 2011). Thus, within this framework of species as natural entities in space and time an identifiable species taxon can serve as a hypothesis of a species entity.

In freshwater gastropods high levels of morphological disparity and taxonomic diversity are frequently correlated, but often only because traditionally disparity was equated with diversity, as has been exemplified for limnic Cerithioidea, such as e.g. the Mediterranean melanopsids (Glaubrecht 1993, 1996, 2004), the Southeast Asian pachychilids as well as Australian thiarids; see Glaubrecht (2004, 2009, 2010, 2011) and Glaubrecht et al. (2009) for review and additional references.

As has been discussed by the latter author with focus on freshwater gastropods, the widely adopted typological practice during the 19th and way into the 20th century of naming allopatric populations, in isolated fashion and often based on single specimens only, as if representing putatively distinct (morpho-) species, has led to a plethora of species and subspecies names. Freshwater gastropods were found to exhibit a pronounced individual conchological variability, which has been attributed to the environmental conditions of their habitats that widely fluctuate on a temporal and spatial scale (e.g. Rensch 1929, 1934, Dillon 2000, Glaubrecht 1993, 1996, 2004, 2009, 2010, 2011). However, even the most pronounced of these conchological features, such as e.g. shell size, shape and sculpture as well as colour, hardly allow for definite species identifications. In limnic gastropods the shells are notoriously phenotypically plastic and variable environmental conditions can produce substantial modifications. Consequently, even marked differences in shell shape, size and sculpture do not necessarily indicate the presence of more than one species. Nevertheless, the former typological perception as to conchological variabil-

ity in molluscs in general and freshwater gastropods in particular, has resulted in long lists of synonyms, causing a considerable amount of taxonomic redundancy in many cases, and, consequently, resulted in an unwanted inflation of biodiversity, as is now also evident from our studies on Thiaridae from Southeast Asia,

In the course of the systematic revision of these thiarids, based on an evolutionary systematic approach (see Glaubrecht 2010) combining morphological and molecular genetic data, not only the number of species in general can be reduced. These investigations also provide the basis for an evaluation of phenotypical (i.e. conchological) plasticity in these gastropods, to be distinguished from genotypical diversity as yet another indication of a differentiation process within and among populations that reflect incipient or other stages of speciation. Here phenotypical plasticity is understood as the ability to express different phenotypes depending on the biotic or abiotic environment within one species (Agrawal 2001), in contrast to a truly speciational process.

However, an assessment of the significance of distinct phenotypic traits is in general lacking, as is an understanding of the genetic basis of phenotypical variation in particular for gastropods. For the limnic pomatiopsid *Oncomelania hupensis*, Davis and Ruff (1973) were able to show that apparently a single mutation in only one gene is sufficient for producing axially ribbed shells in a smooth-shelled population, suggesting that a relatively simple underlying genetic mechanisms (likely controlled by a few genes) might be responsible for gastropod shell traits. In a natural experimental situation in *Oncomelania* from the Miao River in the Yangtze floodplain in China, Davis et al. (1999) found that ribbing is indeed genetically controlled by a single gene with multiple alleles and suggested this to be an adaptation for dealing with annual flooding and survival by water transport. However, understanding the mechanisms that generate phenotypic variation such as shell sculpture and shape and being evolutionary relevant (i.e. inherited and selected with an adaptive value) still remains a fundamental challenge for contemporary evolutionary biology.

Owing to the earlier typological approach that resulted in the traditional overestimation of taxonomical diversity due to conchological disparity, but also in context of the genetically apparently closely related but morphologically highly distinct thiarids found across the distributional ranges throughout Southeast Asia and Australasia, we have to ask whether we are indeed dealing with actually many diverse species as separate evolutionary entities rather than only few, though highly polymorphic species with maybe several sympatric morphs exhibiting different ecophenotypical adaptations in shell response to the many variable environments where thiarids are usually to be found.

## Shell morphology

In the present study, we examined phenotypically distinguishable shell morphs of yet another thiarid from Thailand, traditionally assigned to *Tarebia granifera*, in reference to samples from Timor Leste as known type locality of the nominal species, using biometry and geometric morphometrics in combination with phylogeographical analyses of molecular genetics and reproductive strategy.

We found *Tarebia* to be widespread in almost all freshwater bodies throughout Thailand, with a wide range of conchological variants or morphs, of which some closely resemble the types and topotypical material of *granifera* collected on Timor. While in Thailand *Tarebia* has been reported with only one species by Brandt (1974), distinct shell morphologies allow to distinguish phenotypically disparate morphs. Some of these have even been formally named as distinct species (albeit from other regions in Asia), based on ornamental features such as tubercles and/or nodules as well as the formation of elevated spiral ridges prominent in particular on the last body whorls. For example, the name *lineata* (as well as *lateritia*, traditionally used for Philippine forms) have frequently been applied to morphs and/or populations in the Oriental region. Subba Rao (1989) discussed that *T. lineata* was often synonymised with *T. granifera*, or treated as its variety (e.g. Benthem-Jutting 1959), although it is readily distinguished from the latter by the presence of the very distinct spiral ridges. Also, Appleton et al. (2009) for invading populations of *T. granifera* in South Africa described two distinct morphological variants found at different locations, among them also one with pronounced spiral ridges.

Applying a drainage-based phylogeographical as well as a biometrical approach, we were unable to find for the populations in Thailand any correlation of the morphs distinguished in this study based on discernable shell features as well as overall "Gestaltwahrnehmung" with any criteria deducible from our observations given above, neither with geographical occurrence or preferred habitat and substrate nor with the molecular genetic substructuring detected (see below). So, all available evidence points at the coexistence of different morphologies or disparate phenotypes in *Tarebia granifera* in this part of mainland Southeast Asia. However, in the absence of any of the discussed parameters or factors to be causally correlated with these morphological differences we are left with the hypothesis that they either qualify for reflecting phenotypical plasticity correlated with ecological variables in the habitat of the individual populations studied, and/or, alternatively, being correlated with the parthenogenetic reproduction discussed further below.

## Biometry and geometric morphometrics

Biometric analyses are found useful tools for the study of characteristics that shape morphologically distinct entities, thus allowing to look into evolutionary pattern (e.g. Bocxlaer and Schultheiß 2010, Maaß and Glaubrecht 2012). Geometric analyses are used in addition to traditional morphometrics in order to compare in detail different populations and relationships among variable groupings (e.g. Rohlf and Marcus 1993, Sheets et al. 2006).

Although there are some differences in the biometric parameters and in the geometric morphometrics of Thai

*Tarebia*, it is generally impossible to delimit distinct entities (in the sense of being at least indicative of the existence as biological species) based on these features, as all of them largely overlap (Figs 6, 7). Thus, our morphometric data do not support the distinction of *T. lineata* or other morphs from the nominal *T. granifera*, based on shell size and/or form. In addition, the same holds true for the two distinct molecular clades separated by mitochrondrial DNA sequences used in our study, for which we failed to find any diagnostic features in shell morphology or other phenotypical characteristics.

The measurement of shell height of *T. granifera* showed that they are within the size range previously reported as to vary between 6 to 44 mm (e.g. Abbott 1952, Brandt 1974). Also Bradstreet and Rogowsky (2012) reported on specimens of *T. granifera* to exhibit the same overall shape with an elongately or ovate-conoidal shell with the size index (L3W/W) in the order of 0.54–2.65 mm (Fig. 6d). Isnaningsih et al. (2017) found the shape of *T. granifera* from the Indonesian islands of Lombok, as well as from Banten and Maros, to be for the ratio of shell height to width 1.29–3.02 mm.

The results of geometric morphometrics revealed the overall shell shape of *T. granifera* from Thailand to be very similar to, and virtually undistinguishable from, conspecifics from Timor Leste (Fig. 6e). Thus, although *T. granifera* exhibit shell polymorphism this intra- and interpopulational variability in its shell characteristics does not allow for species-specific differentiation, as it was found, for example, in the thiarid *Melanoides* (e.g. Facon et al. 2003, Genner et al. 2004, Sorensen et al. 2005, Yousif et al. 2009).

## Phylogenetic analyses

In contrast to shell morphology (morphs A–C, or *lineata* vs. *granifera* phenotypes), we found based on molecular genetics strong indication as to the distinction of at least two natural entities within *Tarebia* in Thailand. As our analyses revealed, there is a most pronounced separation of two distinct mtDNA clades in this taxon, marked on the one hand by long branches in the resulting phylogenetic tree connecting these two clades, and on the other hand by very shorter branches within each of them (Figs 4, 5).

Therefore, our analyses would potentially allow for a more narrow species delimitiation within what has been to date traditionally treated in Thailand as *T. granifera* only (Brandt 1974). At the same time, the two clades correspond with a geographical separation into a northern and southern group. This is also reflected in ecology insofar, as both show a preference in altitude (see Fig 4, insert). However, we propose that the latter reflects rather the occurrences in higher mountainous regions in the north than in the south of Thailand than a truly differential habitual preference. In contrast, the two genetically distinct lineages do neither match with features in shell morphology or biometry nor with differences in their reproductive strategies.

However, the p-distance of 13.8 % for *cox1* and 10 % for 16S sequences has to be considered relatively high, hinting potentially at the existence of two genetically distinct species. However, a definite decision as to this species question in *Tarebia* in Thailand should remain open until the geographical distribution of genetically characterized populations of *T. granifera* and other congeneric forms is completely resolved and better understood within the entire autochthonous range in the Oriental region. Thus, it should be the privilege of a more comprehensive and in-depth analysis of the biogeographical situation based on an ongoing molecular genetic study (Glaubrecht unpubl. data).

## Historical biogeography

While we found representatives of clade A in the northern tributaries of rivers such as the Chao Phraya and Mae Klong that run into the Gulf of Thailand, with only few others occurring at some localities in the south of Thailand (Figs 4, 8, 9a,b), those in clade B were found in the Salween River and the headwaters of Ping, Wang, Yom and Nan River. Accordingly, *Tarebia* snails from clade A are more frequent in the central to southern part of the country, whereas those from clade B are more frequent in the northern part. This overall geographic picture allows to attribute clade A as an element of the Sundaic region, given that it extends even further south and also comprises the Timor group (thus rendering it the nominal *granifera*), while clade B is mainly distributed in the Indochinese region (Figs 1, 4).

However, although being more frequent in the northern provinces, some representatives of clade B also occur in more southern locations, such as in the provinces Surat Thani (SUT 0516137), Nakhon Si Thammarat (SUT 0516139) and Phatthalung (SUT 0516138). We anticipate that this might reflect occurrences of passive dispersal, potentially via aquatic plant or other material or even transport by birds, rather than vicariance via the influence of sea level or tidal flows in drainage systems. The results of the median-joining haplotypes network and bGMYC analysis (Fig. 5) reveal that clade A and B exhibit many steps separating these two groups, and have low probabilities of conspecificity between clade A and B (p=0–0.05).

As we found in our molecular analyses this major split of clade A and B in Thai *Tarebia* to be as old as most likely c. 5.32 million years ago (Fig. 5d), it is worthwhile to look for a possible biogeographic explanation of the above distribution. In general, distinct faunal and floral assemblages are biogeographically restricted by barriers to dispersal such as characteristic geomorphological boundaries, even when individual taxa among each of the biota on either side often vary and may not all reflect the same discrete pattern. As Bruyn et al. (2005) pointed out, historical biogeography while providing crucial insights into the relationship between biological diversity and earth history, as a consequence has its limitations. However, patterns of intraspecific molecular variation may show unambiguous evidence for such historical divides, and can be used to test competing biogeographic hypotheses, such as e.g. the dispersal-vicariance debate, see e.g.

Glaubrecht (2000) and Glaubrecht and Rintelen (2003) for limnic gastropods).

For the distributional pattern found in *Tarebia* in Thailand, a vicariant hypothesis can be formulated using a major biogeographic transition zone between the Sundaic and Indochinese biota, located just north of the Isthmus of Kra. It is interpreted as the result of Neogene marine transgressions that breached this isthmus in two locations for prolonged periods of time, i.e. more than 1 million year duration, as was shown e.g. by phylogeographic analyses of a freshwater decapod crustacean, the giant freshwater prawn *Macrobrachium rosenbergii* (cf. Bruyn et al. 2005). In his review Parnell (2013) examined, based on the relevant geological, geographical, climatic, biogeographic and sea-level data, the available evidence on the Isthmus of Kra as being a significant biogeographic divide on the Thai-Malay Peninsula and, thus, of mainland Southeast Asia. It is believed to be of the same scale as, e.g., the 'Wallace's Line', albeit it remains less well-known and less well-studied, with its location and cause being still enigmatic. Dejtaradol et al. (2016) reported that population boundaries in birds did not coincide with the Isthmus of Kra, but instead were located north of the Thai-Malay Peninsula in Central Thailand, while only one of four divides represented an Indochinese-Sundaic transition. They supposed that different phylogeographical patterns among target species were presumably shaped by different ecological preferences in Pleistocene palaeohabitats. They found in bulbuls, as we suggest in analogy here for thiarid gastropods, Pliocene Indochinese-Sundaic lineage divergence, for which they hypothesized that it coincides with strong vegetational changes on the Peninsula shaping two phytogeographical transitions. As distribution limits of bird species roughly coincide with these transition zones, the avifaunal Thai-Malay transition represents apparently a broad zone rather than a sharp boundary.

While the separation of *Tarebia* and *Thiara* hint at a Late Miocene splitting event (anticipated to have occurred somewhere in the Indo-Malayan insular region of the Sunda and Sahul shelfs), our molecular and distributional data on *T. granifera* (Figs 4, 5d) suggest, with its two lineages in the north and south along the Thai peninsular mainland, to roughly correlate with a Late Miocene/Early Pliocene event (5.5–4.5 Mya). Thus, the separation of clade A and B can be hypothesized as resulting from a later marine transgression in the area to the north of today's Isthmus of Kra that may have produced high sea-level stands with a seaway that dissected the Thai-Malay Peninsula for durations longer than one million years; see Bruyn et al (2005) and literature therein for further details as to the relevant geological data and discussion.

The fact that today the distributional boundaries of the two *Tarebia* populations in clade A and B do not coincide exactly with the position of the Isthmus of Kra, but are instead placed further to the north, could in this case be attributed to later palaeo-drainage differentiation in connection with orogenesis or other tectonic events in the mountainous central and northern regions of Thailand, as it was discussed using relevant geological and available biogeographical data, for example, from fishes and gastropods in Glaubrecht and Köhler (2004). Thus, although being today located north of the Thai-Malay Peninsula in Central Thailand, the Isthmus of Kra and late Miocene/early Pliocene marine transgression might have caused in the freshwater thiarids of this region the separation of the Indochinese and Sundaic lineage within what has been regarded as *Tarebia granifera* to date.

**Reproductive biology1**

*Tarebia* snails are all viviparous, i.e. they incubate embryos and later ontogenetic stages in an extra-uterine structure, called the subhemocoelic brood pouch, located at the back of the head in the female's body running alongside and below, but being independent of the pallial organs (e.g. the gonoduct), and formed apparently by an invagination of the genital grove found in other oviparous cerithioidean gastropods (Glaubrecht 1996, 1999, 2006, 2011, Glaubrecht et al. 2009). Based on histology, for *T. granifera* Glaubrecht (1996) described an eu-viviparous strategy, involving matrotrophy (i.e. the nourishment by the female) of the progeny that develop in the subhemocoelic "marsupium" from early to late embryos and subsequently build their multi-whorled shells before hatching as crawling juveniles. This strategy, also known as typical for other thiarids such as e.g. *Melanoides*, is in contrast to an ovo-viviparous mode, reported e.g. for *Thiara amarula* and some other Australian thiarids, such as *Stenomelania aspirans* (see Schütt and Glaubrecht 1999, Glaubrecht et al. 2009, Maaß and Glaubrecht 2012).

In the Thai populations of *Tarebia*, as well as those from Timor, we found most if not all ontogenetic stages contained at the same time in the female's marsupium, from early embryos to late embryos and shelled juveniles, in all morphs (A–C), both molecular genetic clades (A and B) and specimens from all drainage systems, without a clear-cut differentiation of this reproductive strategy. In particular, the ontogeny of *T. granifera* in Thailand is not obviously correlated to specific drainage systems, no matter where these water bodies eventually drain. Therefore, we conclude that *Tarebia* throughout its distributional range covered here is eu-viviparous, with only very few representatives in some populations (see Figs 8, 9, and more details above) that were found to only possess late and/or even early embryonic stages, respectively. This is in contrast to a pronounced correlation as to reproductive biology in Thiaridae from Australia (Glaubrecht et al. 2009), where all ovo-viviparous taxa that release veligers paradoxically exhibit very restricted distributional ranges in the Jardinian biogeographical region only. It is also in contrast to differences in the Thai thiarid *Melanoides jugicostis* (see Dechruksa et al. 2013), that was found to lack viviparous populations at least in some geographical regions and during some time of the year.

As in this later case, it could be hypothesized that any environmental factor might affect the reproductive strategy also in *Tarebia*. However, our analysis of represen-

tative climatic charts for the two parameters temperature and precipitation revealed no clear regional pattern of brood pouch content, as no correlation with the various ontogenetic stages were found across all locations in Thailand where *T. granifera* was sampled (see Figs 8, 9, 10, 11a). However, as reported above (see Figs 11b, 12a) some populations in rivers in the northwest (Pai, Moei, Ping), that were sampled essentially in the first half of the year (i.e. particularly early in the rainy season from April to June) exhibit a considerable amount of non-gravid specimens. The same might be true for some populations sampled during the early rainy season (April–July) in the Gulf of Thailand drainages, and to a lesser extent, too, in samples collected in May in the Andaman Sea drainages. It can be provisionally deduced from these data, that there might be a tendency for *Tarebia* females to be gravid especially during and after the end of the main or great rainy season in the second half of the year (and potentially during the dry season). In contrast to this temporal (spatial) hypothesis, we do not explain the frequency of non-gravid specimens as being indicative of the varying existence of males, as their occurrence would alternatively be regionally specific (in the northwest) and seasonal (little rainy season early in the year), which we doubt.

As in most (if not all) thiarids, *Tarebia* apparently lacks males in most populations, as we failed to find positive evidence for their existence. Parthenogenetic reproduction has gained much interest in the past in evolutionary biology, not only with respect to the origin of sex. Clonal reproduction in natural populations has obviously many advantages over sexual modes, with growth rates in the former often being much accelerated over the latter, as all individuals within the population are able to contribute (Maynard Smith 1978). In addition, these clones are considered instrumental in fast colonization of new habitats and areas, as even a single female can give rise to a new population (Baker 1955). Nevertheless, most faunas are dominated by sexually reproducing species, with asexual organisms being in the minority (Bell 1982).

Also in malacology there are some classical case studies, such as the New Zealand freshwater hydrobiid *Potamopyrgus antipodarum* (Jokela et al. 2003) or the thiarid *Melanoides tuberculata* (Jacob 1957, 1958, Berry and Kadri 1974, Ben-Ami and Heller 2005). However, in both cases reproduction is not exclusively parthenogenetic. In populations of *Melanoides tuberculata,* for example, the frequency of males was found to vary between 40 % in the French West Indies (Samadi et al. 1998) and up to 66 % in Israel (Livshits and Fishelson 1983, Heller and Farstey 1990).

It would be tempting to anticipate a similar phenomenon of *T. granifera* in Thailand and Timor Leste here from the varying frequencies (with up to 17.40 %) of non-gravid specimens. However, none ad hoc feature such as e.g. shell morphology between male and female could be differentiated in these aphallic Cerithioideans. So, in the present study we assumed not only any brood pouch-bearing snail to be female but also those without brood pouch as being non-gravid females rather than being rare males, for the reasons discussed above in connection with regional and/or climatic differences.

### Species concepts in parthenogenetic *Tarebia1*

Given the prediction supported here that thiarid gastropods reproduce largely (if not completely) via parthenogenesis, the application in particular of the biological species concept is not made easy in case of thiarids. Morrison (1954) in discussing the enormous shell variability in thiarids in context with parthenogenesis, noted wisely that "wise indeed is the scientist who can tell whether a clone is a species or not, and be right every time, in the case of the Thiaridae". This was shown, for example, for *Melanoides tuberculata* (Jacob 1957, 1958, Berry and Kadri 1974, Facon et al. 2003). Therefore, Stoddart (1985) preferred to apply instead of the biological species concept that of the evolutionary species (ESC) following Wiley (1978, 1981), as in his opinion this concept "stresses the relevance of the process of speciation to species definitions and provides the most appropriate framework for the taxonomy of asexual organisms". Although this statement is debatable for several reasons, admittedly, the biological species concept (BSC) is also not without problems in application to *Tarebia*, as it explicitly uses the reproductive criterion in sexually reproducing organisms. The BSC was introduced by Mayr (1942) and since then widely discussed; see literature survey with references updated and discussion with respect to limnic gastropods e.g. in Glaubrecht (2004, 2009, 2010, 2011, Glaubrecht et al. 2009).

In case of the thiarids it remains to be seen in how far they are actually prone exclusively to parthenogenesis. For example, for populations of *Melanoides tuberculata* in Israel Ben-Ami and Heller (2005) reported sexually as well as asexually reproducing individuals, thus contradicting the general assumption that indeed all thiarids reproduce via apomixis. Apparently, at least in *M. tuberculata* there are both modes realized, securing the exchange of genetic information by sexual reproduction as was shown in earlier allozyme studies (Livshits and Fishelson 1983) and excluding the possibility of gynogenesis, i.e. parthenogenesis with the development of eggs to be induced by contact with sperm, though. Given the fact that we have (albeit indirect) evidence for the presence of males at least in low frequency, as is evident from published records, e.g. on *Thiara amarula* (see Healy and Glaubrecht 2018), as well as unpublished data, we here anticipate at least the occasional sexual reproduction in Thiaridae. As their species either maintain low levels of males in some populations, or by other means switch between asexual and sexual reproduction, there is no objection to not applying species concepts grounded on the reproductive criterion, as explicitly done under the BSC.

## Conclusion

In view of the pronounced phenotypic plasticity reported herein for the Thai *Tarebia granifera*, it should be asked, in addition or alternatively to environmental factors, in

how far this conchologically expressed variation is correlated to or even caused by these, at least frequently, parthenogenetically reproducing thiarids. Resulting in monoclonal lineages, populations of morphologically varying freshwater snails with partly or potentially completely parthenogenetic females hitherto have erroneously been treated as species under the traditional typological approach (not only in malacology). However, this simplistic and often non-comprehensive approach has most likely underestimated natural variation and intraspecific disparity by, at the same time, overestimating taxonomic diversity, resulting in taxonomic redundancy as an underrated phenomenon in evolutionary biology.

The development of an accurate and rapid method for the detection of males in aphallic thiarids, in order to evaluate the frequency of parthenogenesis in individual populations and species or higher-level taxa, respectively, remain an essential desideratum in biosystematics research on these snails. In addition, it remains to be analysed thoroughly whether and in how far there is a correlation of partially or completely parthenogenetic populations with parasite infections by digenic trematodes, for example, in the thiarids *Melanoides tuberculata* (see Krailas et al. 2011, 2012, 2014), in *M. jugicostis* (see Dechruksa et al. 2013) and *Tarebia granifera* (Veeravechsukij et al. 2018).

Our preliminary analyses of the brood pouch content in the latter species under study here revealed that infected females tend to have fewer embryos than non-infected specimens, which might be a hint to the influence of parasite load on the reproductive mode of this major intermediate host. Therefore, given the human infection aspects of these trematode-carrying gastropods, our study not only has implication for human health in Thailand. We also hope that with studying trematode infections in the various conchologically disparate and molecular genetically distinct lineages of *Tarebia* we will eventually gain deeper insights into the complex evolutionary interplay of various trematode parasites and their snail hosts mediating infections in the human population.

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## References

Abbott RT (1952) A study of an intermediate snail host (*Tarebia granifera*) of the oriental lung fluken (*Paragonimus*). Proceedings of the United States National Museum 102: 71–115. https://doi.org/10.5479/si.00963801.102-3292.71

Adams DC, Otárola-Castillo E (2013) geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods in Ecology and Evolution 4: 393–399. https://doi.org/10.1111/2041-210X.12035

Adams H, Adams A (1854) The genera of recent Mollusca; arranged according to their organization. In three volumes. Vol. I. Part X. Van Voorst, London.

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294: 321–326. https://doi.org/10.1126/science.1060701

Appleton CC, Nadasan DS (2002) Frist report of *Tarebia granifera* (Lamarck, 1816) (Gastropod: Thiaridae) from Africa. Journal of Molluscan Studies 68: 399–402. https://doi.org/10.1093/mollus/68.4.399

Appleton CC, Forbes AT, Demetriades NT (2009) The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa. Zoologische Mededelingen 83: 525–536.

Baimai V (2010) Biodiversity in Thailand. The Journal of the Royal Institute of Thailand 2: 107–114.

Baker HG (1955) Self-compatibility and establishment after 'longdistance' dispersal. Evolution 9: 347–349.

Bell G (1982) The masterpiece of nature. The evolution and genetics of sexuality. University of California Press, Berkeley, Los Angeles.

Ben-Ami F, Heller J (2005) Spatial and temporal patterns of parthenogenesis and parasitism in the freshwater snail *Melanoides tuberculata*. Journal of Evolutionary Biology 18: 138–146. https://doi.org/10.1111/j.1420-9101.2004.00791.x

Benthem-Jutting WSS (1937) Non marine Mollusca from Nias Island. Miscellanea Zoologica Sumatrana 84/85: 1–17.

Benthem-Jutting WSS (1959) Catalogue of the non-marine mollusca of Sumatra and of its satellite islands. Beaufortia 7: 41–191.

Berry AJ, Kadri ABH (1974) Reproduction of the Malayfreshwater cerithiacean gastropod *Melanoides tuberculata*. Journal of Zoology 172: 369–381. https://doi.org/10.1111/j.1469-7998.1974.tb04113.x

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A software platform for Bayesian evolutionary analysis. PLoS Computational Biology 10: e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Bocxlaer BV, Schultheiß R (2010) Comparison of morphometric techniques for shapes with few homologous landmarks based on machine-learing approaches to biological discrimination. Paleobiology 36: 497–515. https://doi.org/10.1666/08068.1

Brandt AM (1974) The non-marine aquatic Mollusca of Thailand. Archiv für Molluskenkunde 105: 1–423.

Bradstreet J, Rogowski DL (2012) Native springsnails and the invasive red-rim Melania snail (*Melanoides tuberculata*), species habitat associations and life history investigations in the San Solomon Spring complex, Texas. Final Report the endangered species program grant no. tx e-121-r. Department of Natural Resources Management Texas Tech University, 81 pp.

Brot A (1874–1879) Die Melaniaceen (Melanidae) in Abbildungen nach der Natur mit Beschreibungen. Systematisches Conchylien-Cabinet von Martini und Chemnitz 1 (24): 1–488. [pls 1–49]

Brown DS (1994) Freshwater snails of Africa and their medical importance. Second edition. Taylor & Francis, London, Bristol.

Bruyn MD, Nugroho E, Hossain MM, Wilson JC, Mather PB (2005) Phylogeographic evidence for the existence of an ancient biogeographic barrier: the Isthmus of Kra Seaway. Heredity 94: 370–378. https://doi.org/10.1038/sj.hdy.6800613

Chessel D, Dufour AB, Thioulouse J (2004) The ade4 package-I: One-table methods. R news 4: 5–10.

Davis GM, Wilke T, Yi Z, Xu Z, Qiu C, Spolsky C, Qiu, D, Li Y, Xia M, Feng Z (1999) Snail-*Schistosoma, Paragonimus* interactions in China: population ecology, genetic diversity, coevolution, and emerging diseases. Malacologia. 41: 355–377.

Davis GM, Ruff MD (1973) *Oncomelania hupensis* (Gastropoda, Hydrobiidae): hybridisation, genetics, and transmission of *Schistosoma japanicum*. Malacological Review 6: 181–197.

Dechruksa W, Krailas D, Ukong S, Inkapatanakul W, Dangprasert T (2007) Trematode infections of freshwater snails family Thiaridae in Khek River. Southeast Asian Journal of Tropical Medicine and Public Health 38: 1016–1028.

Dechruksa W, Krailas D, Glaubrecht M (2013) Evaluating the status and identity of "*Melania*" *jugicostis* Hanley & Theobald, 1876 an enigmatic thiarid gastropod in Thailand (Caenogastropoda, Cerithioidea). Zoosystematics and Evolution 89: 293–310. https://doi.org/10.1002/zoos.201300015

Dejtaradol A, Renner SC, Karapan S, Bates PJJ, Moyle RG, Päckert M (2016) Indochinese-Sundaic faunal transition and phylogeographical divides north of the Isthmus of Kra in Southeast Asian bulbuls (Aves: Pycnonotidae). Journal of Biogeography 43(3): 471–483. https://doi.org/10.1111/jbi.12662

Dinno A (2017) Package "dunn.test" vers. 1.3.5. https://cran.r-project.org/web/packages/dunn.test/dunn.test.pdf [accessed: 31 May 2018]

Dillon RT (2000) The ecology of freshwater molluscs. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511542008

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatic 5: 113. https://doi.org/10.1186/1471-2105-5-113

Facon B, Pointier JP, Glaubrecht M, Poux C, Jarne SP, David P (2003) A molecular phylogeography approach to biological invasions of the new world by parthenogenetic Thiarid snails. Molecular Ecology 12: 3027–3039. https://doi.org/10.1046/j.1365-294X.2003.01972.x

Folmer O, Black M, Hoeh W, Lut R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Fox J, Weisberg S (2011) An R Companion to Applied Regression. 2nd ed. Sage, Thousand Oaks CA.

Genner MJ, Michel E, Erpenbeck D, De Voogd N, Witte F, Pointier JP (2004) Camouflaged inversion of Lake Malawi by an Oriental gastropod. Molecular Ecology 13: 2135–2142. https://doi.org/10.1111/j.1365-294X.2004.02222.x

Gimnich F (2015) Molecular approaches to the assessment of biodiversity in limnic gastropods (Cerithioidea, Thiaridae) with perspectives on a Gondwanian origin. Unpublished PhD thesis, Humboldt University, Berlin.

Glaubrecht M (1993) Mapping the diversity: geographical distribution of the freshwater snail *Melanopsis* (Gastropoda: Cerithioidea: Melanopsidae) with focus on its systematics in the Mediterranean Basin. Mitteilungen aus dem Hamburger Zoologischen Museum und Institut 90: 41–97.

Glaubrecht M (1996) Evolutionsökologie und Systematik am Beispiel von Süß- und Brackwasserschnecken (Mollusca: Caenogastropoda: Cerithioidea): Ontogenese-Strategien, paläontologische Befunde und Historische Zoogeographie. Backhuys Publishers, Leiden.

Glaubrecht M (1999) Systematics and the evolution of viviparity in tropical freshwater gastropods (Cerithioidea: Thiaridae sensu lato): An overview. Courier Forschungs-Institut Senckenberg 215: 91–96.

Glaubrecht M (2000) A look back in time: Toward an historical biogeography as synthesis of systematic and geologic patterns outlined with limnic gastropods. Zoology: Analysis of Complex Systems 102: 127–147.

Glaubrecht M (2002) The "experience" of nature: From Salomon Müller to Ernst Mayr, or The insights of travelling naturalists toward a zoological geography and evolutionary biology. Verhandlungen zur Geschichte und Theorie der Biologie 9: 245–282.

Glaubrecht M (2004) Leopold von Buch's legacy: treating species as dynamic natural entities, or why geography matters. American Malacological Bulletin 19(1/2): 111–134.

Glaubrecht M (2006) Independent evolution of reproductive modes in viviparous freshwater Cerithioidea (Gastropoda, Sorbeoconcha) a brief review. Basteria 69 (Supplement 3): 28–32.

Glaubrecht M (2009) On "Darwinian Mysteries" or molluscs as models in evolutionary biology: from local speciation to global radiation. American Malacological Bulletin 27: 3–23. https://doi.org/10.4003/006.027.0202

Glaubrecht M (2010) Evolutionssystematik limnischer Gastropoden. Habilitationsschrift, Humboldt University, Berlin.

Glaubrecht M (2011) Towards solving Darwin's "mystery": Speciation and radiation in lacustrine and riverine freshwater gastropods. American Malacological Bulletin 29: 187–216. https://doi.org/10.4003/006.029.0211

Glaubrecht M, Köhler F (2004) Radiating in a river: systematics, molecular genetics and morphological differentiation of viviparous freshwater gastropods endemic to the Kaek River, central Thailand (Cerithioidea, Pachychilidae). Biological Journal of the Linnean Society 82: 275–311. https://doi.org/10.1111/j.1095-8312.2004.00361.x

Glaubrecht M, RintelenTv (2003) Systematics, molecular genetics and historical zoogeography of the viviparous freshwater gastropod *Pseudopotamis* (Cerithioidea, Pachychilidae): a relic on the Torres Strait Islands, Australia. Zoologica Scripta 32(5): 415–435. https://doi.org/10.1046/j.1463-6409.2003.00127.x

Glaubrecht M, Brinkmann N, Pöppe J (2009) Diversity and disparity 'down under': Systematics, biogeography and reproductive modes of the 'marsupial' freshwater Thiaridae (Caenogastropoda, Cerithioidea) in Australia. Zoosystematics and Evolution 85: 199–275. https://doi.org/10.1002/zoos.200900004

Golubchik T, Wise MJ, Easteal S, Jermiin LS (2007) Mind the gaps: evidence of bias in estimates of multiple sequence alignments. Molec-

ular Biology and Evolution 24: 2433–2442. https://doi.org/10.1093/molbev/msm176

Healy J, Glaubrecht M (2018) Ultrastructure of spermatophoral sperm in the freshwater gastropod *Thiara amarula* (Linnaeus, 1758) (Cerithioidea, Thiaridae): potential taxonomic features including eusperm nuclear content differentiation. Journal of Molluscan Studies 84: 310–323. https://doi.org/10.1093/mollus/eyy018

Heller J, Farstey V (1990) Sexual and parthenogenetic populations of the freshwater snail *Melanoides tuberculata* in Israel. Israel Journal of Zoology 37: 75–87.

Hui W, Gel YR, Gastwirth JL (2008) lawstat: an R package for law, public policy and biostatistics. Journal of Statistical Software 28: 1–26. https://doi.org/10.18637/jss.v028.i03

Isnaningsih NR, Basukriadi A, Marwoto M (2017) The morphology and ontogenetic of *Tarebia granifera* (Lamarck, 1822) from Indonesia (Gastropoda: Cerithioidea: Thiaridae). Treubia 44: 1–14. https://doi.org/10.14203/treubia.v44i0.2914

Jacob J (1957) Cytological studies of Melaniidae (Mollusca) with special reference to parthenogenesis and polyploidy. I. Oogenesis of the parthenogentic species of *Melanoides* (Prosobranchia-Gastropoda). Transactions of the Royal Society of Edinburgh 63: 341–352. https://doi.org/10.1017/S0080456800009534

Jacob J (1958) Cytological studies of Melaniidae (Mollusca) with special reference to parthenogenesis and polyploidy. II. A study of meiosis in the rare males of the polyploid race of *M. tuberculata* and *M. lineatus*. Transactions of the Royal Society of Edinburgh 63: 433–444. https://doi.org/10.1017/S0080456800009571

Jokela J, Lively CM, Dybdahl MF, Fox JA (2003) Genetic variation in sexual and clonal lineages of a freshwater snail. Biological Journal of the Linnean Society 79: 165–181. https://doi.org/10.1046/j.1095-8312.2003.00181.x

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 32: 772–780. https://doi.org/10.1093/molbev/mst010

Köhler F, Glaubrecht M (2001) Toward a systematic revision of the Southeast Asian freshwater gastropod *Brotia* H. Adams, 1866 (Cerithioidea: Pachychilidae): an account of species from around the South China Sea. Journal of Molluscan Studies 67: 281–318. https://doi.org/10.1093/mollus/67.3.281

Köhler F, Glaubrecht M (2006) A systematic revision of the Southeast Asian freshwater gastropod *Brotia* (Cerithioidea: Pachychilidae). Malacologia 48: 159–251.

Köhler F, Glaubrecht M (2010) Uncovering an overlooked radiation: molecular phylogeny and biogeography of Madagascar's endemic river snails (Caenogastropoda: Pachychilidae: *Madagasikara* gen. nov.). Biological Journal of the Linnean Society 99: 867–894. https://doi.org/10.1111/j.1095-8312.2009.01390.x

Krailas D, Namchote S, Rattanathai P (2011) Human intestinal flukes *Haplorchris taichui* and *Haplorchris pumilio* in their intermediate hosts, freshwater snails of the families Thiaridae and Pachychilidae, in southern Thailand. *Zoosystematics and Evolution* 87: 349–360. https://doi.org/10.1002/zoos.201100012

Krailas D, Janecharut T, Tharapoom K, Inkapatanakul W (2012) Surveillance of Helminthiasis at Water Resource Development Area in order to Maximize of Usage and Sustainability: A Case Study of the Communities of Pasak Cholasid Dam. The official report to the Commission on Higher Education, Ministry of Education, Thailand, 1–231. [In Thai]

Krailas D, Namchote S, Koonchornboon T, Dechruksa W, Boonmekam D (2014) Trematode infections obtained from freshwater snail *Melanoides tuberculata* (Müller, 1774) in Thailand. Zoosystematics and Evolution, 90(1): 57–86. https://doi.org/10.3897/zse.90.7306

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lamarck JBPA de Monet de (1816) Encyclopédie méthodique. Tableau Encyclopédique et méthodique des trois règnes de la nature. Vingt-troisième partie. Liste des objets representés dans les planches de cette livraison. V. Agasse, Paris, 391–488.

Lamarck JBPA de Monet de (1822) Histoire naturelle des animaux sans vertèbres, présentant les caractères généraux et particuliers de ces animaux, leur distribution, leurs classes, leurs familles, leurs genres, et la citation des principales espèces qui s'y rapportent; précédée d'une introduction offrant la détermination des caractères essentiels de l'animal, sa distinction du végétal et des autres corps naturels, enfin, l'exposition des principes fondamentaux de la zoologie. Tome sixième. 2me. Partie. L'auteur, Paris, 232 pp.

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Lekagul B, Round PD (1991) A guide to the Birds of Thailand. Darnsutha Press, Thailand, 1–457.

Livshits G, Fishelson L (1983) Biology and reproduction of the freshwater snail *Melanoides tuberculata* (Gastropoda: Prosobranchia) in Israel. Israel Journal of Zoology 32: 21–35.

Lydeard C, Holznagel WE, Glaubrecht M, Ponder WF (2002) Molecular phylogeny of a circum-global, diverse gastropod superfamily (Cerithioidea: Mollusca: Caenogastropoda): pushing the deepest phylogenetic limits of mitochondrial LSU rDNA sequences. Molecular Phylogenetics and Evolution 22(3): 399–406. https://doi.org/10.1006/mpev.2001.1072

Luo A, Qiao H, Zhang Y, Shi W, Ho SYW, Xu W, Zhang A, Zhu C (2010) Performance of criteria for selecting evolutionary models in phylogenetics: a comprehensive study based on simulated datasets. BMC Evolutionary Biology 10: 242. https://doi.org/10.1186/1471-2148-10-242

Maaß N, Glaubrecht M (2012) Comparing the reproductive biology of three "marsupial", eu-viviparous gastropods (Cerithioidea, Thiaridae) from drainages of Australia's monsoonal north. Zoosystematics and Evolution 88: 293–315. https://doi.org/10.1002/zoos.201200023

Maynard Smith J (1978) The evolution of sex. Cambridge University Press, Cambridge.

Mayr E (1942) Systematics and the Origin of Species. Columbia University Press, New York.

Mendiburu F (2010) Agricolae: Statistical Procedures for Agricultural Research. Faculty of Economics and Planning, La Molina Agrarian University, La Molina.

Mermod G (1952) Les types de la collection Lamarck au Muséum de Genève, mollusques vivants 3. Revue Suisse de Zoologie 59 (2): 23–97. https://doi.org/10.5962/bhl.part.75362

Morrison JPE (1954) The relationships of old and New World melanians. Proceedings of the United States National Museum 103: 357–393.

Parnell J (2013) The biogeography of the Isthmus of Kra region: a review. Nordic Journal of Botany 31(1): 1–15. https://doi.org/10.1111/j.1756-1051.2012.00121.x

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55: 595–609. https://doi.org/10.1080/10635150600852011

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, automatic barcode gap discovery for primary species delimitation. Molecular Ecology 21: 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*: https://doi.org/10.1093/sysbio/syy032

Rensch B (1929) Das Prinzip geographischer Rassenkreise und das Problem der Artbildung. Borntraeger, Berlin.

Rensch B (1934) Süsswassermollusken der deutschen limnologischen Sunda-Expedition. Archiv für Hydrobiologie Supplement 8: 203−254.

Reid NM, Carstens BC (2012) Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BioMedCentral Evolutionary Biology 12: 196. https://doi.org/10.1186/1471-2148-12-196

Rintelen T, Wilson AB, Meyer A, Glaubrecht M (2004) Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. Proceedings of the Royal Society of London B 271: 1541–1549. https://doi.org/10.1098/rspb.2004.2842

Rohlf FJ (2017a) *TpsUtil 1.74*. Department of Ecology & Evolution and Anthropology, State Univesity of Stony Books.

Rohlf FJ (2017b) *tpsDIG2 2.30*. Department of Ecology & Evolution and Anthropology, State Univesity of Stony Books.

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. Trends in Ecology and Evolution 8: 129–132. https://doi.org/10.1016/0169-5347(93)90024-J

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

RStudio Team (2016) *RStudio*. Boston, MA: Integrated Development for R. RStudio, Inc. http://www.rstudio.com/ [Accessed: 31 May 2018]

Samadi S, Artiguebielle E, Estoup A, Pointier JP, Silvain JF, Heller J, Cariou ML, Jarne P (1998) Density and varibility of dinukleotide microsatellites in the parthenogenetic polyploid snail *Melanoides tuberculata*. Molecular Ecology 7: 1233–1236. https://doi.org/10.1046/j.1365-294x.1998.00405.x

Schütt S, Glaubrecht M (1999) *Thiara amarula* (Linné, 1758) (Caenogastropoda: Thiaridae) in Australia - new evidence on the anatomy of the reproductive system in a viviparous freshwater mollusc. Courier Forschungs-Institut Senckenberg 215: 181–188.

Schwenk K, Brede N, Streit B (2008) Introduction. Extent, process and evolutionary impact of interspecific hybridization in animals. Philosophical Transactions of the Royal Society London 363(B): 2805–2810. https://doi.org/10.1098/rstb.2008.0055

Sheets HD, Corvino KM, Panasiewicz JM, Morris SR (2006) Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. Frontiers in Zoology 3: 1–15. https://doi.org/10.1186/1742-9994-3-15

Sorensen LV, Jorgensen A, Kristensen TK (2005) Molecular diversity and phylogenetic relationships of the gastropod genus *Melanoides* in Lake Malawi. African Zoology 40: 179–191. https://doi.org/10.1080/15627020.2005.11407317

Starmühler F (1976) Ergebnisse der Österreichischen Indopazifik-Expedition 1971 des 1. Zoologischen Institutes der Universität Wien: Beiträge zur Kenntnis der Süßwassergastropoden pazifischer Inseln. Annalen des Naturhistorischen Museums in Wien 80 B: 473–656.

Stoddart JA (1985) Analysis of species lineages of some Australian thiarids (Thiaridae, Prosobranchia, Gastropoda) using the evolutionary species concept. Journal Malacological Society of Australia 7(1–2): 7–16. https://doi.org/10.1080/00852988.1985.10673972

Strong EE, Colgan DJ, Healy JM, Lydeard C, Ponder WF, Glaubrecht M (2011) Phylogeny of the gastropod superfamily Cerithioidea using morphology and molecules. Zoological Journal Linnean Society 162: 43–89. https://doi.org/10.1111/j.1096-3642.2010.00670.x

Sukumaran J, Holder MT (2010) Dendropy: a Python library for phylogenetic computing. Bioinformatics 26: 1569–1571. https://doi.org/10.1093/bioinformatics/btq228

Subba Rao NV (1989) Handbook freshwater molluscs of India. Zoological Survey of India, Calcutta.

Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony *and other methods. Version 4.0b10. Sinauer Associates, Sunderland.

Ukong S, Krailas D, Dangprasert T, Channgarm P (2007) Studies on the morphology of cercariae obtained from freshwater snails at Erawan Waterfall, Erawan National Park, Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health 38: 302–312.

Veeravechsukij B, Namchote S, Neiber MT, Glaubrecht M, Krailas D (2018) Exploring the evolutionary potential of parasites: Larval stages of pathogen digenic trematodes in their limnic thiarid host *Tarebia granifera* in Thailand. Zoosystematics and Evolution 94(2). https://doi.org/10.3897/zse.94.28793

Wiley EO (1978) The evolutionary species concept reconsidered. Sytematic Zoology 27: 17–26.

Wiley EO (1981) Phylogenetics – The Theory and Practice of Phylogenetic Systematic. Wiley, New York.

Wilson AB, Glaubrecht M, Meyer A (2004) Ancient lakes as evolutionary reservoirs: evidence from thalassoid gastropods of Lake Tanganyika. Proceedings of the Royal Society of London B 271: 529–536. https://doi.org/10.1098/rspb.2003.2624

Winnepenninckx B, Backeljau T, DeWachter R (1993) Extraction of high molecular weight DNA from molluscs. Trends in Genetics 9: 407. https://doi.org/10.1016/0168-9525(93)90102-N

Wolmarans CT, de Kock K (2006) The current status of freshwater molluscs in the Kruger National Park. Koedoe - African Protected Area Conservation and Science 49: 39–44.

Yousif F, Ibrahim A, Sleem S (2009) Morphological and Genetic Analyses of *Melanoides tuberculata* Population in Egypt. Global Journal of Molecular Science 4: 112–117.

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD thesis, University of Texas at Austin.

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# Diversity and conservation of seasonal killifishes of the *Hypsolebias fulminantis* complex from a Caatinga semiarid upland plateau, São Francisco River basin, northeastern Brazil (Cyprinodontiformes, Aplocheilidae)

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## Abstract

A high concentration of endemic species of seasonal killifishes has been recorded for a small area encompassing the highland plateaus associated with the upper section of the Carnaúba de Dentro River drainage and adjacent drainages of the middle section of the São Francisco River basin, northeastern Brazil. The present study is primarily directed to the taxonomy of the *H. fulminantis* species complex in this region, and describes habitat decline and extirpation of natural killifish populations recorded in field studies between 1993 and 2017. Both morphological characters and molecular species delimitation methods using single-locus models (GMYC and bPTP) support recognition of two closely related endemic species, *H. fulminantis* and *H. splendissimus* Costa, **sp. n.** The new species is distinguished from other congeners of the *H. fulminantis* complex by having a red pectoral fin in males, well-developed filamentous rays on the tips of the dorsal and anal fins in adult males, and the second proximal radial of the dorsal fin between the neural spines of the 8$^{th}$ and 9$^{th}$ vertebrae in males. Most recent field inventories indicated possible local extinction of populations of *H. fulminantis* and *H. splendissimus* in the studied area, but additional field studies should be made in other parts of the upper Carnaíba de Dentro River basin to evaluate the current conservation status of these species.

## Introduction

In the last three decades, field studies of cynolebiine killifishes in temporary pools of the Caatinga, a semi-arid phytogeographical province of northeastern Brazil, have continuously revealed spectacular species diversity (e.g., Costa 2001, 2007, 2014; Costa et al. 2012, 2018a). Over 50 valid species of the two killifish genera occurring in the Caatinga, *Cynolebias* Steindachner, 1876 and *Hypsolebias* Costa, 2006, are endemic to the main river basins of the region, with a greater concentration of species in the São Francisco River basin (e.g., Costa et al. 2018b). Like other African and South American seasonal aplocheiloid killifishes, cynolebiine killifishes of the Caatinga are uniquely found in temporary pools formed during the rainy seasons, a specific kind of aquatic habitat that was not sampled by ichthyologists until the first studies of seasonal killifishes in the region (e.g., Costa and Brasil 1990, 1991, 1993). The life cycles of seasonal killifish are conditioned by irregular rainy seasons in the region mostly occurring between November and May, as well as by long dry periods, sometimes extending over a year, when species survive in resistant eggs buried in the pool substrate (Wourms 1972; Costa 1995).

In spite of the great morphological diversity exhibited by different endemic lineages of seasonal killifishes, several cryptic species have been recently recognised in the Caatinga using molecular species delimitation analyses

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(e.g., Costa et al. 2012, 2014, 2018a). These studies have identified distinct cryptic species inhabiting the same drainage of the São Francisco River basin, showing that most seasonal killifish species exhibit a very restricted distribution range (Costa et al. 2012, 2018a). However, while field studies have been conducted to estimate killifish species diversity in the region, drastic anthropic modifications in seasonal killifish habitats of some Caatinga areas have caused extinction of several populations (Costa 2002, 2017; Costa et al. 2012, 2018a).

An uncommonly high concentration of endemic species of seasonal killifishes has been recorded for a small area encompassing the highland plateaus associated with the upper sections of the Carnaúba de Dentro and the Verde Pequeno river drainages, in the middle section of the São Francisco River basin (Costa and Brasil 1993; Costa et al. 1996; Costa and Nielsen 2004; Costa 2006a, 2014, 2017). This area is characterised by a series of plains located at slightly different altitudes, between 500 and 630 m above sea level (asl), separated from each other by an undulating relief and drained by temporary rivers and streams. Among the eight species reported for this area, two species, *Hypsolebias fulminantis* (Costa & Brasil, 1993) and *Hypsolebias carlettoi* (Costa & Nielsen, 2004) are members of a clade endemic to the Caatinga, which was named as J'-clade by Costa (2006b) and then diagnosed by the presence of a distinctive anteromedial process on the second hypobranchial, directed toward the second basibranchial (Costa 2006b: fig. 17c). Species of this clade are also unique among congeners by the highly contrasting colouration of the unpaired fins in males, consisting of intense bright blue marks over a red background, and the presence of intense red pigmentation on the trunk in males. The J'-clade also includes *H. shibattai* Nielsen, Martins, Araujo & Suzart, 2014, a species closely related to *H. fulminantis*, and a group known as the *Hypsolebias magnificus* species complex that comprises *H. gardneri* Costa, 2018, *H. hamadryades* Costa, 2018, *H. harmonicus* (Costa, 2010), *H. magnificus* (Costa & Brasil, 1991), and *H. picturatus* (Costa, 2000) (Costa 2007, 2010; Nielsen et al. 2014; Costa et al. 2018a). *Hypsolebias fulminantis* and *H. shibattai* form a consistent subclade, herein named the *H. fulminantis* species complex, easily diagnosed by the presence of narrow metallic blue lines parallel to the fin rays on all unpaired fins, in contrast to metallic blue dots or transverse blue stripes on the unpaired fins in other species of the group (Costa 2007). In addition, in species of this complex the opercular region and the anteroventral portion of the flank is intense yellow ochre in males, instead of pale golden as in other congeners of the J'-clade.

*Hypsolebias fulminantis* since its description in 1993, has become a popular aquarium fish due to the colouration exhibited by males. It was often collected by aquarists and amateur ichthyologists and consequently appears in numerous aquarium fish websites. However, field studies in the region have shown a sharp decline in natural populations (person. observation by WJEMC). In the past, *H. fulminantis* was frequently sampled around the town of Guanambi, in pools situated in the northeastern and southern parts of the town's periphery, at altitudes between about 525 and 555 m asl. However, during field studies in January 2010, after a severe environmental change in the region caused by the expansion of the Guanambi urban area, it was noted that all temporary pools sampled in previous years, inhabited by *H. fulminantis*, had been extirpated. On the other hand, new populations of seasonal killifishes were found just west from Guanambi, including a population with specimens similar to *H. fulminantis* but exhibiting some distinct morphological traits, suggesting that they may be a new species, which is here supported by molecular species delimitation methods. The objectives of this paper are to describe the new species and to provide a report on distribution and conservation of species of the *H. fulminantis* complex in the upper Carnaíba de Dentro River basin based on field studies made between February 1993 and March 2017.

## Material and methods

### Specimens

Methods for fish capture, euthanasia, fixation, and preservation in collections follow methods described by Costa et al. (2018a) for other seasonal killifishes of the Caatinga, which were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 065/18). Collections were made with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit numbers: 34270-4, 20618-1, 57099-1). Preserved specimens listed in this paper are deposited in the ichthyological collections of: Museu de Zoologia, Universidade de São Paulo, São Paulo (MZUSP), and Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ). In lists of material, the abbreviation C&S indicates specimens prepared for osteological analysis and preserved in glycerine (see below), and DNA indicates specimens fixed and preserved in 98 % ethanol. List of specimens used in the molecular analysis and their respective GenBank accession numbers appears in Table 1. Comparative material is listed in Costa (2007, 2010) and Costa et al. (2018a).

### Morphological data

Descriptions of colouration in living fish were based on photographs of both sides of individuals. Photographs were taken in small aquaria about 24 hours or less after collections. Additional direct observations were made with fish in small transparent plastic bottles just after collection. Measurements and counts follow Costa (1988). Measurements are presented as percentages of standard length (SL), except for those related to head morphology, which are expressed as percentages of head length. Measurements were made only in specimens fixed in 10 %

**Table 1.** List of specimens used in the molecular analysis, with their respective catalog numbers, coordinates of the collecting site, and GenBank accession numbers for cytb sequences. Asterisk indicates sequences not published previously.

| Species | Catalog number | Coordinates | Cytb |
|---|---|---|---|
| *Hypsolebias carlettoi* | UFRJ 6780.1 | 14°13'42"S, 42°55'12"W | MH909076* |
| *Hypsolebias carlettoi* | UFRJ 6780.2 | 14°13'42"S, 42°55'12"W | MH048856 |
| *Hypsolebias carlettoi* | UFRJ 6780.3 | 14°13'42"S, 42°55'12"W | MH909078* |
| *Hypsolebias carlettoi* | UFRJ 6780.4 | 14°13'42"S, 42°55'12"W | MH909079* |
| *Hypsolebias fulminantis* | UFRJ 6726.1 | 14°12'21"S, 42°45'42"W | MH048854 |
| *Hypsolebias fulminantis* | UFRJ 6726.2 | 14°12'21"S, 42°45'42"W | MH909075* |
| *Hypsolebias hellneri* | UFRJ 6700.4 | 15°04'49"S, 44°04'40"W | MH909072* |
| *Hypsolebias splendissimus* | UFRJ 6778.1 | 14°12'54"S, 42°50'22"W | MH909080* |
| *Hypsolebias splendissimus* | UFRJ 6778.2 | 14°12'54"S, 42°50'22"W | MH909081* |
| *Hypsolebias splendissimus* | UFRJ 6778.3 | 14°12'54"S, 42°50'22"W | MH909082* |
| *Hypsolebias splendissimus* | UFRJ 6778.4 | 14°12'54"S, 42°50'22"W | MH909083* |
| *Hypsolebias picturatus* | UFRJ 6708.1 | 11°28'03"S, 43°17'10"W | MH048868 |

formalin for a period of 10 days, and then transferred to 70 % ethanol; specimens fixed in 98 % ethanol, and consequently having slightly deformed body by dehydration, were not measured. Fin-ray counts include all elements. At least four specimens of each species, two males and two females, were cleared and stained for osteological examination using Taylor and Van Dyke's (1985) protocol. Terminology for osteological structures followed Costa (2006b), for frontal squamation Hoedeman (1958), and for cephalic neuromast series Costa (2001). Meristic data were taken from all available specimens, except osteological characters that were taken only from cleared and stained (C&S) specimens.

### DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissue of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. A fragment of the mitochondrial DNA gene cytochrome b (cytb) was amplified using the primers L14724 and H15149 (Kocher et al. 1989; Meyer et al. 1990). Polymerase chain reaction (PCR) was performed in 15 µl reaction mixtures containing 5× Green GoTaq Reaction Buffer (Promega), 3.2 mM $MgCl_2$, 1 µM of each primer, 75 ng of total genomic DNA, 0.2 mM of each dNTP and 1 U of Taq polymerase. The thermocycling profile was: (1) 1 cycle of 4 minutes at 94 °C; (2) 35 cycles of 1 minute at 92 °C, 1 minute at 44–54 °C and 1 minute at 72 °C; and (3) 1 cycle of 4 minutes at 72 °C. In all PCR reactions, negative controls without DNA were used to check for contaminations. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 10 µl reaction volumes containing 1 µl BigDye 2.5, 1.55 µl 5× sequencing buffer (Applied Biosystems), 2 µl of the amplified products (10–40ng), and 2 µl primer. The thermocycling profile was: (1) 35 cycles of 10 seconds at 96 °C, 5 seconds at 54 °C and 4 minutes at 60 °C. The sequencing reactions were purified and denatured and the samples were run on an ABI 3130 Genetic Analyzer. Sequences were edited using MEGA 6 (Tamura et al. 2013) and aligned using ClustalW (Chenna et al. 2003); alignments were subsequently translated into amino acids residues to check premature stop codons or indels. List of specimens used in the molecular analysis and their respective GenBank accession numbers appear in Table 1.

### Phylogenetic analysis and species delimitation

Analyses were conducted with a short cytb fragment (416 bp) that has been efficiently used for delimitating cryptic species of different aplocheiloid killifish groups (Sonnenberg 2007; Van der Zee and Sonnenberg 2011; Costa et al. 2012, 2014, 2018a). Terminal taxa were 10 specimens of the three species of the J'-clade endemic to the upper Carnaíba de Dentro River drainage; out-groups comprised one species of the *H. magnificus* complex (*H. picturatus* (Costa, 2000)), and *H. hellneri* (Berkenkamp, 1993), the sister group of the J'-clade (Costa et al. 2017), which was used to root the phylogeny. The best-fit model of sequence evolution was calculated by jModelTest 2.1.7 (Darriba et al. 2012), which indicated the general-time reversible model with a gamma frequency distribution of categories among sites (GTR + G). Bayesian reconstruction was performed with BEAST v.1.8 (Drummond et al. 2012), using an uncorrelated relaxed lognormal model and other parameters set as default; the MCMC length was 30,000,000 runs with sampling every 1,000 runs. The quality of the MCMC chains was evaluated in Tracer 1.5 (Rambaut, et al. 2013); a 25% burn-in was removed and the final tree was obtained using TreeAnnotator v.1.5 from BEAST v.1.8 package; support values of the Bayesian inference (BI) analysis were calculated by posterior probability. Two different single-locus models for species delimitation were used: the Generalized Mixed Yule-Coalescent (GMYC), independently applying single and multiple-threshold (Fujisawa and Barraclough 2013), and the Bayesian implementation of Poisson Tree Process (bPTP), using both Maximum likelihood and Bayesian solutions (Zhang et al. 2013), with 500,000 Markov chain Monte Carlo (MCMC) generations, thin-

ning set to 100 and a burn-in of 25% initial samples. All analyses were carried on the Exelixis Lab's web server (GMYC at http://species.h-its.org/gmyc/; bPTP at http://species.h-its.org/ptp/).

## Conservation data

Descriptions of field data relative to habitat conservation were made during collecting trips between 1994 and 2017 (February 1994, February 1999, May 1999, January 2002, January 2005, May 2009, January 2010, January 2017, and April 2017).

## Results

The phylogenetic analysis generated a tree with most branches supported by highest posterior probability values (Fig. 1). This analysis strongly supports *H. carlettoi* as being more closely related to species of the *H. fulminantis* complex than to *H. picturatus*, which is a member of the *H. magnificus* complex (Costa et al. 2018a). All methods of species delimitation yielded identical results, supporting *H. fulminantis* and the population from the pool just west of Guanambi as a distinct species, which is described below.

### *Hypsolebias splendissimus* Costa sp. n.

http://zoobank.org/77B46AC3-448F-4CBF-94E0-487D303E36A4
Figs 2–3, Table 2

**Holotype.** UFRJ 6909, male, 42.7 mm SL; Brazil: State of Bahia state: Municipality of Guanambi: temporary pool close to road BR-030, about 1.5 km W from the confluence between the Poço do Magro River and the Carnaíba de Dentro River, São Francisco River basin, and about 3 km W of the town of Guanambi, 14°12'54" S 42°50'22" W, altitude about 505 m asl; W. J. E. M. Costa et al., 30 January 2010.

**Paratypes.** UFRJ 6779, 1 male, 43.3 mm SL, 2 females, 28.5–30.2 mm SL; UFRJ 6910, 1 male, 42.7 mm SL, 3 females, 26.7–30.5 mm SL (C&S); UFRJ 6778, 2 males, 33.7–36.2 mm SL, 6 females, 28.4 – 29.5 mm SL (DNA); collected with holotype.

**Diagnosis.** *Hypsolebias splendissimus* differs from *H. fulminantis* and *H. shibattai* by having: pectoral fin red in males (vs. hyaline in *H. fulminantis* and *H. shibattai*), well-developed filamentous rays on the tips of the dorsal and anal fins in adult males (vs. filamentous rays absent or rudimentary, poorly visible), and the second proximal radial of the dorsal fin between the neural spines of the 8th and 9th vertebrae in males (vs. between the neural spines of the 6th and 7th vertebrae). Also distinguished from *H. shibattai* by having the dorsal-fin origin posterior to the anal-fin origin in males (vs. anterior), distinctive red bars restricted to the anterior portion of the flank males (vs. extending over the whole flank), and absence of contact organs on the pectoral fin in males (vs. present).

**Table 2.** Morphometric data of *Hypsolebias splendissimus*.

| | Holotype | Paratypes | |
|---|---|---|---|
| | Male | Males (2) | Females (5) |
| Standard length (mm) | 42.7 | 42.9–43.3 | 27.0–30.5 |
| **Percent of standard length** | | | |
| Body depth | 36.0 | 35.0–36.8 | 35.8–38.8 |
| Caudal peduncle depth | 16.1 | 15.5–16.0 | 14.9–15.6 |
| Pre-dorsal length | 45.2 | 47.3–48.0 | 58.0–62.3 |
| Pre-pelvic length | 42.2 | 42.7–43.6 | 49.9–52.0 |
| Length of dorsal-fin base | 42.9 | 39.6–40.1 | 24.3–28.8 |
| Length of anal-fin base | 42.1 | 40.0–43.8 | 23.2–26.7 |
| Caudal-fin length | 40.2 | 40.0–41.3 | 34.7–37.8 |
| Pectoral-fin length | 28.2 | 28.8–29.1 | 24.1–25.7 |
| Pelvic-fin length | 10.7 | 10.4–11.6 | 10.6–12.2 |
| Head length | 27.1 | 26.5–27.7 | 28.3–31.1 |
| **Percent of head length** | | | |
| Head depth | 109.9 | 111.9–114.3 | 102.5–97.6 |
| Head width | 63.9 | 67.1–68.7 | 65.2–74.4 |
| Snout length | 14.7 | 12.9–15.5 | 13.8–14.8 |
| Lower jaw length | 19.9 | 17.7–18.0 | 14.9–16.5 |
| Eye diameter | 28.4 | 29.3–32.0 | 31.3–37.1 |

**Description.** Morphometric data appear in Table 2. Body relatively deep, compressed. Greatest body depth at vertical just anterior to pelvic-fin base. Dorsal and ventral profiles of head and trunk slightly convex, approximately straight on caudal peduncle. Head narrow, sub-triangular in lateral view. Jaws short, teeth numerous, conical, irregularly arranged; outer teeth hypertrophied, inner teeth small and numerous. Vomerine teeth absent. Gill-rakers on first branchial arch 2 + 10–11, gill-rakers short, straight, without denticles.

Dorsal and anal fins pointed in males, with two or three filaments on tip, rounded, without filaments, in females. Caudal fin rounded. Pectoral fin sub-lanceolate, posterior tip reaching vertical between base of 5th and 7th anal-fin rays in males, reaching between anus and urogenital papilla in females. Pelvic fin small, tip reaching base of 3rd anal-fin ray in males, reaching between urogenital papilla and anal-fin origin in females; pelvic-fin bases medially united. Dorsal-fin origin on vertical between base of 2nd and 4th anal-fin rays in males, between base of 4th and 6th anal-fin rays in females. Dorsal-fin rays 19–22 in males, 15–16 in females; anal-fin rays 21 in males, 18–19 in females; caudal-fin rays 23–24; pectoral-fin rays 12–13; pelvic-fin rays 6. No contact organs on fins. Second proximal radial of dorsal fin between neural spines of 8th and 9th vertebrae in males, between neural spines of 11th and 12th vertebrae in females; first proximal radial of anal fin between pleural ribs of 8th and 9th vertebrae in males, between pleural ribs of 9th and 10th vertebrae in females; total vertebrae 26–27.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation

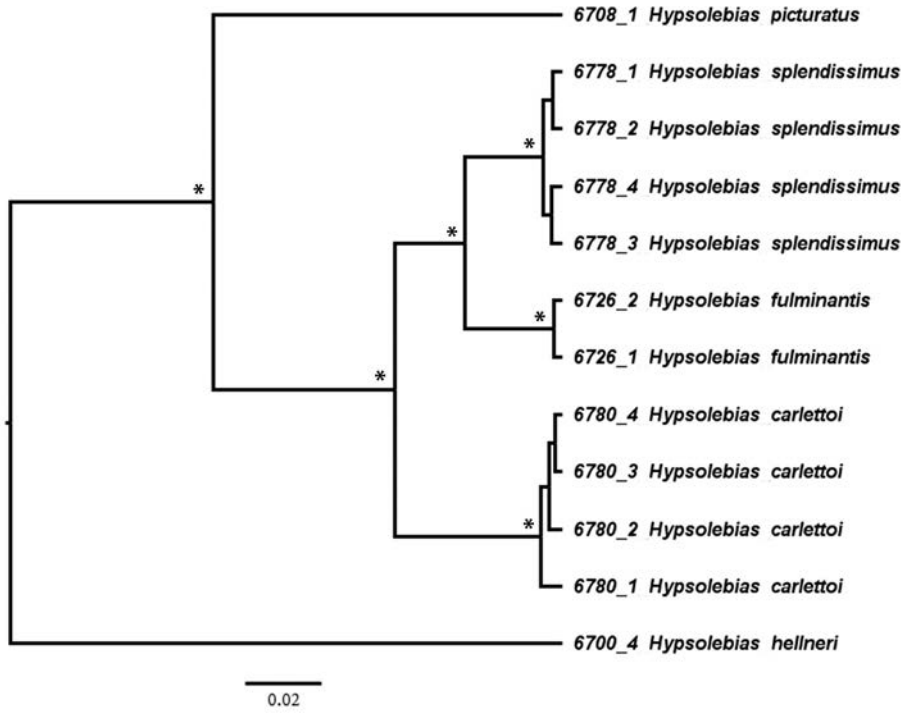


**Figure 1.** Bayesian phylogeny used to delimit species endemic to the upper Carnaíba de Dentro River drainage, inferred by using sequences of the mitochondrial gene cytochrome b, 416 bp. Posterior probability values below 95% are not depicted; asterisk above nodes represents maximum value of posterior probability (100 %); numbers before species names are catalogue numbers for voucher specimens.

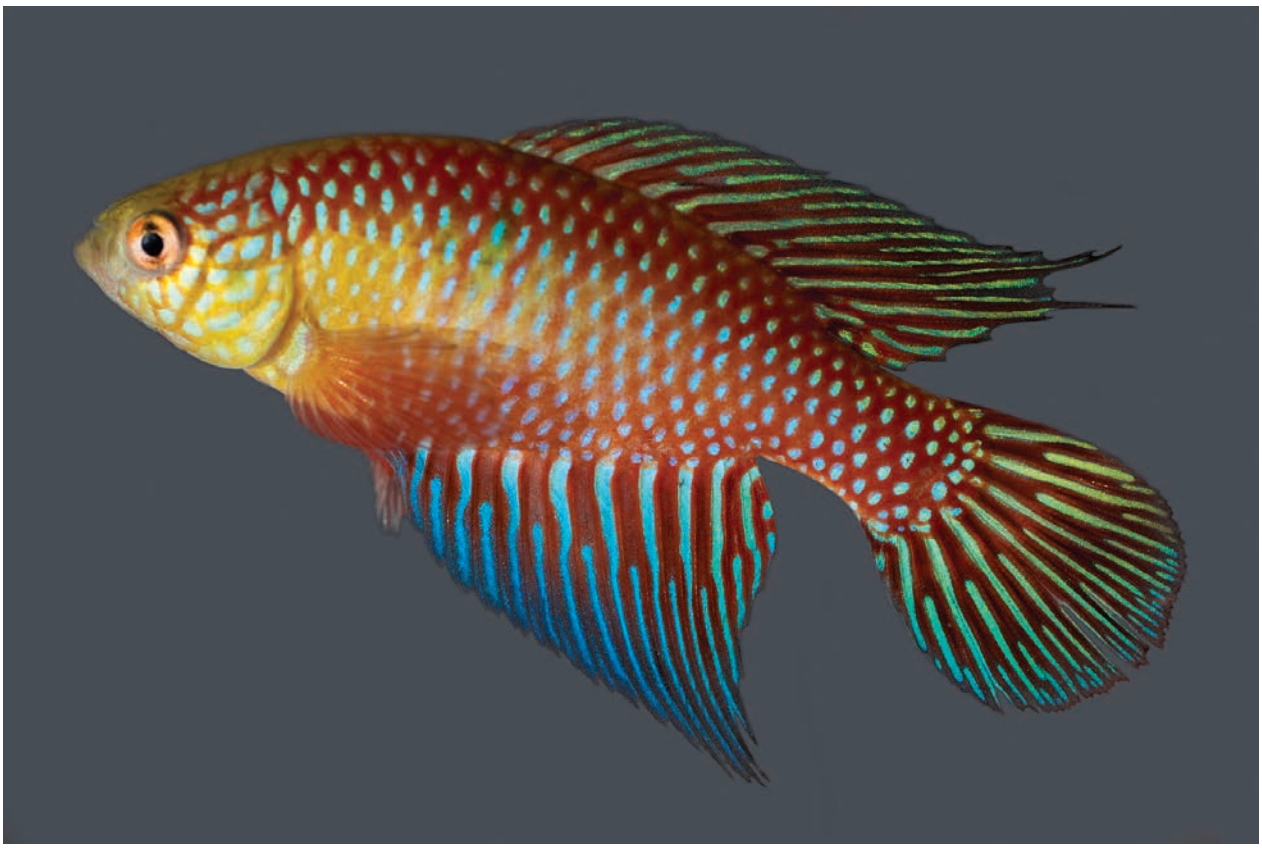

**Figure 2.** *Hypsolebias splendissimus* Costa sp. n., live holotype, UFRJ 6909, male, 42.7 mm SL. Photograph by W.J.E.M. Costa.

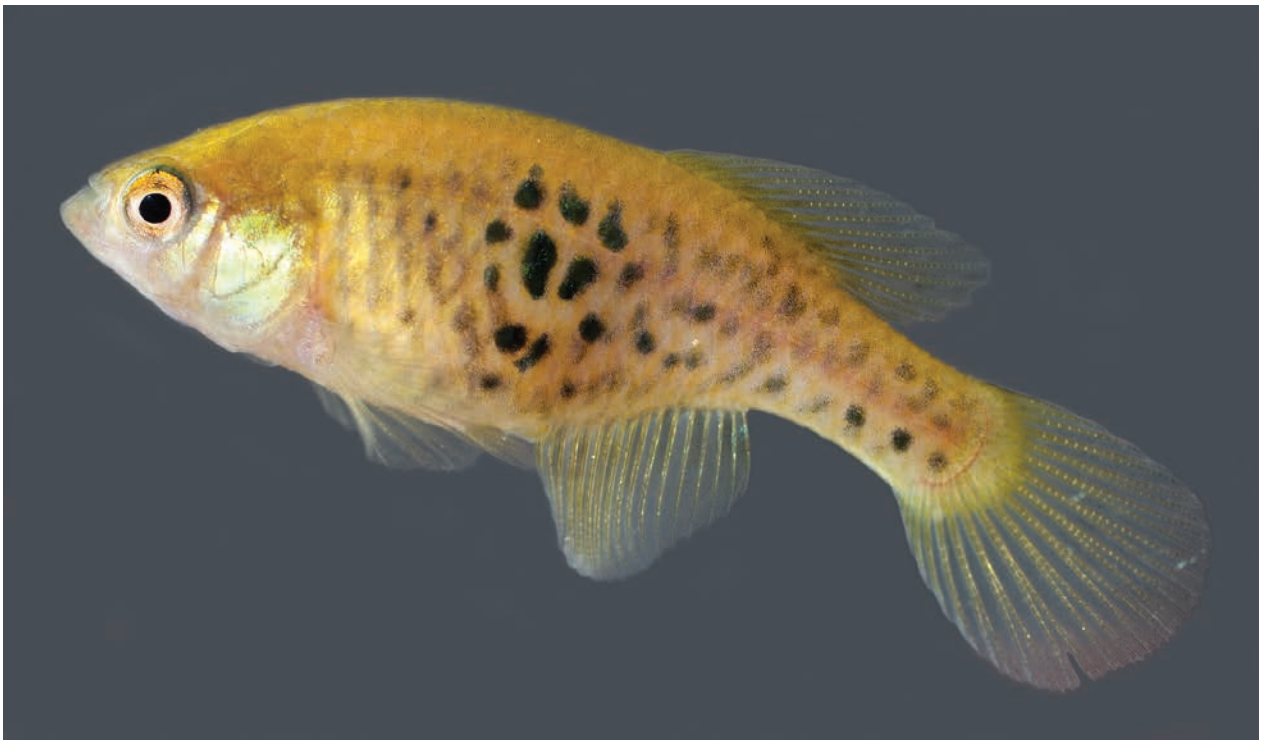

**Figure 3.** *Hypsolebias splendissimus* Costa sp. n., live paratype, UFRJ 6779, female, 28.5 mm SL. Photograph by W.J.E.M. Costa.

extending over anterior 20 % of caudal-fin base; scales slightly extending on middle part of anal-fin base in males. Frontal scales E-patterned. Longitudinal series of scales 25–26; transverse series of scales 11; scale rows around caudal peduncle 12. One prominent contact organ on each flank scale in males. Cephalic neuromasts: supraorbital 12–16; parietal 2; anterior rostral 1, posterior rostral 1; infraorbital 3 + 22–24; preorbital 3–4; otic 2, post-otic 2; supratemporal 1; median opercular 1, ventral opercular 1–2; pre-opercular 15–16; mandibular 10–13; lateral mandibular 4, paramandibular 1.

**Colouration in life.** ***Males***. Flank intense red to pink on middle portion and metallic yellow ochre on anteroventral part; small, vertically elongated bright blue spot on centre of each scale; central portion of flank often with distinctive red bars, alternating with faint green bars, sometimes inconspicuous. Dorsum pale yellowish brown, venter yellowish white. Side of head metallic light blue, with red scale margins on dorsal portion and intense metallic yellow ochre on opercular, post-orbital and infra-orbital regions; snout and jaws light grey. Iris light yellow to pale orange, with dark brown bar through orbit centre. Unpaired fins red, with alternating short and long metallic blue lines to greenish golden lines, depending on angle of light incidence, parallel to fin rays; dorsal and anal fin filaments dark grey to black. Pelvic fin red with light blue rays. Pectoral fin red.

***Females.*** Flank light brownish grey, to yellowish grey on dorsal portion and pale golden on anteroventral portion; two or three oval black spots on antero-central portion of flank; smaller specimens, about 28 mm SL or less, with dark grey bars, often interrupted; larger specimens above 28 mm SL, with dark grey spots on whole flank, often arranged in vertical rows, becoming dark grey to black around antero-central spots. Dorsum yellowish grey, venter white. Side of head yellowish grey, pale greenish golden on opercular and post-orbital regions; jaws light grey. Iris light yellow to pale orange, with dark brown bar through orbit centre. Fins yellowish hyaline.

**Colouration in alcohol.** Trunk and head pale brown, with faint grey bars on anterior portion of flank in males, and grey spots on flank in females. Fins grey in males, hyaline in females. No vestige of red pigmentation and blue iridescent marks.

**Distribution, habitat and conservation.** *Hypsolebias splendissimus* is known from a single collection at the type locality, a temporary pool in a flat plains area about 1.5 km W from the confluence between the Poço do Magro and Carnaíba de Dentro rivers, middle São Francisco River basin, Bahia, Brazil (14°12'54" S 42°50'22" W, altitude about 505 m asl; Fig. 4). At the time of the type series collection (30 January 2010) the pool was about 100 m long and 30 m wide, with a maximum depth of about 0.5 m. All individuals of *H. splendissimus* were concentrated in one part of the pool, near its margin, in an area about 100 $m^2$, that was densely populated by shrubs and aquatic plants, forming a distinctive shaded habitat. This site was visited again in January 2017, but the entire pool had been drained and landfilled by bulldozers and the new species was not found again.

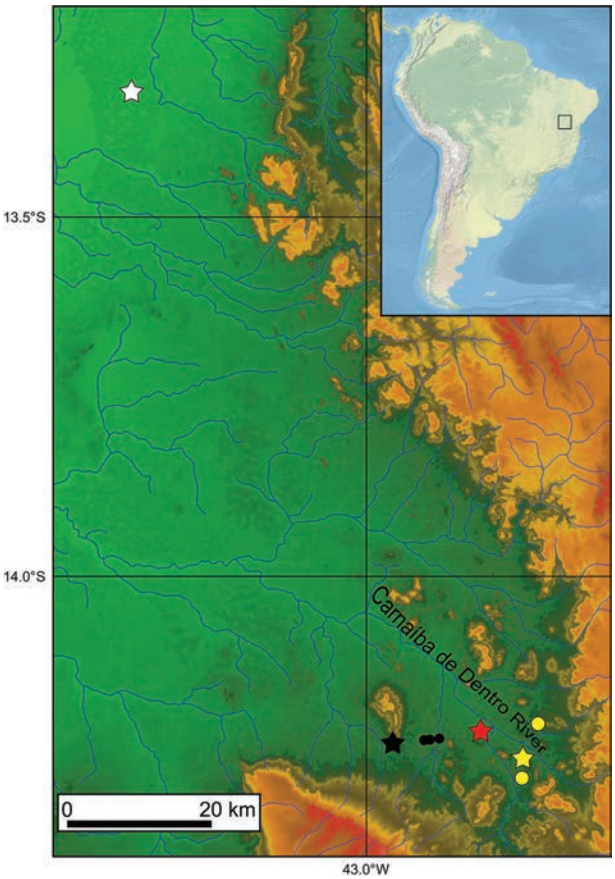


**Figure 4.** Geographical distribution of species of the *Hypsolebias* J'-clade in the upper Carnaíba de Dentro River drainage (yellow, *H. fulminantis*; red, *H. splendissimus*; black, *H. carlettoi*) and *H. shibattai* (white); stars indicate type localities.

**Etymology.** From the Latin *splendissimus* (very splendid), an allusion to the bright colours in males of the new species, which is among the most colourful South American aplocheiloid killifishes.

### *Hypsolebias fulminantis* (Costa & Brasil, 1993)

Figs. 5, 6

*Cynolebias fulminantis* Costa & Brasil, 1993: 194 (type locality: swamp near Guanambi [road BR-122], Estado da Bahia, northeastern Brazil [14°15'16"S, 42°46'56"W, altitude about 555 m]; MZUSP 43674).

**Diagnosis.** *Hypsolebias fulminantis* is a member of the *H. fulminantis* complex, differing from *H. splendissimus* by: the presence of hyaline pectoral fins in males (vs. red), presence of rudimentary or absence of filamentous rays on the tips of the dorsal and anal fins in adult males (vs. well-developed filamentous rays present), and the second proximal radial of the dorsal fin situated between the neural spines of the 6th and 7th vertebrae in males (vs. between the neural spines of the 8th and 9th vertebrae); and from *H. shibattai* by having the dorsal-fin origin posterior to the anal-fin origin in males (vs. anterior); distinctive red bars restricted to the anterior portion of the flank males (vs. extending over the whole flank); and absence of contact organs on the pectoral fin in males (vs. present).

**Distribution, habitat and conservation.** *Hypsolebias fulminantis* has been recorded from several localities in the upper Carnaíba de Dentro River basin, close to the town of Guanambi, in altitudes between 525–555 m asl (Fig. 4). These pools were shallow, maximum depth about 0.5 m, with their surface between about 15 and 300 $m^2$, and always densely occupied by aquatic plants, except in parts where recent anthropic modifications were recorded. *Hypsolebias fulminantis* was always found close to the pool margins, in shadier places. In 1994, this kind of habitat was abundant in the region, but some decline was already recorded in 1999 (Costa 2002). Previously unsampled pools inhabited by *H. fulminantis* were found in January 2002 and January 2005. After an intense expansion of the urban area, field studies in May 2009, January 2010, and January and April 2017 failed to find any specimen of *H. fulminantis* in the region.

**Remarks.** For a full description, see Costa (2007) based on types and other specimens collected in the type locality area.

**Material examined.** Brazil: State of Bahia: Municipality of Guanambi: São Francisco River basin, upper Carnaíba de Dentro River drainage: MZUSP 43674, holotype, male, 38.9 mm SL; MZUSP 43675, 2 paratypes; UFRJ 685, 2 paratypes; UFRJ 686, 3 paratypes; Guanambi, road BR-122, 14°15'16"S, 42°46'56"W, altitude about 555 m; G. C. Brasil, 1 Jan. 1992. – UFRJ 6068, 6; UFRJ 6069, 2; UFRJ 6726, 3; Guanambi, road BR-030, 14°12'21"S, 42°45'42"W, altitude about 545 m; W. J. E. M. Costa et al., 13 Jan. 2005. – UFRJ 4802, 1; temporary pool about 4.5 km S from Guanambi, Rio road BR-122,14°16'49"S, 42°47'01"W, altitude about 525 m; W. J. E. M. Costa et al., 11 Feb. 1999. – UFRJ 4847, 2; same locality as UFRJ 4802; W. J. E. M. Costa et al., 4 May 1999. – UFRJ 3809, 6; UFRJ 5864, 4 (C&S); temporary pool 4.5 km S from Guanambi; A. L. F. Cyrino et al., 27 Jan. 1996.

## Discussion

*Hypsolebias splendissimus* is presently known from a single locality just 8 km west from the geographical area inhabited by *H. fulminantis* (Fig. 4). Their distribution areas are situated in neighbouring sub-drainages of the upper section of the Carnaíba de Dentro River drainage, at slightly different altitudes, about 505 m asl at the type locality of *H. splendissimus* and between 525 and 555 m asl at the localities from where *H. fulminantis* has been recorded. Despite their geographical proximity, both morphological characters (see diagnosis above) and molecular data (Fig. 1) support recognition of them as two different species.

Field studies in the Caatinga have shown that *H. carlettoi* is also endemic to the upper Carnaíba de Dentro River drainage, but it was never found in sympatry with

**Figure 5.** *Hypsolebias fulminantis*, UFRJ 4847, male, 44.0 mm SL. Photograph by W.J.E.M. Costa.

*H. fulminantis* or *H. splendissimus*. Its distribution range is situated in a different subdrainage of the Upper Carnaíba de Dentro River drainage, the Mutula River subdrainage, and is separated by a distance of about 7 km from the type locality of *H. splendissimus* and about 15 km from the recorded geographical range of *H. fulminantis* (Fig. 4). In morphological analyses, *H. carlettoi* was considered to be more closely related to species of the *H. magnificus* complex than to *H. fulminantis* by exhibiting a red pectoral fin in males, contrasting with the hyaline pectoral fin in males of *H. fulminantis* and *H. shibattai*, which would be a plesiomorphic condition for cynolebiine killifishes (Costa 2006b, 2007). However, molecular analyses indicated that *H. carlettoi* is more closely related to *H. fulminantis* than to species of the *H. magnificus* complex (Costa et al. 2018a), a finding that is also corroborated here (Fig. 1), refuting the presence of red pectoral fins as an unambiguous synapomorphy for a subclade of the J'-clade including only *H. carlettoi* and species of the *H. magnificus* complex. The presence of a red male pectoral fin only in *H. splendissimus* among species of the *H. fulminantis* complex may be tentatively interpreted as a plesiomorphic condition for the J'-clade lost in *H. fulminantis* and *H. shibattai*, in which the pectoral fin is always hyaline in contrast to red as in the remaining congeners of the J'-clade. This colour pattern character thus suggests that *H. fulminantis* is more closely related to *H. shibattai* that is endemic to a distant area, about 115 km from the area herein studied (Fig. 4), than to *H. splendissimus* that inhabits a neighbouring area. However, molecular data for *H. shibattai* are not available, making interpretations about relationships among these three species weak.

The present study reports an accentuated decline in seasonal killifish habitats in the upper Carnaíba de Dentro River drainage around the town of Guanambi, possibly causing local extinction of *H. fulminantis* and *H. splendissimus*. However, most parts of the Carnaíba de Dentro River drainage are still not easily accessible and field studies to detect the occurrence of seasonal killifishes have never been conducted. So at this time it is not possible to evaluate the conservation status of *H. fulminantis* and *H. splendissimus*. On the other hand, satellite images indicate that these unsampled areas are extensively modified for agriculture, an environmental impact that usually has negatively affected seasonal killifish habitats (Costa 2002). Species of the J'-clade are particularly vulnerable, since they are only found in shaded parts of the pool and consequently habitat deforestation results in quick extirpation of species, even when open vegetation parts of the pool are not destroyed (Costa et al. 2018a). This study indicates the urgency to conduct additional inventories of the seasonal killifishes found in the temporary pools of the Carnaíba de Dentro River drainage to accurately establish their distribution and conservation status.

**Figure 6.** *Hypsolebias fulminantis*, UFRJ 4847, female, 34.0 mm SL. Photograph by W.J.E.M. Costa.


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## References


Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31: 3497–3500.

Costa WJEM (1988) Sistemática e distribuição do complexo de espécies *Cynolebias minimus* (Cyprinodontiformes, Rivulidae), com a descrição de duas espécies novas. Revista Brasileira de Zoologia 5: 557–570. https://doi.org/10.1590/S0101-81751988000400004

Costa WJEM (1995) Pearl killifishes, the Cynolebiatinae: systematics and biogeography of the neotropical annual fish subfamily (Cyprinodontiformes: Rivulidae). TFH, Neptune City, 128 pp.

Costa WJEM (2001) The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. Ichthyological Exploration of Freshwaters 12: 333–383.

Costa WJEM (2002) Peixes anuais brasileiros: diversidade e conservação. Editora da UFPR, Curitiba, 238 pp.

Costa WJEM (2006a) Three new species of seasonal killifishes of the *Simpsonichthys antenori* species group (Teleostei: Cyprinodontiformes: Rivulidae) from the rio São Francisco basin, Brazil. Zootaxa 1306: 25–39.

Costa WJEM (2006b) Descriptive morphology and phylogenetic relationships among species of the Neotropical annual killifish genera *Nematolebias* and *Simpsonichthys* (Cyprinodontiformes: Aplocheiloidei: Rivulidae). Neotropical Ichthyology 4: 1–26. http://dx.doi.org/10.1590/S1679-62252006000100001

Costa WJEM (2007) Taxonomic revision of the seasonal South American killifish genus *Simpsonichthys* (Teleostei: Cyprinodontiformes: Aplocheiloidei). Zootaxa 1669: 1–134.

Costa WJEM (2010) *Simpsonichthys harmonicus*, a new seasonal killifish from the São Francisco River basin, northeastern Brazil (Cyprinodontiformes: Rivulidae). Ichthyological Exploration of Freshwaters 21: 73–78.

Costa WJEM (2014) Six new species of seasonal killifishes of the genus *Cynolebias* from the São Francisco river basin, Brazilian Caatinga, with notes on *C. porosus*. Ichthyological Exploration of Freshwaters 25: 79–96.

Costa WJEM (2017) Description of two endangered new seasonal killifish species of the genus *Cynolebias* from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes, Aplocheilidae). Zoosystematics and Evolution 93: 333–341. https://doi.org/10.3897/zse.93.20906

Costa WJEM, Amorim PF, Bragança PHN (2014) Species limits and phylogenetic relationships of red-finned cryptic species of the seasonal killifish genus *Hypsolebias* from the Brazilian semi-arid Caatinga (Teleostei: Cyprinodontiformes: Rivulidae). Journal of Zoological Systematics and Evolutionary Research 52: 52–58. https://doi.org/10.1111/jzs.12041

Costa WJEM, Amorim PF, Mattos JLO (2012) Species delimitation in annual killifishes from the Brazilian Caatinga, the *Hypsolebias flavicaudatus* complex (Cyprinodontiformes: Rivulidae): implications for taxonomy and conservation. Systematics and Biodiversity 10: 71–91. http://dx.doi.org/10.1080/14772000.2012.664177

Costa WJEM, Amorim PF, Mattos JLO (2017) Molecular phylogeny and timing of diversification in South American Cynolebiini season-

al killifishes. Molecular Phylogenetics and Evolution. https://doi.org/10.1016/j.ympev.2017.07.020

Costa WJEM, Amorim PF, Mattos JLO (2018a) Cryptic species diversity in the *Hypsolebias magnificus* complex, a clade of endangered seasonal killifishes from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes, Aplocheilidae). ZooKeys 777: 141–158. https://doi.org/10.3897/zookeys.777.25058

Costa WJEM, Amorim PF, Mattos JLO (2018b) Synchronic historical patterns of species diversification in seasonal aplocheiloid killifishes of the semi-arid Brazilian Caatinga. PLoS ONE 13(2) e0193021. https://doi.org/10.1371/journal.pone.0193021

Costa WJEM, Brasil GC (1990) Description of two new annual fishes of the genus *Cynolebias* (Cyprinodontiformes: Rivulidae) from the São Francisco basin, Brazil. Ichthyological Exploration of Freshwaters 1: 15–22.

Costa WJEM, Brasil GC (1991) Three new species of *Cynolebias* (Cyprinodontiformes: Rivulidae) from the São Francisco basin, Brazil. Ichthyological Exploration of Freshwaters 2: 55–62.

Costa WJEM, Brasil GC (1993) Two new species of *Cynolebias* (Cyprinodontiformes: Rivulidae) from the São Francisco basin, Brazil, with notes on phylogeny and biogeography of annual fishes. Ichthyological Exploration of Freshwaters 4: 193–200.

Costa WJEM, Cyrino ALF, Nielsen DTB (1996) Description d'une nouvelle espèce de poisson annuel du genre *Simpsonichthys* (Cyprinodontiformes: Rivulidae) du bassin du rio São Francisco, Brésil. Revue Française d'Aquariologie et Herpetologie 23: 17–20.

Costa WJEM, Nielsen DTB (2004) *Simpsonichthys carlettoi* (Cyprinodontiformes: Rivulidae) a new annual fish from the Rio São Francisco basin, north-eastern Brazil. Aqua Journal of Ichthyology and Aquatic Biology 8: 125–130.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. https://doi.org/10.1093/molbev/mss075

Fujisawa T, Barraclough TG (2013) Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. Systematic Biology 62: 702–724. https://doi.org/10.1093/sysbio/syt033

Hoedeman JJ (1958) The frontal scalation pattern in some groups of toothcarps (Pisces, Cyprinodontiformes). Bulletin of Aquatic Biology 1: 23–28.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the Natural Academy of Sciences USA 86: 6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Meyer A, Kocher TD, Basasibwaki P, Wilson AC (1990) Monophyletic origin of Lake Victorian cichlids fishes suggested by mitochondrial DNA sequences. Nature 347: 550–553. https://doi.org/10.1038/347550a0

Nielsen DTB, Martins M, Araujo LM, Suzart RR (2014) *Hypsolebias shibattai*, a new species of annual fish (Cyprinodontiformes: Rivulidae) from the rio São Francisco basin, northeastern Brazil. Aqua International Journal of Ichthyology 20: 27–34.

Rambaut A, Suchard MA, Xie D, Drummond AJ (2013) Tracer v1.5. http://beast.bio.ed.ac.uk/Trace

Sonnenberg R (2007) Description of three new species of the genus *Chromaphyosemion* Radda, 1971 (Cyprinodontiformes: Nothobranchiidae) from the coastal plains of Cameroon with a preliminary review of the *Chromaphyosemion splendopleure* complex. Zootaxa 1591: 1–38.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–109. http://sfi.mnhn.fr/cybium/numeros/1985/92/01-Taylor%5b92%5d107-119.pdf

Van der Zee J, Sonnenberg R (2011) *Aphyosemion musafirii* (Cyprinodontiformes: Nothobranchiidae), a new species from the Tshopo Province in the Democratic Republic of Congo, with some notes on the *Aphyosemion* of the Congo Basin. Bonn Zoological Bulletin 60: 73–87.

Wourms JP (1972) Developmental biology of annual fishes: III. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. Journal of Experimental Zoology 182: 389–414. https://doi.org/10.1002/jez.1401820310

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29: 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

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# Type specimens of fossil "Architectibranchia" and Cephalaspidea (Mollusca, Heterobranchia) in the Academy of Natural Sciences of Philadelphia

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## Abstract

The type specimens of fossil "Architectibranchia" and Cephalaspidea (Mollusca: Heterobranchia) deposited in the Academy of Natural Sciences of Philadelphia, USA, are listed herein. The collection includes types of circa 60 species, from the families: Acteonellidae, Acteonidae, Bullidae, Cylichnidae, Haminoeidae, Philinidae, Retusidae, Rhizoridae, Ringiculidae and Scaphandridae. The catalogue is presented in systematic order, with information on the original description, type locality, type stratum and age, catalog number in the collection, and current taxonomic status. Further taxonomic notes are offered when pertinent. Several species are illustrated here for first time. The new combinations *Roxania hornii* (Gabb, 1864) and *Volvulella minutissima* (Gabb, 1860) are proposed. Some species that have previously been assigned to Acteonidae are revised here, resulting in the following new combinations: *Odostomia milium* (Lea, 1846), *Chrysallida sculpta* (Lea, 1846) and *Pyrgulina angulata* (Lea, 1846). The list of taxa is also presented in other arrangements (alphabetically by specific epithets and by authorship and date) to facilitate locating information.

## Introduction

The collection of fossil mollusks in the Academy of Natural Sciences of Philadelphia (ANSP; Philadelphia, PA, USA) houses about 80,000 lots, mostly of gastropods. Despite containing fossils from diverse localities worldwide, most stems from the Mesozoic and Cenozoic eras of the USA. The collection counts with original material (including type specimens) of several 19th and early 20th century paleontologists, such as Timothy A. Conrad, William M. Gabb, Henry C. Lea, Isaac Lea and Henry A. Pilsbry.

It is an international consensus that all museums should publish inventories of their type specimens. Since many invertebrate species have convoluted taxonomic histories often with inadequate descriptions and illustrations (or no illustration at all), and little modern taxonomic analysis, it is deemed that such catalogues will benefit or prompt future studies.

Some catalogues have been published regarding the Recent mollusks of the ANSP collection, focusing on specific authors or families (Borrero and Rosenberg 2015, Callomon 2015, Snyder and Callomon 2015), and the entire collection is presently searchable online. A catalogue of the ANSP invertebrate fossil types was published by Richards (1968), as well as more specific ones dealing with type specimens of a single author (e.g., Moore 1962, for Conrad's types) or a restricted period (e.g., Johnson 1905, for the Cretaceous). These catalogues sometimes fail to cite the type specimens of some species, might present conflicting information, and, due to their broad scope, offer very

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little extra information on the species' taxonomy. Therefore, we present here an annotated catalogue of the fossil "Architectibranchia" and Cephalaspidea gastropod types housed in the ANSP Invertebrate Paleontology collection. When necessary, new combinations are proposed. Moreover, some species that have previously been assigned to Acteonidae are revised, resulting in new combinations.

## Material and methods

The present catalogue offers information on the original description of each species, the ANSP catalog number for the type material lots, type locality and stratum, age and current taxonomical status (following the most recent revisions, where they exist). At least one type specimen (holotype, lectotype or syntype) of each nominal species is figured here, with further type specimens figured only when they add information. Several species are figured here for the first time.

The type catalogue is arranged in three ways to facilitate locating information: (1) by current systematic position (with additional information and comments and, when necessary, proposed new combinations); (2) alphabetically by specific epithet; (3) by authorship and date of the nominal species.

Some species whose types are housed in the ANSP collection were originally classified in Architectibranchia or Cephalaspidea, but clearly do not belong to them. They are listed further below and, as some of them have never received a taxonomical reassessment, they are reclassified here.

The classification used here follows Bouchet et al. (2005, 2017), complemented (when available) by taxonomic revisions that deal specifically with the fossil taxa. The taxa represented in the ANSP collection are: Architectibranchia (Acteonellidae, Acteonidae, Ringiculidae) and Cephalaspidea (Bullidae, Cylichnidae, Haminoeidae, Philinidae, Retusidae, Rhizoridae, Scaphandridae).

Type localities and strata are provided as precisely as possible and when the original descriptions were not very precise, we added information from previous authors, specimens labels, or our own research (new information is always clearly indicated as such). Moreover, some locality and formation names were updated to conform to current conventions; the original names are also indicated. Likewise, the age of the strata are given with as much resolution as possible. However, several localities have not been studied in detail since then; in these cases, a coarser age span (e.g., period) is indicated.

Previous catalogues and species lists (e.g., Palmer 1937, Moore 1962, Richards 1968) often refer to the type specimens in different manners, without discussing the reasoning behind their choice. Herein, we indicated these previous assesments in quotation marks. One issue can be generalized here, though, regarding the use of the word "holotype" by previous catalogues when confronted by a single specimen in the collection. This practice is erroneous and all the original specimens are considered syntypes (even if there is only a single one) herein, unless explicetely indicated on the original description that a single specimen was available (in which case, it is a holotype).

Unfortunately, some of the types supposedly housed in the ANSP collection could not be found in the present study and are thus considered lost. They are: *Acteon costellatus* Conrad, 1833; *Acteon modicellus* Conrad, 1860; *Bulla mortoni* Forbes, 1845 and *Retusa sulcata fossilis* Pilsbry, 1922.

## Systematic list of taxa

**List of taxa by systematic arrangement**

**Heterobranchia**
**Superfamily Acteonoidea d'Orbigny, 1843**
**Family Acteonidae d'Orbigny, 1843**
**Genus *Acteocina* Gray, 1847**

### *Acteocina cederstromi* Richards, 1947

Figure 1A–B

*Acteocina cederstromi* Richards, 1947: 34, pl. 11, fig. 9.

**Type locality.** Bacons Castle, Virginia, depth 115 ft. (ca. 35 m); stratum: Yorktown Formation; age: Late Miocene to Middle Pliocene.

**Type material.** Holotype, ANSP IP16771 (as "type" in Richards 1968: 113).

**Current taxonomic status.** *Acteocina candei* (d'Orbigny, 1841) (Campbell 1993).

### *Acteocina chowanensis* Richards, 1947

Figure 1C–D

*Acteocina chowanensis* Richards, 1947: 34, pl. 11. fig. 10.

**Type locality.** Edenton, well 11 (as "well 3" in original description), depth 46–58 ft. (ca. 14–17.5 m), U. S. Marine Base, North Carolina, USA; stratum: Chowan River Formation; age: Pliocene.

**Type material.** Holotype, ANSP IP16754 (as "type" in Richards 1968: 113).

**Current taxonomic status.** *Acteocina canaliculata* (Say, 1826) (Mikkelsen and Mikkelsen 1984).

### *Acteocina crassiplica* (Conrad, 1848)

Figure 1E–F

*Bulla crassiplica* Conrad, 1848a: 282.

**Type locality.** Dr. Smith's plantation, 6 miles northeast of Vicksburg, Warren County, Mississippi, USA; stratum: Vicksburg Group; age: Oligocene.

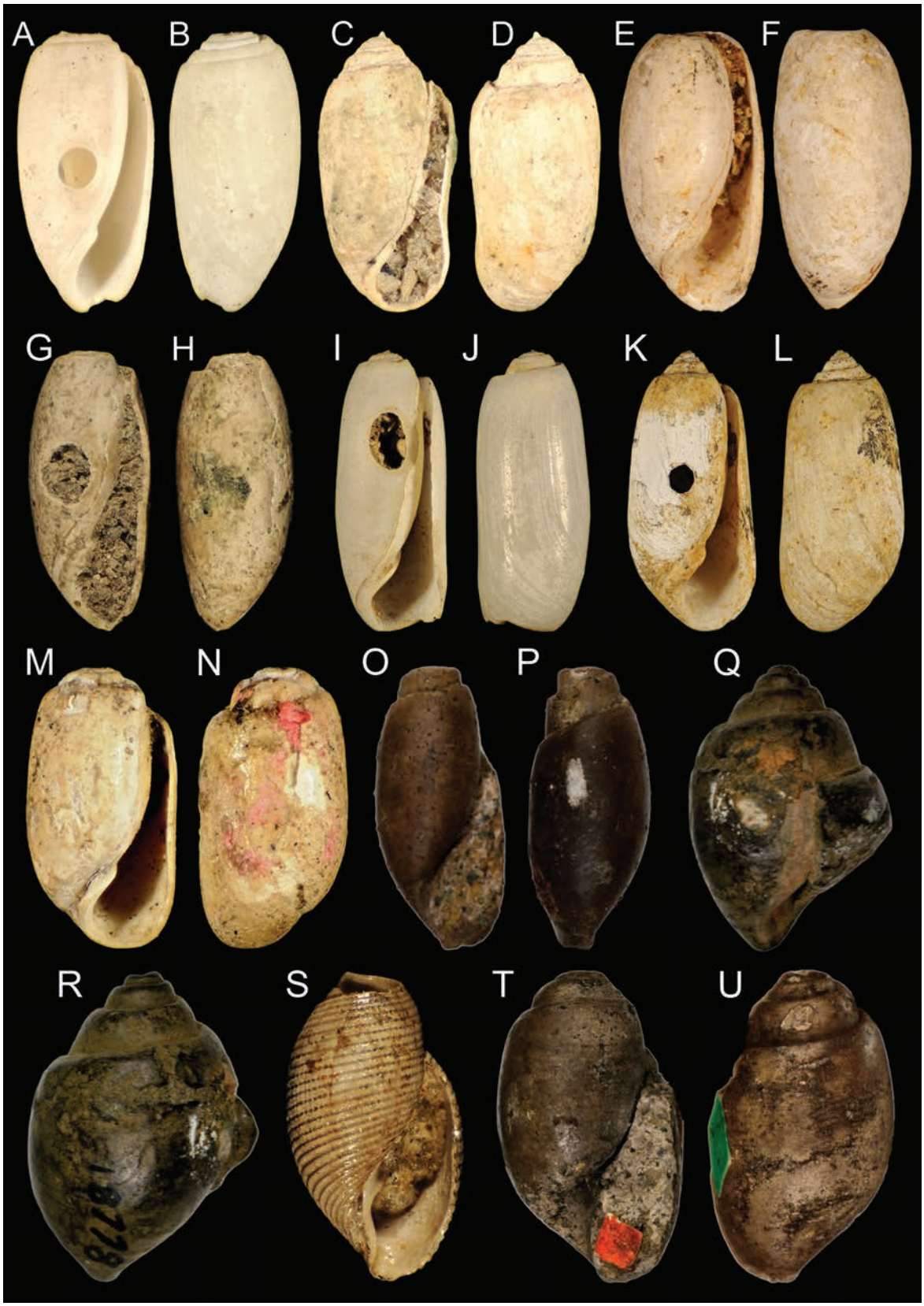


**Figure 1.** Types. **A–B.** Holotype of *Acteocina cederstromi*, H 4.0 mm, ANSP IP16771. **C–D.** Holotype of *Acteocina chowanensis*, H 3.8 mm, ANSP IP16754. **E–F.** Lectotype of *Acteocina crassiplica*, H 6.0 mm, ANSP IP13412. **G–H.** Paratype of *Acteocina kirkwoodiana*, H 5.0 mm, ANSP IP15935. **I–J.** Holotype of *Acteocina puruha*, H 7.6 mm, ANSP IP13684. **K–L.** Holotype of *Acteocina subbullata*, H 9.9 mm, ANSP IP3193. **M–N.** Syntype of *Acteocina weatherlli*, H 4.6 mm, ANSP IP14431. **O–P.** Syntype of *Acteon biplicatus*, H 17.2 mm, ANSP IP19466; also, syntype of *A. gabbana*. **Q–R.** Syntype of *Acteon cretacea*, H 19.1 mm, ANSP IP18778. **S.** Holotype of *Acteon elegans*, H 6.0 mm, ANSP IP6011. **T–U.** Syntype of *Acteon forbesiana*, H 9.2 mm, ANSP IP18777.

**Type material.** Lectotype, ANSP IP13412 (designation in Moore 1962: 51, Richards 1968: 119, MacNeil and Dockery 1984: 240); paralectotypes, ANSP 13413, 6 shells (as "paratype" in Richards 1968: 119, MacNeil and Dockery 1984: 240).

**Current taxonomic status.** *Acteocina crassiplica* (Conrad, 1848) (MacNeil and Dockery 1984).

### *Acteocina kirkwoodiana* Richards & Harbison, 1944

Figure 1G–H

*Acteocina kirkwoodiana* Richards & Harbison, 1944: 9, figs 1, 5–6.

**Type locality.** Brandywine Lighthouse well, depth 385 feet [ca. 117 m], Delaware Bay, New Jersey, USA; stratum: Kirkwood Formation; age: Miocene.

**Type material.** Paratype, ANSP IP15935, 1 shell (Richards and Harbison 1944: 14, fig. 1, 5; as "paratypes" in Richards and Harbison 1944: figs 1, 5; as "cotype" in Richards 1968: 148).

**Current taxonomic status.** —*Acteocina kirkwoodiana* Richards & Harbison, 1944.

### *Acteocina puruha* Pilsbry & Olsson, 1941

Figure 1I–J

*Acteocina puruha* Pilsbry & Olsson, 1941: 13, pl. 8, fig. 1.

**Type locality.** Punta Blanca, Ecuador; stratum: Canoa Formation; age: Late Pliocene.

**Type material.** Holotype, ANSP IP13684 (as "type" in Richards 1968: 179).

**Current taxonomic status.** *Acteocina puruha* Pilsbry & Olsson, 1941.

### *Acteocina subbullata* Pilsbry & Johnson, 1917

Figure 1K–L

*Acteocina subbullata* Pilsbry & Johnson, 1917: 150–151.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Holotype, ANSP IP3193 (as "type" in Richards 1968: 192).

**Current taxonomic status.** *Acteocina bullata* (Kiener, 1834) (Woodring 1970).

### *Acteocina weatherlli* (Lea, 1833)

Figure 1M–N

*Acteon weatherlli* Lea, 1833: 213, pl. 6, fig. 224.

**Type locality.** Monmouth Co., Deal, New Jersey, USA; stratum: uncertain; age: Miocene(?).

**Type material.** Syntype, ANSP IP14431, 1 shell (as "type" in Richards 1968: 205).

**Current taxonomic status.** *Acteocina canaliculata* (Say, 1826) (Mikkelsen and Mikkelsen 1984 as *A. wetherlli* [sic]).

## Genus *Acteon* Montfort, 1810

### *Acteon biplicatus* (Gabb, 1860)

Figure 1O–P

*Acteonina biplicata* Gabb, 1860a: 93, pl. 2, fig. 13 [non d'Orbigny].

**Type locality.** —New Jersey, USA; stratum: "Lower Green Marls" (Navesink Formation); age: Cretaceous.

**Type material.** Syntypes, ANSP IP19466 (as "type" in Richards 1968: 107), 1 shell, ANSP 19467, 1 shell.

**Current taxonomic status.** *Acteon gabbana* Whitfield, 1892 (Whitfield 1892, Richards and Ramsdell 1962).

### *Acteon costellatus* Conrad, 1833

*Acteon costellatus* Conrad, 1833b: 45.

**Type locality.** Claiborne Bluff, Alabama River, Monroe County, Alabama, USA; stratum: Gosport Sand (uppermost Claiborne Group); age: Eocene.

**Type material.** Lost (Harris 1895a: 13, Palmer 1937: 501, Moore 1962: 50).

**Current taxonomic status.** *Acteon costellatus* Conrad, 1833 is considered a *species inquirenda* (Salvador and Cunha 2016).

### *Acteon cretacea* Gabb, 1862

Figure 1Q–R

*Acteon cretacea* Gabb, 1862: 318.

**Type locality.** Crosswicks, New Jersey, USA; stratum: "Lower Green Marls" (Navesink Formation); age: Cretaceous.

**Type material.** Syntypes, ANSP IP18778, 2 shells (as "types" in Richards 1968: 120).

**Current taxonomic status.** *Acteon cretacea* Gabb, 1862 (Richards and Ramsdell 1962, Richard and Shapiro 1963).

### *Acteon elegans* (Lea, 1833)

Figure 1S

*Monoptygma elegans* Lea, 1833: 203, pl. 6, fig. 217.

**Type locality.** Monroe Co., Claiborne Bluff, Alabama, USA; stratum: Gosport Sand (uppermost Claiborne Group); age: Eocene.

**Type material.** Holotype, ANSP IP6011 (as "lectotype" [error] in Palmer 1937: 499; as "type" in Richards 1968: 128).

**Current taxonomic status.** *Acteon pomilius* Conrad, 1833 (Palmer and Brann 1966).

### *Acteon forbesiana* Whitfield, 1892

Figure 1T–U

*Actaeon forbesiana* Whitfield, 1892: 157, pl. 19, figs 17–22.

**Type locality.** Walnford, New Jersey, USA; stratum: "Lower Green Marls" (Navesink Formation); age: Cretaceous.

**Type material.** Syntypes, ANSP IP18777, 4 shells (as "types" in Richards 1968: 113).

**Current taxonomic status.** *Acteon cretacea* Gabb, 1862 (Weller 1907, Richards and Ramsdell 1962).

### *Acteon gabbana* Whitfield, 1892

Figure 1O–P

*Actaeon gabbana* Whitfield, 1892: 156, pl. 19, figs 23–25.

**Type locality.** Tinton Falls, New Jersey, USA; stratum: "Lower Green Marls" (Navesink Formation); age: Cretaceous.

**Type material.** Syntypes (also of *Acteonina biplicata* Gabb, 1860), ANSP 19466 (as "type" in Richards 1968: 135), 1 shell, ANSP IP19467, 1 shell.

**Current taxonomic status.** *Acteon gabbana* Whitfield, 1892 (Weller 1907, Richards and Ramsdell 1962), nom. nov. pro *Acteonina biplicata* Gabb, 1860 [non d'Orbigny].

### *Acteon glans* Lea, 1846

Figure 2A

*Acteon glans* Lea, 1846: 256, pl. 36, fig. 58.

**Type locality.** Petersburg, Dinwiddie County, Virginia, USA; stratum: Yorktown Formation; age: Late Miocene to Middle Pliocene.

**Type material.** Holotype, ANSP IP1517.

**Current taxonomic status.** *Acteon glans* Lea, 1846.

### *Acteon idoneus* Conrad, 1833

Figure 2B–C

*Acteon idoneus* Conrad, 1833b: 45.

**Type locality.** Claiborne Bluff, Alabama River, Alabama, USA; stratum: Gosport Sand (uppermost Claiborne Group); age: Eocene.

**Type material.** Syntypes, ANSP IP30547 (as "lectotype" in Palmer 1937: 500; as "lectotype?" in Moore 1962: 65), 1 shell, ANSP IP53814, 5 shells (all as "syntypes" in Richards 1968: 144). The designation as lectotype by Palmer (1937) is likely mistaken (Moore 1962; Richards 1968).

**Current taxonomic status.** *Acteon idoneus* Conrad, 1833 (Palmer 1937, Palmer and Brann 1966, Dockery 1977).

### *Acteon lineatus* Lea, 1833

Figure 2D–E

*Acteon lineatus* Lea, 1833: 112, pl. 4, fig. 97.

**Type locality.** Claiborne, Alabama, USA; stratum: uncertain [likely Gosport Sand (uppermost Claiborne Group)]; age: Eocene.

**Type material.** Lectotype, ANSP IP5541, 1 shell (designation by Palmer 1937: 500; as "holotype" in Richards 1968: 152); paralectotypes, ANSP IP5442, 1 shell, ANSP IP5443, 1 shell, ANSP IP5444, 1 shell (each as "paratype" in Richards 1968: 152).

**Current taxonomic status.** *Acteon idoneus* Conrad, 1833 (Palmer 1937, Palmer and Brann 1966).

### *Acteon modicellus* Conrad, 1860

*Actaeon modicellus* Conrad, 1860: 287.

**Type locality.** Tippah County, Mississippi, USA; stratum: "dark gray marl"; age: Late Cretaceous.

**Type material.** Lost (Sohl 1964).

**Current taxonomic status.** *Acteon modicellus* Conrad, 1860, nomen dubium (Sohl 1964).

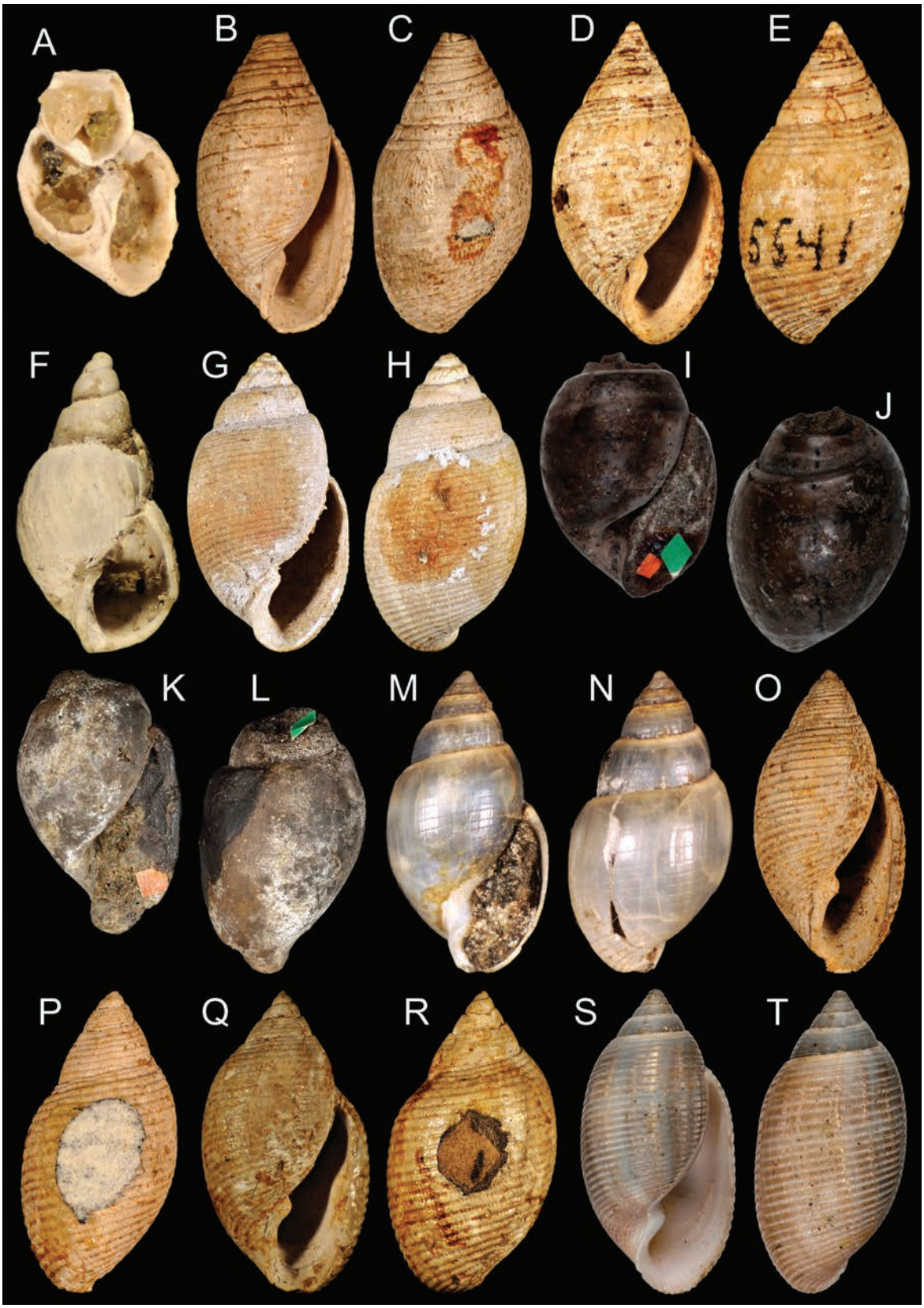


**Figure 2.** Types. **A.** Holotype of *Acteon glans*, H 1.9 mm, ANSP IP1517. **B–C.** Syntype of *Acteon idoneus*, H 11.1 mm, ANSP IP30547. **D–E.** Lectotype of *Acteon lineatus*, H 8.6 mm, ANSP IP5541. **F.** Holotype of *Acteon nitens*, H 4.0 mm, ANSP IP1516. **G–H.** Syntype of *Acteon novellus*, H 12.0 mm, ANSP IP1600. **I–L.** Syntype of *Acteon ovoidea*, H 23 mm, ANSP IP56167. 32–33. Same, H 21.0 mm, ANSP IP56166; also, holotype of *A. subovoides*. **M–N.** Lectotype of *Acteon politus*, H 8.2 mm, ANSP IP4266. **O–P.** Syntype of *Acteon pomilius*, H 10.1 mm, ANSP IP30546. **Q–R.** Syntype of *Acteon punctatus*, H 8.8 mm, ANSP IP5537. **S–T.** Holotype of *Acteon subtornatilis*, H 18.1 mm, ANSP IP3183.

### *Acteon nitens* Lea, 1846
Figure 2F

*Acteon nitens* Lea, 1846: 257, pl. 36, fig. 60.

**Type locality.** Petersburg, Dinwiddie County, Virginia, USA; stratum: Yorktown Formation; age: Late Miocene to Middle Pliocene.

**Type material.** Holotype, ANSP IP1516.

**Current taxonomic status.** *Acteon nitens* Lea, 1846.

### *Acteon novellus* Conrad, 1834
Figure 2G–H

*Acteon novellus* Conrad, 1834: 142.

**Type locality.** Suffolk, Virginia, USA; stratum: uncertain; age: Miocene/Pliocene(?).

**Type material.** Syntypes, ANSP IP1600, 1 shell (as "holotype" in Gardner 1948: 277, pl. 38, figs 24, 26), ANSP IP79768, 2 shells (all as "syntypes" in Moore 1962: 80; as "types" in Richards 1968: 165).

**Current taxonomic status.** —*Acteon novellus* Conrad, 1834 (Moore 1962).

### *Acteon ovoidea* Gabb, 1862
Figure 2I–L

*Actaeon ovoidea* Gabb, 1862: 319.

**Type locality.** New Jersey, USA; stratum: "Lower Green Marls" (Navesink Formation); age: Cretaceous.

**Type material.** Syntypes, ANSP IP56166, 1 shell, ANSP IP56167, 1 shell.

**Current taxonomic status.** *Acteon cretacea* Gabb, 1862 (Weller 1907, Richards and Ramsdell 1962).

### *Acteon politus* (Gabb, 1869)
Figure 2M–N

*Ringinella polita* Gabb, 1869: 174–175, 231, pl. 28, fig. 60.

**Type locality.** Colusa County, California, USA; stratum: Shasta Group (Shasta Formation); age: Cretaceous.

**Type material.** Lectotype, ANSP IP4266 (designation in Stewart 1926: 431, fig. 18); paralectotype, ANSP IP79514, 11 shells (as "types lot" in Richards 1968: 176).

**Current taxonomic status.** *Acteon politus* (Gabb, 1869) (Anderson 1958).

### *Acteon pomilius* Conrad, 1833
Figure 2O–P

*Acteon pomilius* Conrad, 1833b: 45.

**Type locality.** Claiborne Bluff, Alabama River, Monroe County, Alabama, USA; stratum: Gosport Sand (uppermost Claiborne Group); age: Eocene.

**Type material.** Syntypes, ANSP IP30546 (as "lectotype?" in Moore 1962: 88), ANSP IP53815, 5 shells (all as "syntypes" in Richards 1968: 177).

**Current taxonomic status.** *Acteon pomilius* Conrad, 1833 (Palmer and Brann 1966).

### *Acteon punctatus* Lea, 1833
Figure 2Q–R

*Acteon punctatus* Lea, 1833: 111, pl. 4, fig. 96.

**Type locality.** Claiborne Bluff, Alabama River, Monroe County, Alabama, USA; stratum: Gosport Sand (uppermost Claiborne Group); age: Eocene.

**Type material.** Syntypes, ANSP IP5537, 1 shell (as "holotype" in Richards 1968: 179, and in Palmer and Brann 1966: 482), ANSP IP5538, 1 shell, ANSP IP5539, 1 shell, ANSP IP5540, 1 shell (as "paratype" in Richards 1968: 179).

**Current taxonomic status.** Valid as *Acteon pomilius punctatus* Lea, 1833 (Palmer and Brann 1966), but possible synonym of *Acteon pomilius* Conrad, 1833 (Harris 1895a).

### *Acteon subovoides* Whitfield, 1892
Figure 2I–L

*Actaeon subovoides* Whitfield, 1892: 155, pl. 19, figs 14–16.

**Type locality.** New Jersey, USA; stratum: "Lower Green Marls" (Navesink Formation); age: Cretaceous.

**Type material.** Holotype, ANSP IP56166 [also a syntype of *Actaeon ovoidea* Gabb, 1862].

**Current taxonomic status.** *Acteon cretacea* Gabb, 1862 (Weller 1907, Richards and Ramsdell 1962).

### *Acteon subtornatilis* Pilsbry & Johnson, 1917
Figure 2S–T

*Acteon subtornatilis* Pilsbry & Johnson, 1917: 150.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Holotype, ANSP IP3183 (as "type" in Pilsbry 1922: 310, 431, pl. 23, fig. 15; Richards 1968: 193).

**Current taxonomic status.** *Acteon subtornatilis* Pilsbry & Johnson, 1917 (Pilsbry 1922).

**Genus *Nucleopsis* Conrad, 1860**

***Nucleopsis subvaricatus* (Conrad, 1860)**
Figure 3A–B

*Acteonina subvaricata* Conrad, 1860: 294, pl. 47, fig. 22.

**Type material.** Lectotype, ANSP IP30692 (designation in Palmer 1937: 503, pl. 90, fig. 18; see also Moore 1962: 100, Salvador and Cunha 2016: figs 2A–E); paralectotypes, ANSP IP30693, 2 shells (Richards 1968: 193; Salvador and Cunha 2016: figs 2F–G).

**Type locality.** Claiborne, Alabama, USA; stratum: likely Gosport Sand (uppermost Claiborne Group); age: Eocene.

**Current taxonomic status.** *Nucleopsis subvaricatus* (Conrad, 1860) (Salvador and Cunha 2016).

**Genus *Rictaxis* Dall, 1871**

***Rictaxis andersoni* (Conrad, 1847)**
Figure 3C–D

*Actaeon andersoni* Conrad, 1847: 287.

**Type locality.** Vicinity of Vicksburg, Warren County, Mississippi, USA; stratum: Vicksburg Group; age: Oligocene.

**Type material.** Holotype, ANSP IP13411 (Conrad 1848b: 117, pl. 11, fig. 37, Moore 1962: 38, Richards 1968: 100, MacNeil and Dockery 1984: 232, pl. 39, fig. 10).

**Current taxonomic status.** *Rictaxis andersoni* (Conrad, 1847) (MacNeil and Dockery 1984).

***Rictaxis oryza* (Gabb, 1872)**
Figure 3E–F

*Actaeonidea oryza* Gabb, 1872: 245.

**Type locality.** Cibao region, Dominican Republic; stratum: uncertain [likely either Cercado or Gurabo Formations]; age: Miocene/Pliocene.

**Type material.** Syntype, ANSP IP3181 (as "type" in Pilsbry 1922: 310, pl. 23, fig. 12; Richards 1968: 167).

**Remarks.** Type species of genus *Actaeonidea* Gabb, 1872, by monotypy.

**Current taxonomic status.** *Rictaxis oryza* (Gabb, 1872) (Pilsbry 1922, Woodring 1970).

**Genus *Tornatellaea* Conrad, 1860**

***Tornatellaea bella* Conrad, 1860**
Figure 3G–H

*Tornatellaea bella* Conrad, 1860: 294, pl. 47, fig. 23

**Type locality.** Alabama(?), USA; stratum: uncertain [likely Gosport Sand (uppermost Claiborne Group)]; age: Eocene.

**Type material.** Lectotype, ANSP IP30691 (designation by Palmer 1937: 502, pl. 90, fig. 21; as "holotype" in Richards 1968: 177; see also Moore 1962: 41, Salvador and Cunha 2016: fig. S1N–O).

**Remarks.** Type species of genus *Tornatellaea*, by monotypy.

**Current taxonomic status.** *Tornatellaea bella* Conrad, 1860 (Salvador and Cunha 2016).

***Tornatellaea impressa* (Gabb, 1864)**
Figure 3I–J

*Acteon impressus* Gabb, 1864: 142, pl. 21, fig. 106.

**Type locality.** North fork of Cottonwood Creek, Shasta County, California, USA; stratum: uncertain [likely Shasta Formation]; age: Early Cretaceous.

**Type material.** Lectotype, ANSP IP4286 (designation by Stewart 1926: 434, pl. 24, fig. 8); paralectotypes, ANSP IP79476, 6 shells (Richards 1968: 144).

**Current taxonomic status.** *Tornatellaea impressa* (Gabb, 1864) (Anderson 1958).

**Genus *Volvaria* Lamarck, 1801**

***Volvaria reticulata* Johnson, 1899**
Figure 3K–L

*Volvaria reticulata* Johnson, 1899: 71, pl. 1, fig. 1.

**Type locality.** Moseley's Ferry, Brazos River, Burleson Co., Texas, USA; stratum: Stone City Beds (middle Claiborne Group); age: Middle Eocene.

**Type material.** Holotype, ANSP IP6467 (as "type" in Richards 1968: 182).

**Current taxonomic status.** *Volvaria reticulata* Johnson, 1899 (Palmer and Brann 1966).

**Family Acteonellidae Gill, 1871 †**
**Genus *Acteonella* d'Orbigny, 1843**

***Acteonella oviformis* Gabb, 1869**
Figure 3M–N

*Acteonella oviformis* Gabb, 1869: 173, 232, pl. 28, fig. 58.

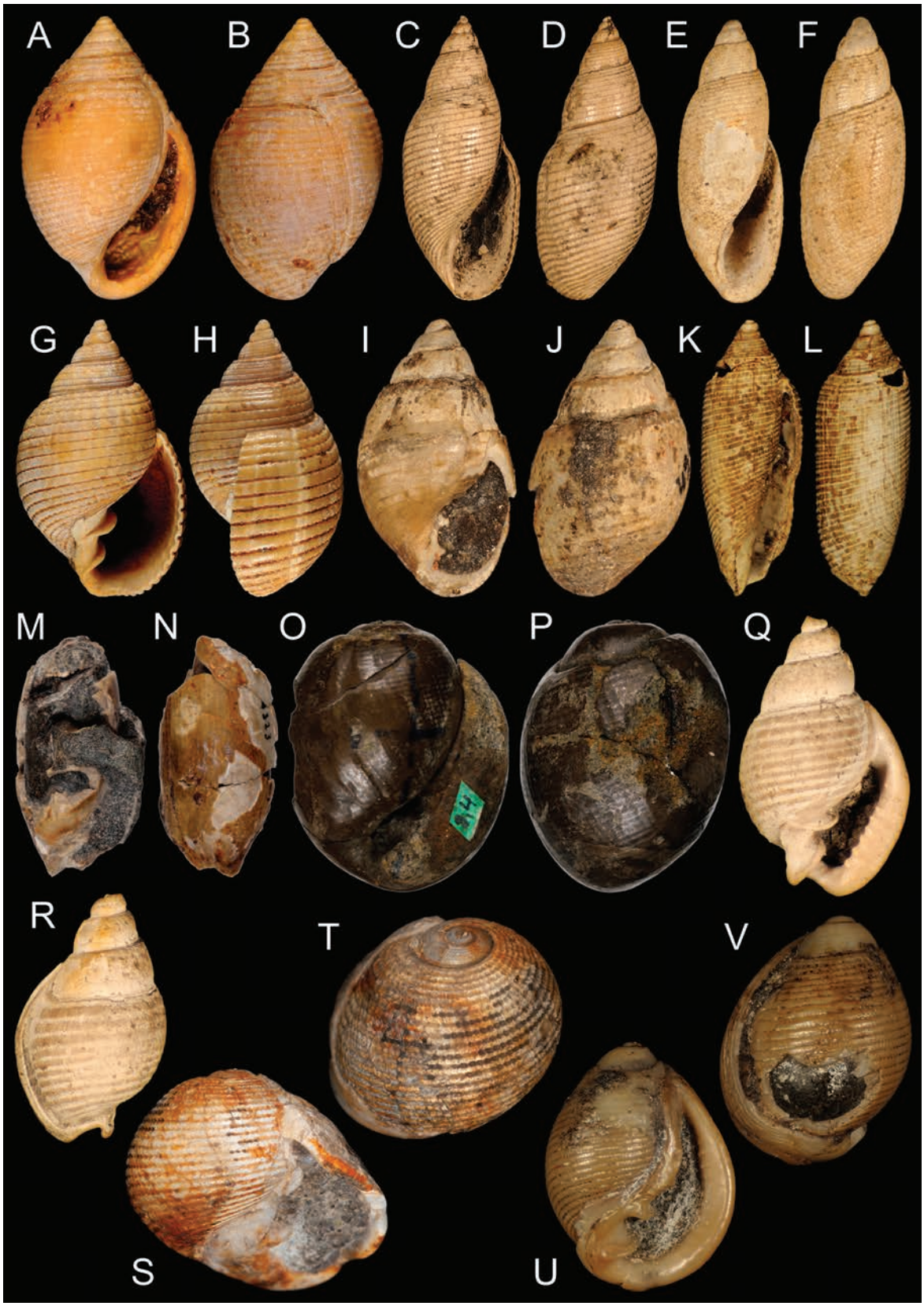


**Figure 3.** Types. **A–B.** Lectotype of *Nucleopsis subvaricatus*, H 10.8 mm, ANSP IP30692. **C–D.** Holotype of *Rictaxis andersoni*, H 10.5 mm, ANSP IP13411. **E–F.** Syntype of *Rictaxis oryza*, H 6.8 mm, ANSP IP3181. **G–H.** Lectotype of *Tornatellaea bella*, H 12.5 mm, ANSP IP30691. **I–J.** Lectotype of *Tornatellaea impressa*, H 10.7 mm, ANSP IP4286. **K–L.** Holotype of *Volvaria reticulata*, H 7.2 mm, ANSP IP6467. **M–N.** Holotype of *Acteonella oviformis*, H 42.1 mm, ANSP IP4323. **O–P.** Syntype of *Avellana bullata*, H 25.4 mm, ANSP IP289. **Q–R.** Syntype of *Avellana costata*, H 4.5 mm, ANSP IP691. **S–T.** Lectotype of *Biplica obliqua*, H 9.9 mm, ANSP IP4263. **U–V.** Lectotype of *Biplica mathewsonii*, H 11.6 mm, ANSP IP4262.

**Type locality.** Cottonwood Creek, Shasta County, California, USA; stratum: uncertain [either Shasta or Chico Formations]; age: Cretaceous.

**Type material.** Holotype, ANSP IP4323 (Stewart 1926: 432, pl. 21, fig. 13; Richards 1968: 168).

**Current taxonomic status.** *Acteonella oviformis* Gabb, 1869 (Anderson 1958).

**Superfamily Ringiculoidea Philippi, 1853**
**Family Ringiculidae Philippi, 1853**
**Genus *Avellana* d'Orbigny, 1843**

***Avellana bullata* (Morton, 1834)**
Figure 3O–P

*Tornitella*? *bullata* Morton, 1834: 48, pl. 5, fig. 3.

**Type locality.** Merchantville, New Jersey, USA; stratum: uncertain [likely Navesink Formation]; age: Cretaceous.

**Type material.** Syntypes, ANSP IP289, 1 shell, ANSP IP19702, 1 shell (as "type" in Whitfield 1892: 163, pl. 20, figs 3–4; Richards 1968: 108; Richards and Ramsdell 1962: 93).

**Current taxonomic status.** *Avellana bullata* (Morton, 1834) (Richards and Ramsdell 1962).

***Avellana costata* (Johnson, 1898)**
Figure 3Q–R

*Cinulia costata* Johnson, 1897: 264 [nomen nudum].
*Cinulia costata* Johnson, 1898: 462, fig. 1.

**Type locality.** Mount Laurel well, New Jersey, USA; stratum: uncertain; age: Cretaceous.

**Type material.** Syntypes, ANSP IP691, 1 shell, ANSP IP79408, 2 shells (as "type" in Richards and Ramsdell 1962: 94).

**Current taxonomic status.** *Avellana costata* (Johnson, 1898) (Richards and Ramsdell 1962).

**Genus *Biplica* Popenoe, 1957**

***Biplica obliqua* (Gabb, 1864)**
Figure 3S–T

*Cinulia obliqua* Gabb, 1864: 111, pl. 19, fig. 64.

**Type locality.** Tuscan Springs, Tehama Co., California, USA; stratum: uncertain; age: Late Cretaceous.

**Type material.** Lectotype, ANSP IP4263 (designation by Stewart 1926: 436, pl. 24, fig. 14; see also Richards 1968: 166; Popenoe 1957: 435).

**Current taxonomic status.** *Biplica obliqua* (Gabb, 1864) (Popenoe 1957).

***Biplica mathewsonii* (Gabb, 1864)**
Figure 3U–V

*Cinulia mathewsonii* Gabb, 1864: 111, 225, pl. 19, fig. 65.

**Type locality.** Bull's Head Point, Martinez, California, USA; stratum: uncertain [likely Chico Formation]; age: Cretaceous.

**Type material.** Lectotype, ANSP IP4262 (designation by Stewart 1926: 437, pl. 24, fig. 11).

**Remarks.** Popenoe (1957: 434) points out that Gabb's material from Bull's Head Point could represent a non-Cretaceous locality/horizon or be Paleogene material mixed with Gabb's Cretaceous specimens.

**Current taxonomic status.** *Biplica mathewsonii* (Gabb, 1864) (Popenoe 1957).

**Genus *Cinulia* Gray, 1840**

***Cinulia naticoides* (Gabb, 1860)**
Figure 4A–B

*Actaenia naticoides* Gabb, 1860c: 299, pl. 48, fig. 2.

**Type locality.** Mullica Hill, New Jersey, USA; stratum: uncertain [likely Navesink Formation]; age: Cretaceous.

**Type material.** Syntypes, ANSP IP18784, 2 shells (as "type" in Richards 1968: 164).

**Current taxonomic status.** *Cinulia naticoides* (Gabb, 1860) (Clark 1916, Richards and Ramsdell 1962).

***Cinulia rectilabrum* Gabb, 1869**
Figure 4C–D

*Cinulia rectilabrum* Gabb, 1869: 264, pl. 35, fig. 10.

**Type locality.** Arivechi, Eastern Sonora, Mexico; stratum: uncertain [either Cañada de Tarachi or El Potrero Grande Units]; age: Late Cretaceous.

**Type material.** Syntype(?), ANSP IP4753 (as "type?" in Richards 1968: 181).

**Current taxonomic status.** *Cinulia rectilabrum* Gabb, 1869 (Stanton 1947).

**Genus *Ringicula* Deshayes, 1838**

***Ringicula hypograpta* Brown & Pilsbry, 1912**
Figure 4E–F

*Ringicula hypograpta* Brown & Pilsbry, 1912: 505, text fig. 2.

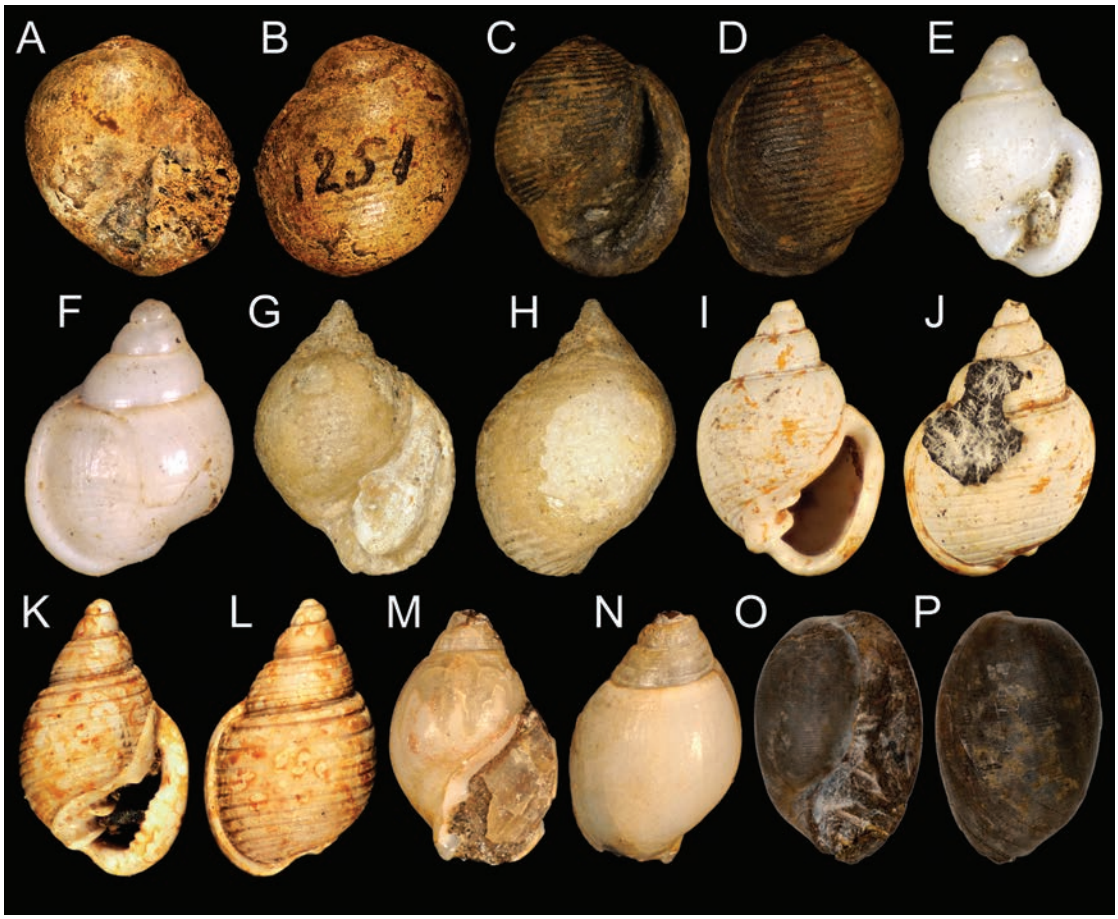


**Figure 4.** Types. **A–B.** Syntype of *Cinulia naticoides*, H 9.0 mm, ANSP IP18784. **C–D.** Syntype(?) of *Cinulia rectilabrum*, H 9.9 mm, ANSP IP4753. **E–F.** Holotype of *Ringicula hypograpta*, H 2.0 mm, ANSP IP3841. **G–H.** Syntype of *Ringicula lata*, H 15.4 mm, ANSP IP30695. **I–J.** Lectotype of *Ringicula mississippiensis*, H 3.7 mm, ANSP IP13414. **K–L.** Syntype of *Ringicula trapaquara*, H 2.3 mm, ANSP IP6468. **M–N**. Lectotype of *"Ringicula" varia*, H 7.1 mm, ANSP IP4264. **O–P.** Holotype of *Roxania hornii* new comb., H 20.1 mm, ANSP IP4232.

**Type locality.** Scott's locality 3, excavation of the lower locks at Gatun, Canal Zone, Panama; stratum: Gatun Formation; age: Late Miocene.

**Type material.** Holotype, ANSP IP3841 (as "type" in Richards 1968: 143).

**Current taxonomic status.** *Ringicula* (*Ringiculella*) *semistriata* d'Orbigny, 1841 (Woodring 1970).

### *Ringicula lata* (Conrad, 1865)

Figure 4G–H

*Actaeon* (*Nucleopsis*) *latus* Conrad, 1865a: 34.

**Type locality.** Alabama(?), USA; stratum: uncertain; age: Early Eocene(?).

**Type material.** Syntype, ANSP IP30695, 1 shell (as "holotype" in Palmer 1937: 502, Richards 1968: 150; as "probable holotype" in Moore 1962: 69; see also Salvador and Cunha 2016: fig. S1K–M).

**Current taxonomic status.** *Ringicula lata* (Conrad, 1865) (Salvador and Cunha 2016).

### *Ringicula mississippiensis* Conrad, 1848

Figure 4I–J

*Ringicula mississippiensis* Conrad, 1848a: 287.

**Type locality.** Dr. Smith's plantation, 6 miles northeast of Vicksburg, Warren County, Mississippi, USA; stratum: Vicksburg Group; age: Oligocene.

**Type material.** Lectotype, ANSP IP13414 (designation by Moore 1962: 77; as "lectotype" in MacNeil and Dockery 1984: 235, Moore 1962: 77; as "holotype" in Richards 1968: 161); paralectotypes, ANSP IP13415, 8 shells (as "paratype" in Richards 1968: 161; MacNeil and Dockery 1984: 235; see also Conrad 1848b: 117, pl. 11, fig. 36).

**Current taxonomic status.** *Ringicula* (*Ringiculella*) *mississippiensis* Conrad, 1848 (MacNeil and Dockery 1984).

***Ringicula trapaquara* Harris, 1895**
Figure 4K–L

*Ringicula trapaquara* Harris, 1895a: 76, pl. 8, fig. 7.

**Type locality.** Moseleys Ferry, Brazos River, Texas; stratum: lower Claiborne Formation; age: Eocene.

**Type material.** Syntypes, ANSP IP6468, 8 shells (as *R. trapaquaria* [sic], "paratype" in Richards 1968: 198).

**Current taxonomic status.** *Ringicula trapaquara* Harris, 1895 (Palmer 1937).

***Ringicula varia* Gabb, 1864**
Figure 4M–N

*Ringicula varia* Gabb, 1864: 112, pl. 29, fig. 222a–b.

**Type locality.** Cow Creek, Shasta County, California, USA; stratum: Chico Formation; age: Cretaceous.

**Type material.** Lectotype, ANSP IP4264 (designation by Stewart 1926: 435, pl. 24, fig. 3; see also Richards 1968: 202).

**Current taxonomic status.** Uncertain, as "*Ringicula*" *varia* Gabb, 1864 (Stewart 1930).

**Order Cephalaspidea P. Fischer, 1883**
**Superfamily Bulloidea Gray, 1827**
**Family Bullidae Gray, 1827**
**Genus *Roxania* Leach, 1847**

***Roxania hornii* (Gabb, 1864), new comb.**
Figure 4O–P

*Bulla hornii* Gabb, 1864: 143, pl. 29, fig. 235.

**Type locality.** Kern County, California, USA; stratum: Tejon Formation; age: Eocene.

**Type material.** Holotype, ANSP IP4232 (Stewart 1926: 439, pl. 29, fig. 9; Richards 1968: 142).

**New taxonomic status.** *Roxania hornii* (Gabb, 1864) new comb. This species was placed in the genus *Abderospira* Dall, 1898 (e.g., Stewart 1926, Keen and Bentson 1944), which is now considered a synonym of *Roxania* (Valdés 2008).

**Genus *Bulla* Linnaeus, 1758**

***Bulla macrostoma* Gabb, 1860**
Figure 5A–B

*Bulla macrostoma* Gabb, 1860c: 301, pl. 48, fig. 15.

**Type locality.** Prairie Bluff, Alabama, USA; stratum: uncertain ("white limestone"); age: Cretaceous.

**Type material.** Holotype, ANSP IP30727 (as "type" in Richards 1968: 155).

**Current taxonomic status.** *Bulla macrostoma* Gabb, 1860 (Stephenson 1914).

**Genus *Bullopsis* Conrad, 1858**

***Bullopsis cretacea* Conrad, 1858**
Figure 5C–D

*Bullopsis cretacea* Conrad, 1858: 334.

**Type locality.** Owl Creek, near Ripley, Tippah County, Mississippi, USA; stratum: "dark gray sandy marl" (Owl Creek Formation); age: Cretaceous.

**Type material.** Holotype, IPANSP 18924 (Conrad 1860: pl. 46, fig. 27; as "type" in Richards 1968: 120).

**Current taxonomic status.** *Bullopsis cretacea* Conrad, 1858 (Stephenson 1955, Sohl 1964).

**Family Retusidae Thiele, 1925**
**Genus *Retusa* Brown, 1827**

***Retusa biforis* Pilsbry & Johnson, 1917**
Figure 5E–F

*Retusa biforis* Pilsbry & Johnson, 1917: 151

**Type locality.** Dominican Republic. Type stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Holotype, ANSP IP3192 (as "type" in Richards 1968: 106).

**Current taxonomic status.** *Retusa biforis* Pilsbry & Johnson, 1917 (Pilsbry 1922).

***Retusa sulcata fossilis* Pilsbry, 1922**

*Retusa sulcata* var. *fossilis* Pilsbry 1922: 311.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Holotype and paratypes, ANSP IP3186 (3 shells; lost).

**Current taxonomic status.** Uncertain, likely valid as *Retusa sulcata fossilis* Pilsbry, 1922.

***Retusa galba* (Conrad, 1833)**
Figure 5G–H

*Volvaria galba* Conrad, 1833a: 34, pl. 18, fig. 2.

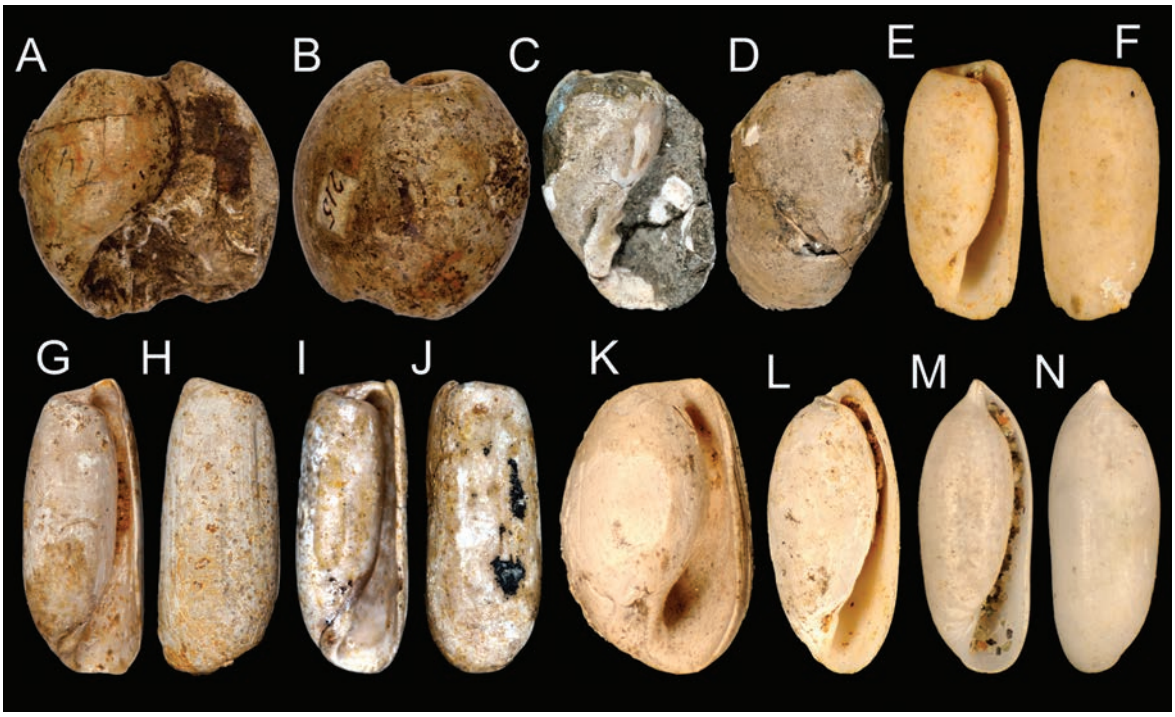


**Figure 5.** Types. **A–B.** Holotype of *Bulla macrostoma*, H 26.2 mm, ANSP IP30727. **C–D.** Holotype of *Bullopsis cretacea*, H 18.0 mm, ANSP IP18924. **E–F.** Holotype of *Retusa biforis*, H 2.9 mm, ANSP IP3192. **G.** Paralectotype of *Retusa galba*, H 14.3 mm, ANSP IP53816. **H.** Lectotype of *Retusa galba*, H 15.0 mm, ANSP IP30548. **I–J.** Lectotype of *Retusa sthillairii*, H 14.0 mm, ANSP IP5486. **K.** Syntype of *Retusa subspissa*, H 4.8 mm, ANSP IP30641. **L.** Holotype of *Volvulella cylichnoides*, H 4.2 mm, ANSP IP3177. **M–N.** Syntype of *Volvulella cylindrica*, H 4.7 mm, ANSP IP3179.

**Type locality.** Claiborne Bluff, Alabama River, Monroe County, Alabama, USA; stratum: Gosport Sand (uppermost Claiborne Group); age: Middle Eocene.

**Type material.** Lectotype, ANSP IP30548 (designation by Palmer and Brann 1966: 878; as "lectotype?" in Moore 1962: 62); paralectotypes, ANSP IP53816, 3 shells, ANSP IP53817, 1 shell, ANSP IP30549, 8 shells (all as "probable syntypes" in Richards 1968: 136).

**Current taxonomic status.** *Retusa* (*Cylichnina*) *galba* (Conrad, 1833) (Palmer and Brann 1966).

### *Retusa sthillairii* (Lea, 1833)

Figure 5I–J

*Bulla St. Hillairii* Lea, 1833: 98, pl. 4, fig. 78.

**Type locality.** Monroe Co., Claiborne Bluff, Alabama, USA; stratum: Gosport Sand (uppermost Claiborne Group); age: Eocene.

**Type material.** Lectotype, ANSP IP5486, 1 shell (designation by Palmer 1937: 481; as "holotype" in Palmer and Brann 1966: 878); paralectotypes, ANSP IP5487, 1 shell, ANSP IP5488, 1 shell, ANSP IP5489, 1 shell, ANSP IP5490, 1 shell, ANSP IP5491, 1 shell, ANSP IP5487, 1 shell (all [including ANSP IP5486] as "types" in Richards 1968: 191).

**Current taxonomic status.** *Retusa galba* (Conrad, 1833) (Palmer and Brann 1966).

### *Retusa subspissa* (Conrad, 1846)

Figure 5K

*Bulla subspissa* Conrad, 1846: 20, pl. 1, fig. 29.

**Type locality.** Calvert Cliffs, Maryland, USA; stratum: Calvert Formation; age: Miocene.

**Type material.** Syntype, ANSP IP30641, 1 shell (as "probable holotype" in Moore 1962: 100; as "type?" in Richards 1968: 193).

**Current taxonomic status.** *Retusa subspissa* (Conrad, 1846), but possible synonym of *Retusa conulus* (Deshayes, 1824) (Martin 1904).

## Family Rhizoridae Dell, 1952
## Genus *Volvulella* Newton, 1891

### *Volvulella cylichnoides* (Pilsbry & Johnson, 1917)

Figure 5L

*Volvula cylichnoides* Pilsbry & Johnson, 1917: 151.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Holotype, ANSP IP3177 (as "type" in Richards 1968: 121); paratype, ANSP IP79027, 1 shell.

**Current taxonomic status.** *Volvulella cylichnoides* (Pilsbry & Johnson, 1917), but could be a synonym of *Retusa yaquensis* Maury, 1917 (Pilsbry 1922).

### *Volvulella cylindrica* (Gabb, 1872)

Figure 5M–N

*Volvula cylindrica* Gabb, 1872: 246 [non Carpenter, 1865; non E.A. Smith, 1871].

**Type locality.** Dominican Republic; type stratum: uncertain [likely either Cercado or Gurabo Formations]; age: Miocene/Pliocene.

**Type material.** Syntype, ANSP IP3179, 1 shell (as "type" in Richards 1968: 121).

**Current taxonomic status.** *Volvulella persimilis* (Mörch, 1875) (Dall 1889, Pilsbry 1922).

### *Volvulella micratracta* Brown & Pilsbry, 1912

Figure 6A

*Volvulella micratracta* Brown & Pilsbry, 1912: 504, text fig. 1.

**Type locality.** Scott's locality 3, excavation of the lower locks at Gatun, Canal Zone, Panama; stratum: Gatun Formation; age: Late Miocene.

**Type material.** Holotype, ANSP IP3842 (as "type" in Richards 1968: 158).

**Current taxonomic status.** *Volvulella micratracta* Brown & Pilsbry, 1912 (Woodring 1970).

### *Volvulella minutissima* (Gabb, 1860), new comb.

Figure 6B

*Volvula minutissima* Gabb, 1860b: 386–387, pl. 67, fig. 52.

**Type locality.** Caldwell County, Texas, USA; stratum: uncertain; age: Eocene.

**Type material.** Syntype, ANSP IP13267, 1 shell (as "type" in Richards 1968: 159).

**New taxonomic status.** *Volvulella minutissima* (Gabb, 1860), new comb. Harris (1895b) considered the species valid. The genus *Volvulella* Newton, 1891 is a replacement name for *Volvula* A. Adams, 1850 non Gistel, 1848 (Valdés 2008).

### *Volvulella ornata* (Pilsbry & Johnson, 1917)

Figure 6C–D

*Volvula ornata* Pilsbry & Johnson, 1917: 151.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Holotype, ANSP IP3178 (as "type" in Richards 1968: 167); paratypes, ANSP IP81665, 6 shells.

**Remarks.** Pilsbry and Johnson (1917) did not clearly indicate which one of their specimens is the holotype, merely indicating the presence of a "Type". Judging by the conventions used elsewhere in their paper, we here consider the holotype to be the specimen measured by these authors. Besides the holotype, Pilsbry and Johnson (1917) mentioned seven specimens, one of which is presently missing.

**Current taxonomic status.** *Volvulella ornata* (Pilsbry & Johnson, 1917) (Pilsbry 1922).

### *Volvulella parallela* (Pilsbry & Johnson, 1917)

Figure 6E

*Volvula parallela* Pilsbry & Johnson, 1917: 151.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Holotype, ANSP IP3188 (as "type" in Richards 1968: 169); paratypes, ANSP IP79026, 2 shells.

**Current taxonomic status.** *Volvulella cylindrica parallela* (Pilsbry & Johnson, 1917) (Woodring 1970).

### *Volvulella tritica* (Olsson & Harbison, 1953)

Figure 6F

*Volvula tritica* Olsson & Harbison, 1953: 163, pl. 25, figs 3–3a.

**Type locality.** St. Petersburg, Pinellas County, Florida, USA; stratum: North St. Petersburg Beds; age: Plio-Pleistocene.

**Type material.** Holotype, ANSP IP19104; paratypes, ANSP IP79270, 2 shells (all as "types" in Richards 1968: 199).

**Current taxonomic status.** *Volvulella tritica* (Olsson & Harbison, 1953) (Portell and Kittle 2010).

## Superfamily Haminoeoidea Pilsbry, 1895
## Family Haminoeidae Pilsbry, 1895
## Genus *Atys* Montfort, 1810

### *Atys cinctorii* Pilsbry & Johnson, 1917

Figure 6G

*Atys cinctorii* Pilsbry & Johnson, 1917: 152.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

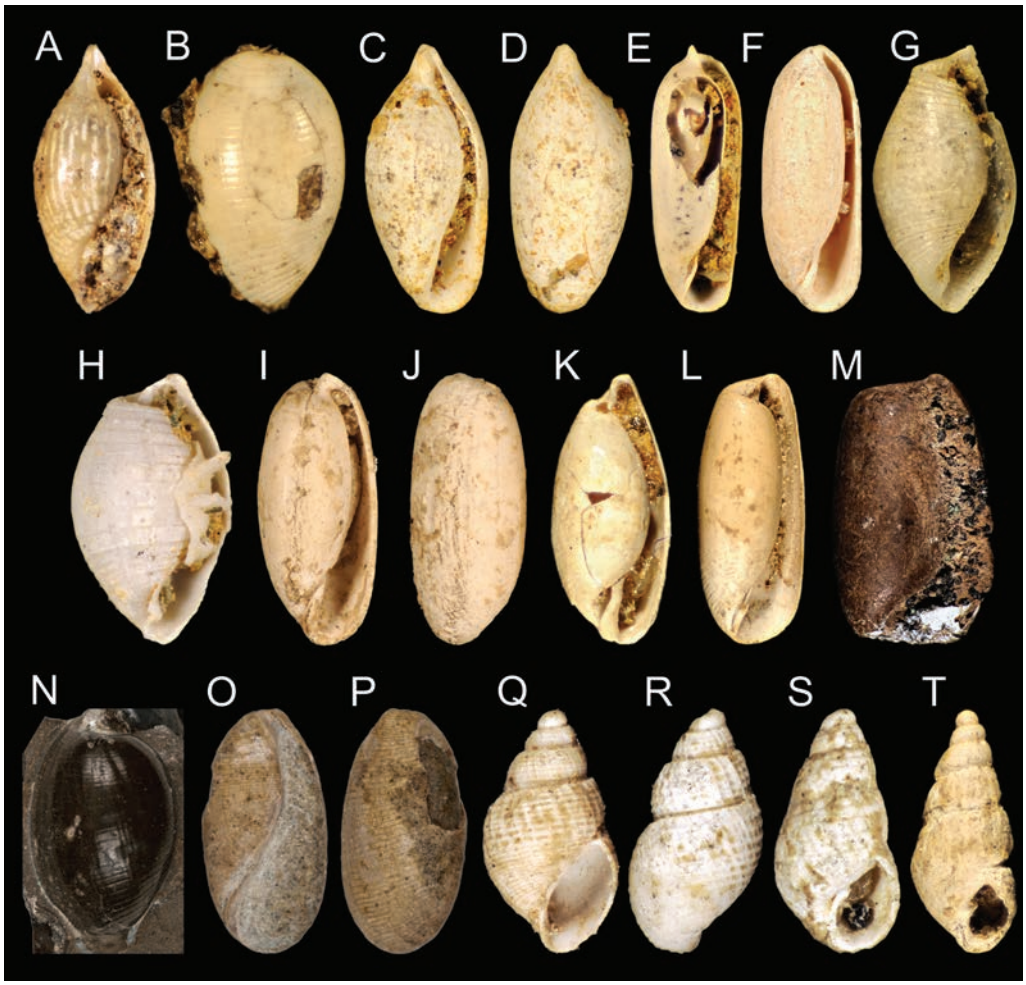


**Figure 6.** Types. **A.** Holotype of *Volvulella micratracta*, H 1.5 mm, ANSP IP3842. **B.** Syntype of *Volvulella minutissima* new comb., H 2.3 mm, ANSP IP13267. **C–D.** Holotype of *Volvulella ornata*, H 3.2 mm, ANSP IP3178. **E.** Holotype of *Volvulella parallela*, H 2.1 mm, ANSP IP3188. **F.** Holotype of *Volvulella tritica*, H 4.1 mm, ANSP IP19104. **G.** Holotype of *Atys cinctorii*, H 2.6 mm, ANSP IP3185. **H.** Syntype of *Atys sulculorum*, H 2.5 mm, ANSP IP3317. **I–J.** Syntype of *Cylichna cylindrus*, H 4.7 mm, ANSP IP1554. **K.** Syntype of *Cylichna dekayi*, H 5.0 mm, ANSP IP6008. **L.** Syntype of *Cylichna kellogii*, H 5.7 mm, ANSP IP13266. **M.** Holotype of *Cylichna recta*, H 8.0 mm, ANSP IP18782. **N.** Syntype of *Bulla occidentalis*, H 10.0 mm, ANSP IP17139. **O–P.** Lectotype of *Cylichna costata*, H 16.0 mm, ANSP IP4338. **Q–R.** Syntype of *Acteon sculptus*, H 1.9 mm, ANSP IP1515. **S.** Syntype of *Acteon milium*, H 2.1 mm, ANSP IP1520. **T**. Holotype of *Acteon angulatus*, H 2.4 mm, ANSP IP1521.

**Type material.** Holotype, ANSP IP3185 (as "type" in Richards 1968: 113).

**Current taxonomic status.** *Atys cinctorii* Pilsbry & Johnson, 1917 (Pilsbry 1922).

### *Atys sulculorum* Pilsbry & Johnson, 1917

Figure 6H

*Atys sulculorum* Pilsbry & Johnson, 1917: 152.

**Type locality.** Dominican Republic; stratum: "Santo Domingan Beds" (either Cercado or Gurabo Formations); age: Miocene/Pliocene.

**Type material.** Syntypes, ANSP IP3317, 2 shells (as "type" in Richards 1968: 194).

**Current taxonomic status.** *Atys doliolum* (Maury, 1917) (Woodring 1970).

### Superfamily Cylichnoidea H. Adams & A. Adams, 1854
### Family Cylichnidae H. Adams & A. Adams, 1854
### Genus *Cylichna* Lovén, 1846

### *Cylichna cylindrus* (Lea, 1846)

Figure 6I–J

*Bulla cylindrus* Lea, 1846: 250–251, pl. 35, fig. 43.

**Type locality.** Petersburg, Dinwiddie County, Virginia, USA; stratum: Yorktown Formation; age: Late Miocene to Middle Pliocene.

**Type material.** Syntypes, ANSP IP1554, 1 shell (Gardner 1948: 279, pl. 38, fig. 27; as "type" in Richards 1968: 122), ANSP IP79382, 1 shell (Gardner 1948: 279, pl. 38, fig. 28).

**Current taxonomic status.** *Cylichna cylindrus* (Lea, 1846) (Gardner 1948).

### *Cylichna dekayi* (Lea, 1833)

Figure 6K

*Bulla dekayi* Lea, 1833: 200, pl. 6, 215.

**Type locality.** Claiborne, Alabama, USA; stratum: Claiborne Group; age: Eocene.

**Type material.** Syntypes, ANSP IP6007, 1 shell (as "holotype" in Richards 1968: 123), ANSP IP6008, 1 shell (as "paratype" in Richards 1968: 123).

**Current taxonomic status.** *Cylichna* (*Mnestia*) *dekayi* (Lea, 1833) (Glibert 1962).

### *Cylichna kellogii* (Gabb, 1860)

Figure 6L

*Bulla kellogii* Gabb, 1860b: 386, pl. 67, fig. 50.

**Type locality.** Texas, USA; stratum: uncertain; age: Eocene.

**Type material.** Syntype, ANSP IP13266, 1 shell (as "type" in Richards 1968: 148).

**Current taxonomic status.** *Cylichna* (*Acrotrema*) *kellogii* (Gabb, 1860) (Gardner 1945).

### *Cylichna recta* (Gabb, 1860)

Figure 6M

*Bulla recta* Gabb, 1860c: 303, pl. 48, fig. 16.

**Type locality.** Burlington County, New Jersey, USA; stratum: "Lower Green Marls" (Navesink Formation); age: Cretaceous.

**Type material.** Holotype, ANSP IP18782 (as "type" in Richards 1968: 181).

**Current taxonomic status.** *Cylichna recta* (Gabb, 1860) (Wade 1926, Richards and Ramsdell 1962).

## Superfamily Philinoidea Gray, 1850
## Family Philinidae Gray, 1850
## Genus *Philine* Ascanius, 1772

### *Philine gabbi* Cossmann, 1895

*Philine* (*Megistostoma*) *gabbi* Cossmann, 1895: 127.

**Type locality.** Martinez, California, USA; stratum: uncertain; age: Eocene.

**Type material.** Syntypes ANSP IP4216 (of *M. striatum* Gabb, 1864), 2 shells (lost).

**Remarks.** This was a new name for *Megistostoma striatum* Gabb, 1864, when transferred to the genus *Philine* [non *striata* Deshayes, 1824]. However, the name *P. gabbiana* (Stoliczka, 1868) has precedence over it (see below).

**Current taxonomic status.** *Philine* (*Megistostoma*) *gabbiana* (Stoliczka, 1868) (Keen and Bentson 1944).

### *Philine gabbiana* Stoliczka, 1868

*Bullaea Gabbiana* Stoliczka, 1868: 434.

**Type locality.** Martinez, California, USA; stratum: uncertain; age: Eocene.

**Type material.** Syntypes ANSP IP4216 (of *M. striatum* Gabb, 1864), 2 shells (lost).

**Remarks.** This was coined as a new name for *Megistostoma striatum* Gabb, 1864, when transferred to the genus *Bullaea* Lamarck, 1801 [non *striata* Deshayes]. *Bullaea* is presently considered a synonym of *Philine*.

**Current taxonomic status.** *Philine* (*Megistostoma*) *gabbiana* (Stoliczka, 1868) (Keen and Bentson 1944).

### *Megistostoma striatum* Gabb, 1864

*Megistostoma striata* [sic] Gabb, 1864: 144, 229, pl. 21, fig. 108.

**Type locality.** Martinez, California, USA; stratum: uncertain; age: Eocene.

**Type material.** Syntypes, ANSP IP4216 (lost), 2 shells (Stewart 1926: 442: pl. 26, fig. 2; as "holotype" and "paratype" in Keen and Bentson 1944: 170; as "holotypes" in Richards 1968: 191).

**Remarks.** Type species of *Megistostoma* Gabb, 1864. The correct epithet is *striatum* (not *striata*), since the ending -*stoma* is neuter.

**Current taxonomic status.** *Philine* (*Megistostoma*) *gabbiana* (Stoliczka, 1868) (Keen and Bentson 1944), new name for *M. striatum* Gabb, 1864 (see above).

## Family Scaphandridae G.O. Sars, 1878
## Genus *Ellipsoscapha* Stephenson, 1941

### *Ellipsoscapha mortoni* (Forbes, 1845)

*Bulla mortoni* Forbes, 1845: 63, fig. A.

**Type locality.** New Jersey, USA; stratum: uncertain [likely Navesink Formation]; age: Cretaceous.

**Type material.** Holotype not found; could be in Charles Lyell's fossil collections (John Sime, pers. comm.), presently in the Natural History Museum (London, UK) and Oxford University Museum of Natural History (Oxford, UK).

**Current taxonomic status.** *Ellipsoscapha mortoni* (Forbes, 1845) (Richards and Ramsdell 1962, Sohl 1964).

***Ellipsoscapha occidentalis* (Meek & Hayden, 1856)**
Figure 6N

*Bulla occidentalis* Meek & Hayden, 1856 (non A. Adams, 1850): 69.

**Type locality.** Yellowstone River (150 miles above its mouth), near Glendive, Montana, USA; stratum: Pierre Shale; age: Late Cretaceous.

**Type material.** Syntypes, ANSP IP17139, 2 shells.

**Current taxonomic status.** *Ellipsoscapha occidentalis* (Meek & Hayden, 1856) (Sohl 1967). Substitution of the junior primary homonym is not mandatory if the conditions of Article 23.9.5 ICZN (1999) are met, but a request for a ruling of the Commission under its plenary powers to validate the junior homonymous name would be necessary.

**Genus *Scaphander* Montfort, 1810**

***Scaphander costatus* (Gabb, 1864)**
Figure 6O–P

*Cylichna costata* Gabb, 1864: 143, pl. 2, fig. 107.

**Type locality.** Martinez, Contra Costa County, California, USA; stratum: Tejon Formation s. l.; age: Eocene.

**Type material.** Lectotype, ANSP IP4338 (designation by Stewart 1926: 437, pl. 27, fig. 3; see also Richards 1968: 118); paralectotypes, ANSP IP79477, 8 shells.

**Remarks.** Type species of subgenus *Mirascapha* Stewart, 1927.

**Current taxonomic status.** *Scaphander* (*Mirascapha*) *costatus* (Gabb, 1864) (Squires 1984).

Species transferred to other gastropod groups

Here are listed the species (with type material in the ANSP collection) that were originally classified in "Architectibranchia" and Cephalaspidea, but that actually do not belong to them. Some of them have already undergone taxonomical revision and are listed concisely in Table 1, while others are reclassified below.

**Panpulmonata**
**Superfamily Pyramidelloidea Gray, 1840**
**Family Pyramidellidae Gray, 1840**
**Genus *Chrysallida* Carpenter, 1856**

***Chrysallida sculpta* (Lea, 1846), new comb.**
Figure 6Q–R

*Acteon sculptus* Lea, 1846: 257, pl. 36, fig. 59.

**Type locality.** Petersburg, Dinwiddie County, Virginia, USA; stratum: Yorktown Formation; age: Late Miocene to Middle Pliocene.

**Type material.** Syntypes, ANSP IP1515, 2 shells (as "types" in Richards 1968: 186).

**Taxonomical reassessment.** This species is better classified in the genus *Chrysallida* due to the following conchological features (Robba 2013): a weak or absent columellar fold, teleoconch sculpture consisting of collabral ribs and spiral cords of similar strength. Most species of *Chrysallida* are strongly sculptured, as the present specimens, with the ribs forming nodes where crossing the spiral cords and tending to fade away near the base.

**Genus *Odostomia* Fleming, 1813**

***Odostomia milium* (Lea, 1846), new comb.**
Figure 6S

*Acteon milium* Lea, 1846: 257, pl. 36, fig. 61.

**Type locality.** Petersburg, Dinwiddie County, Virginia, USA. Type stratum: Yorktown Formation. Age: Late Miocene to Middle Pliocene.

**Type material.** Syntypes, ANSP IP1520, 1 shell, ANSP IP79385, 1 shell (lost, not found in the vial).

**Taxonomical reassessment.** This species is better classified in the genus *Odostomia* due to the following conchological features (Robba 2013): verall ovate-conical shell with moderately convex whorls, suture with a blunt subsutural margin, slit-like umbilicus, and prominent columellar fold.

**Genus *Pyrgulina* A. Adams, 1863**

***Pyrgulina angulata* (Lea, 1846), new comb.**
Figure 6T

*Acteon angulatus* Lea, 1846: 256, pl. 36, fig. 57.

**Type locality.** Petersburg, Dinwiddie County, Virginia, USA. Type stratum: Yorktown Formation. Age: Late Miocene to Middle Pliocene.

**Type material.** Holotype, ANSP IP1521.

**Taxonomical reassessment.** This species is better classified in the genus *Pyrgulina* due to the following conchological features (Robba 2013): elongated (somewhat

**Table 1.** Species (with type material in the ANSP collection) that were originally classified in Architectibranchia and Cephalaspidea, but that after revision were reclassified in other groups. The species are arranged in alphabetical order of the specific epithets, with information on their original description, type specimens in the ANSP collection, current taxonomic status, and references of such status. Abbreviations: Hol = holotype; Lec = lectotype; Pal = Paralectotype(s); Par = paratype(s); Syn = syntype(s).

| Original description | Type material (ANSP) | Current taxonomic status | Family | Reference(s) |
|---|---|---|---|---|
| *calafia*, "*Acteonina*" Stewart, 1926: 432, pl. 21, fig. 12 | 4287 (Hol) | *Paosia calafia* (Stewart, 1926) | Pseudomelaniidae | Squires and Saul (2004) |
| *californica*, *Acteonina* Gabb, 1864: 128, pl. 29, fig. 230a–b | 4259 (Pal) | *Paosia californica* (Gabb, 1864) | Pseudomelaniidae | Squires and Saul (2004) |
| *curta*, *Globiconcha* Gabb, 1862: 319 | 31393 (Hol) | *Tylostoma elevatum* (Shumard, 1853) | Tylostomatidae | Stanton (1947) |
| *elevatus*, *Acteon* Lea, 1833: 113, pl. 4, fig. 98 | 5545 (Lec); 5546 to 5549 (Pal) | *Pyramidella larvata* Conrad, 1833 | Pyramidellidae | Palmer (1937), Palmer and Brann (1966) |
| *globosus*, *Acteon* Lea, 1846: 255, pl. 36, fig. 55 | 1518 (Syn) | *Iselica globosa* (Lea, 1846) | Amathinidae | Lee (2015) |
| *granulatus*, *Acteon* Lea, 1846: 255, pl. 36, fig. 54 | 1533 (Syn) | *Odostomia granulatus* (Lea, 1846) | Pyramidellidae | Holmes (1860) |
| *laevis*, *Actaeon* Lea, 1841: 94, pl. 1, fig. 4 | lost (Hol) | *Odostomia laevis* (Lea, 1847) | Pyramidellidae | Palmer (1937) |
| *linteus*, *Solidulus* Conrad, 1858: 334, pl. 35, fig. 10 | lost (Hol) | *Eoacteon linteus* (Conrad, 1858) | Acteoninidae | Stephenson (1955), Sohl (1964) |
| *magnoplicatus*, *Acteon* Lea, 1841: 94, pl. 1, fig. 5 | 13158 (Hol) | *Odostomia* (*Evalea*) *melanella* (Lea, 1833) | Pyramidellidae | Palmer (1937) |
| *melanellus*, *Acteon* Lea, 1833: 113, pl. 4, fig. 99 | 5550 (Lec); 5551 to 5557 (Pal) | *Odostomia* (*Evalea*) *melanella* (Lea, 1833) | Pyramidellidae | Palmer (1937) |
| *pygmaeus*, ?*Acteon* Lea, 1833: 114, pl. 4, fig. 101 | 5559 (Hol) | *Pyramidella larvata* Conrad, 1833 | Pyramidellidae | Palmer (1937), Palmer and Brann (1966) |
| *simplex*, *Acteon* Lea, 1843: 8 | 1519 (Syn) | *Odostomia simplex* (Lea, 1843) | Pyramidellidae | Ward and Blackwelder (1987) |
| *striatus*, *Acteon* Lea, 1833: 114, pl. 4, fig. 100 | 5558 (Hol) | *Odostomia* (*Evalea*) *melanella alveata* (Lea, 1833) | Pyramidellidae | Palmer (1937), Palmer and Brann (1966) |
| *turbinatus*, *Acteon* Lea, 1843: 256, pl. 36, fig. 56 | 1522 (Syn) | *Odostomia turbinatus* (Lea, 1846) | Pyramidellidae | Ward and Blackwelder (1987) |

turreted) conical shell, convex whorls, distinct subsutural shelf, deep suture, preence of a columellar fold present, teleoconch sculpture consisting of axial ribs (including on the base) overridden by spiral threads.

Campbell (1993: 68) considers *Acteon angulatus* Lea 1846 a synonym of *Melanella angulata* (Lea, 1846) (Eulimidae), which is clearly not the case. The present species lacks the diagnostic conchological features of the latter genus (and of eulimids in general), such as: a high spire, a smooth teleoconch with fine axial marks, and the absence of a columellar fold (Warén 1984, Geiger 2016).

### List of taxa by species-group names

Here is presented a list of the species whose types can be found in the ANSP collection, arranged alphabetically by specific epithet. Species appear first in their original generic allocation and then in their current systematic position. An "*" after the specific epithet indicates that the type material is lost.

***andersoni***, *Actaeon* Conrad, 1848a: 287. ***Rictaxis andersoni* (Conrad, 1848).** Acteonidae.

***bella***, *Tornatellaea* Conrad, 1860: 294. ***Tornatellaea bella* Conrad, 1860.** Acteonidae.

***biforis***, *Retusa* Pilsbry & Johnson, 1917: 151. ***Acteon gabbana* Whitfield, 1892.** Retusidae.

***biplicata***, *Acteonina* Gabb, 1860a: 93. ***Acteocina canaliculata* (Say, 1826).** Acteonidae.

***bullata***, *Tornitella*? Morton, 1834: 48. ***Avellana bullata* (Morton, 1834).** Ringiculidae.

***calafia***, "*Acteonina*" Stewart, 1926: 432. ***Paosia calafia* (Stewart, 1926).** Pseudomelaniidae.

***cederstromi***, *Acteocina* Richards, 1947. ***Acteocina candei* (d'Orbigny, 1841).** Acteocinidae.

***chowanensis***, *Acteocina* Richards, 1947: 34. ***Acteocina canaliculata* (Say, 1826).** Acteocinidae.

***cinctorii***, *Atys* Pilsbry & Johnson, 1917: 152. ***Atys cinctorii* Pilsbry & Johnson, 1917.** Haminoeidae.

***costata***, *Cinulia* Johnson, 1898: 462. ***Avellana costata* (Johnson, 1898).** Ringiculidae.

***costata***, *Cylichna* Gabb, 1864: 143. ***Scaphander* (*Mirascapha*) *costatus* (Gabb, 1864).** Scaphandridae.

***costellatus****, *Acteon* Conrad, 1833b: 45. Status uncertain. Acteonidae.

***crassiplica***, *Bulla* Conrad, 1848a: 282. ***Acteocina crassiplica* (Conrad, 1848).** Bullidae.

***cretacea***, *Acteon* Gabb, 1862: 318. ***Acteon cretacea* Gabb, 1862.** Acteonidae.

***cretacea***, *Bullopsis* Conrad, 1858: 334. ***Bullopsis cretacea* Conrad, 1858.** Bullidae.

***cylichnoides***, *Volvula* Pilsbry & Johnson, 1917: 151. ***Volvulella cylichnoides* (Pilsbry & Johnson, 1917).** Rhizoridae.

***cylindrica***, *Volvula* Gabb, 1872: 246 [non Carpenter, 1865]. ***Volvulella persimilis* (Mörch, 1875).** Rhizoridae.

***cylindrus***, *Bulla* Lea, 1846: 250. ***Cylichna cylindrus* (Lea, 1846).** Cylichnidae.

***dekayi***, *Bulla* Lea, 1833: 200. ***Cylichna* (*Mnestia*) *dekayi* (Lea, 1833).** Cylichnidae.

***elegans***, *Monoptygma* Lea, 1833: 203. ***Acteon pomilius* Conrad, 1833.** Acteonidae.

***forbesiana***, *Actaeon* Whitfield, 1892: 157. ***Acteon cretacea* Gabb, 1862.** Acteonidae

***fossilis****, *Retusa sulcata* Pilsbry, 1922: 311. ***Retusa sulcata fossilis* Pilsbry, 1922.** Retusidae.

***gabbana***, *Actaeon* Whitfield, 1892: 156. ***Acteon gabbana* Whitfield, 1892**. Acteonidae.

***gabbi***, *Philine* (*Megistostoma*) Cossmann, 1895: 127. ***Philine* (*Megistostoma*) *gabbiana* (Stoliczka, 1868).** Philinidae.

***gabbiana***, *Bullaea* Stoliczka, 1868: 434. ***Philine* (*Megistostoma*) *gabbiana* (Stoliczka, 1868).** Philinidae.

***galba***, *Volvaria* Conrad, 1833a: 34. ***Retusa galba* (Conrad, 1833).** Retusidae.

***glans***, *Acteon* Lea, 1846: 256. ***Acteon glans* Lea, 1846.** Acteonidae.

***hornii***, *Bulla* Gabb, 1864: 143. ***Roxania hornii* (Gabb, 1864) new comb.** Scaphandridae.

***hypograpta***, *Ringicula* Brown & Pilsbry, 1912: 505. ***Ringicula* (*Ringiculella*) *semistriata* d'Orbigny, 1841.** Ringiculidae.

***idoneus***, *Acteon* Conrad, 1833b: 45. ***Acteon idoneus* Conrad, 1833.** Acteonidae

***impressus***, *Acteon* Gabb, 1864: 142. ***Tornatellaea impressa* (Gabb, 1864).** Acteonidae.

***kirkwoodiana***, *Acteocina* Richards & Harbison, 1944: 9. ***Acteocina kirkwoodiana* Richards & Harbison, 1944.** Acteocinidae.

***kellogii***, *Bulla* Gabb, 1860b: 386. ***Cylichna* (*Acrotrema*) *kellogii* (Gabb, 1860).** Cylichnidae.

***latus***, *Actaeon* (*Nucleopsis*) Conrad, 1865a: 34. ***Ringicula lata* (Conrad, 1865).** Ringiculidae.

***lineatus***, *Acteon* Lea, 1833: 112. ***Acteon idoneus* Conrad, 1833.** Acteonidae.

***macrostoma***, *Bulla* Gabb, 1860c: 301. ***Bulla macrostoma* Gabb, 1860.** Bullidae.

***mathewsonii***, *Cinulia* Gabb, 1864: 111. ***Biplica mathewsonii* (Gabb, 1864).** Ringiculidae.

***micratracta***, *Volvulella* Brown & Pilsbry, 1912. ***Volvulella micratracta* Brown & Pilsbry, 1912.** Rhizoridae.

***minutissima***, *Volvula* Gabb, 1860b: 386. ***Volvulella minutissima* (Gabb, 1860) new comb.** Rhizoridae.

***mississippiensis***, *Ringicula* Conrad, 1848a: 287. ***Ringicula* (*Ringiculella*) *mississippiensis* Conrad, 1848.** Ringiculidae.

***modicellus****, *Actaeon* Conrad, 1860: 287. ***Acteon modicellus* Conrad, 1860.** Acteonidae.

***mortoni****, *Bulla* Forbes, 1845: 63. ***Haminoea mortoni* Forbes, 1845.** Haminoeidae.

***naticoides***, *Actaenia* Gabb, 1860c: 299. ***Cinulia naticoides* (Gabb, 1860).** Ringiculidae.

***nitens***, *Acteon* Lea, 1846. ***Acteon nitens* Lea, 1846.** Acteonidae.

***novellus***, *Acteon* Conrad, 1834: 142. ***Acteon novellus* Conrad, 1834.** Acteonidae.

***obliqua***, *Cinulia* Gabb, 1864: 111. ***Biplica obliqua* (Gabb, 1864).** Ringiculidae

***occidentalis***, *Bulla* Meek & Hayden, 1856: 69. ***Ellipsoscapha occidentalis* (Meek & Hayden, 1856).** Scaphandridae.

***ornata***, *Volvula* Pilsbry & Johnson, 1917: 151. ***Volvulella ornata* (Pilsbry & Johnson, 1917).** Rhizoridae.

***oryza***, *Acteonidea* Gabb, 1872: 245. ***Rictaxis oryza* (Gabb, 1872).** Acteonidae.

***oviformis***, *Acteonella* Gabb, 1869: 173. ***Acteonella oviformis* Gabb, 1869.** Acteonellidae.

***ovoidea***, *Actaeon* Gabb, 1862: 319. ***Acteon cretacea* Gabb, 1862.** Acteonidae.

***parallela***, *Volvula* Pilsbry & Johnson, 1917: 151. ***Volvulella cylindrica parallela* (Pilsbry & Johnson, 1917).** Rhizoridae.

***polita***, *Ringinella* Gabb, 1869: 174. ***Acteon politus* (Gabb, 1869).** Acteonidae.

***pomilius***, *Acteon* Conrad, 1833b: 45. ***Acteon pomilius* Conrad, 1833.** Acteonidae.

***punctatus***, *Acteon* Lea, 1833: 111. ***Acteon pomilius punctatus* Lea, 1833.** Acteonidae.

***puruha***, *Acteocina* Pilsbry & Olsson, 1941: 13. ***Acteocina puruha* Pilsbry & Olsson, 1941.** Acteocinidae.

***recta***, *Bulla* Gabb, 1860c: 303. ***Cylichna recta* (Gabb, 1860).** Cylichnidae.

***rectilabrum***, *Cinulia* Gabb, 1869: 264. ***Cinulia rectilabrum* (Gabb, 1869).** Ringiculidae.

***reticulata***, *Volvaria* Johnson, 1899: 71. ***Volvaria reticulata* Johnson, 1899.** Acteonidae.

***sthillairii***, *Bulla* Lea, 1833: 98. ***Retusa* (*Cylichnina*) *galba* (Conrad, 1833).** Retusidae.

**striata**, *Megistostoma* Gabb, 1864: 144. ***Philine* (*Megistostoma*) *gabbiana* (Stoliczka, 1868).** Philinidae.

***subbullata***, *Acteocina* Pilsbry & Johnson, 1917: 150. ***Acteocina bullata* (Kiener, 1834).** Acteocinidae.

***subovoides***, *Actaeon* Whitfield, 1892: 155. ***Acteon cretacea* Gabb, 1862.** Acteonidae.

***subspissa***, *Bulla* Conrad, 1846: 20. ***Retusa subspissa* (Conrad, 1846).** Retusidae.

***subtornatilis***, *Acteon* Pilsbry & Johnson, 1917: 150. ***Acteon subtornatilis* Pilsbry & Johnson, 1917.** Acteonidae.

***subvaricata***, *Acteonina* Conrad, 1860: 294. ***Nucleopsis subvaricatus* (Conrad, 1860).** Acteonidae.

***sulculorum***, *Atys* Pilsbry & Johnson, 1917: 152. ***Atys doliolum* (Maury, 1917).** Haminoeidae.

***trapaquara***, *Ringicula* Harris, 1895a: 76. ***Ringicula trapaquara* (Harris, 1895a).** Ringiculidae.

***tritica***, *Volvula* Olsson & Harbison, 1953: 163. ***Volvulella tritica* (Olsson & Harbison, 1953).** Rhizoridae.

***varia***, *Ringicula* Gabb, 1864: 112. ***Ringicula varia* Gabb, 1864.** Ringiculidae.

***weatherlli***, *Acteon* Lea, 1833: 213. ***Acteocina canaliculata* (Say, 1826).** Acteocinidae.

## List of taxa by authorship

Here the list of types is arranged by author (alphabetically) and date; see "References" section for full citation. Species appear in their original generic allocation; see text for current systematic status and placement. An "*" indicates that the type material is lost.

**Brown, A. P. & Pilsbry, H. A.**

1912 ***hypograpta***, *Ringicula*
1912 ***micratracta***, *Volvulella*

**Conrad, T. A.**

1833a ***galba***, *Volvaria*
1833b ***costellatus***, *Acteon**
1833b ***idoneus***, *Acteon*
1833b ***pomilius***, *Acteon*
1834 ***novellus***, *Acteon*
1846 ***subspissa***, *Bulla*
1848a ***andersoni***, *Actaeon*
1848a ***crassiplica***, *Bulla*
1848a ***mississippiensis***, *Ringicula*
1858 ***cretacea***, *Bullopsis*
1860 ***bella***, *Tornatellaea*
1860 ***modicellus***, *Actaeon**
1860 ***subvaricata***, *Acteonina*
1865a ***latus***, *Actaeon* (*Nucleopsis*)

**Cossmann, M.**

1895 ***gabbi***, *Philine* (*Megistostoma*)

**Forbes, E.**

1845 ***mortoni***, *Bulla**

**Gabb, W. M.**

1860a ***biplicata***, *Acteonina*
1860b ***kellogii***, *Bulla*
1860b ***minutissima***, *Volvula*
1860c ***macrostoma***, *Bulla*
1860c ***naticoides***, *Actaenia*
1860c ***recta***, *Bulla*
1862 ***cretacea***, *Acteon*
1862 ***curta***, *Globiconcha*
1862 ***ovoidea***, *Actaeon*
1864 ***costata***, *Cylichna*
1864 ***hornii***, *Bulla*
1864 ***impressus***, *Acteon*
1864 ***mathewsonii***, *Cinulia*
1864 ***obliqua***, *Cinulia*
1864 ***striata***, *Megistostoma*
1864 ***varia***, *Ringicula*
1869 ***oviformis***, *Acteonella*
1869 ***polita***, *Ringinella*
1869 ***rectilabrum***, *Cinulia*
1873 ***cylindrica***, *Volvula*
1873 ***oryza***, *Acteonidea*

**Harris, G. D.**

1895a ***trapaquara***, *Ringicula*

**Johnson, C. W.**

1898 ***costata***, *Cinulia*
1898 ***reticulata***, *Volvaria*

**Lea, H. C.**

1846 ***cylindrus***, *Bulla*
1846 ***glans***, *Acteon*
1846 ***nitens***, *Acteon*

**Lea, I.**

1833 ***dekayi***, *Bulla*
1833 ***elegans***, *Monoptygma*
1833 ***lineatus***, *Acteon*
1833 ***punctatus***, *Acteon*
1833 ***sthillairii***, *Bulla*
1833 ***weatherlli***, *Acteon*

**Meek, F. B. & Hayden, F. V.**

1856 ***occidentalis***, *Bulla*

**Morton, S. G.**

1834 ***bullata***, *Tornitella*?

**Olsson, A. A. & Harbison, A.**

1953 ***tritica***, *Volvula*

**Pilsbry, H. A.**

1922 ***fossilis***, *Retusa sulcata**

**Pilsbry, H. A. & Johnson, C. W.**

1917 ***biforis***, *Retusa*
1917 ***cinctorii***, *Atys*
1917 ***cylichnoides***, *Volvula*
1917 ***ornata***, *Volvula*
1917 ***parallela***, *Volvula*
1917 ***subbullata***, *Acteocina*
1917 ***subtornatilis***, *Acteon*
1917 ***sulculorum***, *Atys*

**Pilsbry, H. A. & Olsson, A. A.**

1941 ***puruha***, *Acteocina*

**Richards, H. G.**

1947 ***cederstromi***, *Acteocina*
1947 ***chowanensis***, *Acteocina*

**Richards, H. G. & Harbison, A.**

1944 ***kirkwoodiana***, *Acteocina*

**Stoliczka, F.**

1868 ***gabbiana***, *Bullaea*

**Whitfield, R. P.**

1892 ***forbesiana***, *Actaeon*
1982 ***gabbana***, *Actaeon*
1982 ***subovoides***, *Actaeon*

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## References

Anderson FM (1958) Upper Cretaceous of the Pacific Coast. Memoirs of the Geological Society of America 71: 1–378. https://doi.org/10.1130/MEM71-p1

Borrero FJ, Rosenberg G (2015) The Paul Hesse Collection at the Academy of Natural Sciences of Philadelphia, with a review of names for Mollusca introduced by Hesse. Proceedings of the Academy of Natural Sciences of Philadelphia 164(1): 43–100. https://doi.org/10.1635/053.164.0109

Bouchet P, Rocroi J-P, Frýda J, Hausdorf B, Ponder W, Valdés Á, Warén A (2005) Classification and nomenclator of gastropod families. Malacologia 47(1–2): 1–397.

Bouchet P, Rocroi JP, Hausdorf B, Kaim A, Kano Y, Nützel A, Parkhaev P, Schrödl M, Strong EE (2017) Revised classification, nomenclator and typification of gastropod and monoplacophoran families. Malacologia 61(1–2): 1–526. https://doi.org/10.4002/040.061.0201

Brown AP, Pilsbry HA (1912) Fauna of the Gatun formation, Isthmus of Panama. II. Proceedings of the Academy of Natural Sciences of Philadelphia 64: 500–519.

Callomon P (2015) On the authorship and types of three Ogasawara helicinids (Mollusca: Gastropoda). Proceedings of the Academy of Natural Sciences of Philadelphia 164(1): 21–29. https://doi.org/10.1635/053.164.0105

Campbell LD (1993) Pliocene molluscs from the Yorktown and Chowan River Formations in Virginia. Virginia Division of Mineral Resources Publication 127, 259 pp.

Clark WB (1916) The Upper Cretaceous deposits of Maryland. In: Clark WB, et al. (Eds) Upper Cretaceous. Maryland Geological Survey Systematic Report 6(1): 23–110.

Conrad TA (1833a) Fossil Shells of the Tertiary Formations of North America. illustrated by figures drawn on stone, from nature. Vol. 1, No. 3. W. P. Gibbons, Philadelphia, 29–38.

Conrad TA (1833b) Fossil Shells of the Tertiary Formations of North America. Illustrated by figures drawn on stone, from nature. Vol. 1, no. 4. W. P. Gibbons, Philadelphia, 39–46.

Conrad TA (1834) Observations on the Tertiary and more recent formations of a portion of the southern states. Descriptions of new Tertiary fossils from the southern states. Journal of the Academy of Natural Sciences of Philadelphia 1st ser. (7): 116–157.

Conrad TA (1846) Descriptions of new species of fossil and recent shells and corals. Proceedings of the Academy of Natural Sciences of Philadelphia 3(1): 19–27.

Conrad TA (1848a) Observations on the Eocene formation, and description of one hundred and five new fossils of that period, from the vicinity of Vicksburg, Mississippi, with an appendix. Proceedings of the Academy of Natural Sciences of Philadelphia 3(11): 280–299.

Conrad TA (1848b) Observations on the Eocene formation, and description of one hundred and five new fossils of that period, from the vicinity of Vicksburg, Mississippi; with an appendix. Journal of the Academy of Natural Sciences of Philadelphia 2nd ser. 1(2): 111–134.

Conrad TA (1858) Observations on a group of Cretaceous Fossil Shells, found in Tippah County, Miss., with descriptions of fifty-six new species. Journal of the Academy of Natural Science of Philadelphia 2(3): 323–336.

Conrad TA (1860) Description of new species of Cretaceous and Eocene fossils of Mississippi and Alabama. Journal of the Academy of Natural Science of Philadelphia 2nd ser. 4: 275–298.

Conrad TA (1865a) Catalogue of the Eocene and Oligocene Testacea of the United States. American Journal of Conchology 1: 1–35.

Conrad TA (1865b) Descriptions of new Eocene shells of the United States. American Journal of Conchology 1: 142–149.

Cossmann M (1895) Essais de paléoconchologie comparée. Vol. 1. Comptoir Géologique, Paris, 161.

Dall WH (1889) Report on the Mollusca (Blake Expedition); pt. 2, Gastropoda and Scaphopoda. Bulletin of the Museum of Comparative Zoology 18: 1–492.

Dockery DT (1977) Mollusca of the Moodys Branch Formation, Mississippi. Bulletin Mississippi Geological, Economic and Topographic Survey 120: 1–212.

Forbes E (1845) On the fossil shells collected by Mr. Lyell from the Cretaceous formations of New Jersey. Quarterly Journal of the Geological Society of London 1: 61–64. https://doi.org/10.1144/GSL.JGS.1845.001.01.14

Gabb WM (1860a) Descriptions of new species of Cretaceous fossils from New Jersey. Proceedings of the Academy of Natural Sciences of Philadelphia 12: 93–95.

Gabb WM (1860b) Description of new species of American Tertiary and Cretaceous fossils. Journal of the Academy of Natural Sciences of Philadelphia 2nd ser. 4(4): 375–406.

Gabb WM (1860c) Descriptions of some new species of Cretaceous fossil. Journal of the Academy of Natural Sciences of Philadelphia 2nd ser. 4(3): 299–305.

Gabb WM (1862) Descriptions of new species of Cretaceous fossils from New Jersey, Alabama and Mississippi. Proceedings of the Academy of Natural Sciences of Philadelphia 13(1861): 318–330.

Gabb WM (1864) Descriptions of the Cretaceous fossils. In: Meek FB, Gabb WM (Eds) Palaeontology of California vol. 1. Geological Survey of California. Caxton Press, Philadelphia, 55–243.

Gabb WM (1869) Description of new, and revision of previously described, Cretaceous fossils. California Geological Survey Paleontology of California 2(section 2, pt. 1): 127–205.

Gabb WM (1872) On the Topography and Geology of San Domingo. Transactions of the American Philosophical Society 15: 49–259. https://doi.org/10.2307/1005417

Gardner J (1916) Upper Cretaceous systematic paleontology: Mollusca. Maryland Geological Survey Systematic Report 6(1–2): 371–733.

Gardner J (1945) Mollusca of the Tertiary Formations of Northeastern Mexico. Geological Society of America Memoirs 11: 1–332. https://doi.org/10.1130/MEM11-p1

Gardner J (1948) Mollusca from the Miocene and Lower Pliocene of Virginia and North Carolina. Part 2: Scaphopoda and Gastropoda. Geological Survey Professional Papers 199B: 179–310.

Geiger DL (2016) *Severnsia strombeulima* n. gen. & sp. from Hawaii (Mollusca, Gastropoda: Caenogastropoda: Eulimidae). Zootaxa 4084(4): 587–589. https://doi.org/10.11646/zootaxa.4084.4.8

Glibert M (1962) Euthyneura et Pulmonata fossiles du Cénozoïque étranger des collections de l'Institut Royal des sciences naturelles de Belgique. Mémoires de l'Institut Royal des sciences naturelles de Belgique 70(2): 1–140.

Gofas S, Bouchet P (2015) *Volvula* – MolluscaBase, World Register of Marine Species. http://www.marinespecies.org/aphia.php?p=taxdetails&id=511917 [accessed 2015–11–05]

Harris GD (1895a) Claiborne fossils. Bulletins of American Paleontology 1(1): 1–52.

Harris GD (1895b) New and otherwise interesting Tertiary Mollusca from Texas. Proceedings of the Academy of Natural Sciences of Philadelphia 47: 45–88, pls. 1–9. https://doi.org/10.5962/bhl.title.17243

Holmes FS (1860) Post-Pleiocene Fossils of South-Carolina. Russel & Jones, Charleston, 122 pp. pls. https://doi.org/10.5962/bhl.title.12267

ICZN (1999) International code of zoological nomenclature. The International Trust for Zoological Nomenclature, London, 306 pp.

Johnson CW (1897) Annual Report of the State Geologist for the year 1897. John L. Murphy Publishing, New Jersey, 368 pp.

Johnson CW (1898) New Cretaceous Fossils from an Artesian Well-Boring at Mount Laurel, N. J. Proceedings of the Academy of Natural Sciences of Philadelphia 50: 461–464.

Johnson CW (1899) New and interesting species in the "Isaac Lea collection of Eocene Mollusca. Proceedings of the Academy of Natural Sciences of Philadelphia 51(1): 71–82.

Johnson CW (1905) List of the types of invertebrate Cretaceous Fossils in the collection of the Academy of Natural Sciences, Philadelphia. Proceedings of the Academy of Natural Sciences of Philadelphia 57: 4–28.

Keen AM, Bentson H (1944) Check List of California Tertiary Marine Mollusca. Geological Society of America Special Papers 56: 1–280. https://doi.org/10.1130/SPE56-p1

Lea HC (1841) Description of some new species of fossil shells from the Eocene, at Claiborne, Alabama. American Journal of Science and Arts 40(1): 92–103.

Lea HC (1843) Descriptions of some new fossil shells, from the Tertiary of Virginia. Proceedings of the American Philosophical Society 3: 162–165.

Lea HC (1846) Descriptions of some new fossil shells, from the Tertiary of Petersburg, Virginia. Transactions of the American Philosophical Society new series 9(9): 229–274. https://doi.org/10.5962/bhl.title.28107

Lea I (1833) Contributions to Geology. Carey, Lea & Blanchard, Philadelphia, 227 pp.

Lee HG (2015) Peregrinations of two little fossils through the annals of gastropod systematics. The Shell-O-Gram 56(3): 3–5.

Lygre F, Schander C (2010) Six new species of pyramidellids (Mollusca, Gastropoda, Pyramidelloidea) from West Africa, introducing the new genus *Kongsrudia*. Zootaxa 2657: 1–17.

MacNeil FS, Dockery TD (1984) Lower Oligocene Gastropoda, Scaphopoda, and Cephalopoda of the Vicksburg Group in Mississippi. Mississippi Department of Natural Resources Bureau of Geology 124: 231–232.

Martin GC (1904) *Gastropoda*. In: Clark WB, Shattuek GB, Dall WM (Eds) The Miocene deposits of Maryland. Maryland Geological Survey, Miocene, Johns Hopkins Press, Baltimore, Maryland, 131–269.

Maury CJ (1917) Santo Domingo type sections and fossils. Bulletins of American paleontology 5(29): 1–251.

Meek FB, Hayden FV (1856) Descriptions of new species of Gastropoda from the Cretaceous Formations of Nebraska Terr. Proceedings of the Academy of Natural Sciences of Philadelphia 8: 63–69.

Mikkelsen PS, Mikkelsen PM (1984) Comparison of *Acteocina canaliculata* (Say, 1826), *A. candei* (d'Orbigny, 1841), and *A. atrata* spec. nov. (Gastropoda: Cephalaspidea). Veliger 27: 164–192.

Moore EJ (1962) Conrad's Cenozoic fossil marine mollusk type specimens at the Academy of Natural Sciences of Philadelphia. Proceedings of the Academy of Natural Sciences of Philadelphia 114(2): 23–120.

Morton SG (1834) Synopsis of the Organic Remains of the Cretaceous Group of the United States. Illustrated by nineteen plates. To which is added an appendix, containing a tabular view of the Tertiary fossils hitherto discovered in North America. Key & Biddle, Philadelphia, 88 pp.

Olsson AA, Harbison A (1953) Pliocene Mollusca of Southern Florida with Special Reference to those from North Saint Petersburg. The Academy of Natural Sciences of Philadelphia Monograph 8, 457 pp.

Palmer KVW (1937) The Claibornian Scaphopoda, Gastropoda, and dibranchiate Cephalopoda of the southern USA. Bulletins of American Paleontology 7(32): 1–548.

Palmer KVW, Brann DC (1966) Catalogue of the Paleocene and Eocene Mollusca of the southern and eastern United States. Part II. Gastropoda. Bulletins of American Paleontology 48(218): 471–1057.

Pilsbry HA (1922) Revision of W. M. Gabb's tertiary Mollusca of Santo Domingo. Proceedings of the Academy of Natural Sciences of Philadelphia 73: 305–435.

Pilsbry HA, Johnson CW (1917) New Mollusca of the Santo Domingan Oligocene. Proceedings of the Academy of Natural Sciences of Philadelphia 69: 150–202.

Pilsbry HA, Olsson AA (1941) A Pliocene fauna from Western Ecuador. Proceedings of the Natural Sciences of Philadelphia 93: 1–79.

Popenoe WP (1957) The Cretaceous gastropod genus *Biplica*, its evolution and biostratigraphic significance. University of California Publications in Geological Sciences 30(6): 425–454.

Portell RW, Kittle BA (2010) Florida Fossil Invertebrates. Part 13. Mollusca. Bermont Formation (Middle Pleistocene). Florida Paleontological Society, Gainesville, 40 pp.

Richards HG (1947) Invertebrate Fossils from Deep Wells along the Atlantic Coastal Plain. Journal of Paleontology 21(1): 23–37.

Richards HG (1968) Catalogue of invertebrate fossil types at the Academy of Natural Sciences of Philadelphia. Special Publication of the Academy of Natural Sciences of Philadelphia 8: 1–222.

Richards HG, Harbison A (1944) Well-boring at Brandywine Lighthouse in Delaware Bay, Part I –Geology and macrofossils. Notulae Naturae 132: 1–14.

Richards HG, Ramsdell RC (1962) A revision and augmentation of Stuart Weller's Cretaceous gastropods of New Jersey. In: Cooke CW, Garner HF, Howell BF, Jeletzky JA, Miller AK, Miller Jr HW, Ramsdell RC, Richards HG, Reeside Jr JB, Roberts HB, Wells JW (Eds) The Cretaceous Fossils of New Jersey, Part II: Gastropoda, Scaphopoda, Nautiloidea, Ammonoidea, Belemnitidae, Crustacea, Vertebrata and Miscellaneous Fossils. State of New Jersey Departament of Conservation and Economic Development (Bulletin 61), Trenton, 1–97.

Richards HG, Shapiro E (1963) An invertebrate macrofauna from the Upper Cretaceous of Delaware. Delaware Geological Survey, Report of Investigations 7: 1–37.

Robba E (2013) Tertiary and Quaternary fossil pyramidelloidean gastropods of Indonesia. Scripta Geologica 144: 1–191.

Salvador RB, Cunha CM (2016) Taxonomic revision of the fossil genera *Bulimactaeon*, *Hemiauricula* (= *Liocarenus*) and *Nucleopsis*, with description of a new Recent genus and species (Gastropoda: Heterobranchia: Acteonidae). Journal of Molluscan Studies 82(3): 472–483. https://doi.org/10.1093/mollus/eyw010

Sohl NF (1964) Neogastropoda, Opisthobranchia and Basommatophora from the Ripley, Owl Creek, and Prairie Bluff Formations. United States Geological Survey Professional Paper 331-B: 153–344. https://doi.org/10.3133/pp331B

Sohl NF (1967) Upper Cretaceous Gastropods from the Pierre Shale at Red Bird, Wyoming. United States Geological Survey Professional Paper 393-B: 97–140.

Squires RL (1984) Megapaleontology of the Eocene Llajas Formation, Simi Valley, California. National Museum of Los Angeles County, Contributions in Science 350: 1–76.

Squires RL, Saul LR (2004) The pseudomelaniid gastropod *Paosia* from the marine Cretaceous of the Pacific Slope of North America and a review of the age and paleobiogeography of the genus. Journal of Paleontology 78(3): 484–500. https://doi.org/10.1666/0022-3360(2004)078<0484:TPGPFT>2.0.CO;2

Stanton TW (1947) Studies of some Comanche pelecypods and gastropods. United States Geological Survey Professional Paper 211: 1–256. https://doi.org/10.3133/pp211

Stephenson LW (1914) Cretaceous deposits of the eastern Gulf region and species of *Exogyra* from the eastern Gulf region and the Carolinas. United States Geological Survey Professional Paper 81: 1–77. https://doi.org/10.5962/bhl.title.61451

Stephenson LW (1955) Owl Creek (Upper Cretaceous) Fossils from Crowley's Ridge Southeastern Missouri. United States Geological Survey Professional Paper 274-E: 97–140.

Stewart RB (1926) Gabb's California fossil type gastropods. Proceedings of the Academy of Natural Sciences of Philadelphia 78: 287–447.

Stewart RB (1930) Gabb's California Cretaceous and Tertiary fossil type lamellibranchs. Special Publications of the Academy of Natural Sciences of Philadelphia 3: 1–314.

Stoliczka F (1868) The Gastropoda. In: Oldham T (Ed.) Cretaceous Fauna of Southern India. Volume 2, Paleontologia Indica, being figures and descriptions of the organic remains procured during the progress of the Geological Survey of India. Memoirs of the Geological Survey of India, Calcutta, 1–497.

Snyder MA, Callomon P (2015) Species named in Fasciolariidae by Tapparone-Canefri and their types (Mollusca: Gastropoda). Proceedings of the Academy of Natural Sciences of Philadelphia 164(1): 37–41. https://doi.org/10.1635/053.164.0108

Valdés Á (2008) Deep-sea "cephalaspidean" heterobranchs (Gastropoda) from the tropical southwest Pacific. Mémoires du Muséum National d'Histoire Naturelle 196: 587–792.

Wade B (1926) The Fauna of the Ripley Formation on Coon Creek, Tennessee. United States Geological Survey Professional Paper 137: 1–272. https://doi.org/10.3133/pp137

Ward LW, Blackwelder BW (1987) Late Pliocene and Early Pleistocene Mollusca from the James City and Chowan River Formations at Lee Creek Mine. Smithsonian Contributions to Paleobiology 61: 113–283.

Warén A (1984) A generic revision of the family Eulimidae (Gastropoda, Prosobranchia). Journal of Molluscan Studies suppl 13: 1–96.

Weller S (1907) A Report on the Cretaceous Paleontology of New Jersey. Volume 4 of the Paleontology Series. N. J. Trenton, New Jersey, 871 pp.

Whitfield RP (1892) Gasteropoda and Cephalopoda of the Raritan Clays and Greensand Marls of New Jersey. Monographs of the United States Geological Survey 18: 1–402. https://doi.org/10.5962/bhl.title.66984

Woodring WP (1970) Geology and paleontology of canal zone and adjoining parts of Panama: Description of Tertiary mollusks (gastropods: Eulimidae, Marginellidae to Helminthoglyptidae). United States Geological Survey Professional Paper 306-D: 299–452. https://doi.org/10.3133/pp306D

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# Shadings in digital taxonomic drawings

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## Abstract

This contribution describes how shadings can be applied to taxonomic line drawings created with vector graphics software. The line drawings created with the vector graphic software are saved in vector format and then also in bitmap graphics format. The bitmap version is opened in a bitmap graphics program and the areas for shading selected. A new layer is then created and clouds of pixels are 'sprayed' onto these areas. The pixel clouds are saved and later placed onto the outlines in the vector graphic software as a new layer. The results combine the advantages of vector graphics, perfectly smooth lines and the greyscales of bitmap graphics.

## Introduction

Photography is often used in taxonomic descriptions and works especially well for larger animals. When using a stack of pictures it is possible to combine them with software into one photo of excellent depth of field (for example with CombineZP: http://combinezp.software.informer.com). But for small, translucent animals stacking does not work as well and many details remain invisible in the photo. Here, drawings are still the best choice for a taxonomic description. The use of computers for taxonomic drawings helped to speed up the documentation and at the same time improve the quality of these line drawings. Compared to the traditional use of ink pens the digital drawing method has advantages: e.g. corrections are easy, it is possible to create complex structures like setae in an elegant and rapid way and the arrangement of plates is quickly achieved (Coleman 2003, 2006, 2009). Montesanto (2015, 2016) used bitmap graphic solutions for taxonomic drawings, e.g. GIMP, but the quality of bitmap line drawings as proposed in these papers is inferior compared to vector graphics and the scaling of detailed drawings during the arrangement of plates leads to different line weights. However, bitmap graphics have a great potential when greyscale information is needed, for example for shading. Shading is especially helpful for showing cuticular structures such as carinae, teeth or grooves. However, vector graphics are superior to bitmap graphics in several respects for pure line drawings. The idea of shading directly within vector graphic software was proposed by Bober and Riehl (2014), but was adopted in only a few papers.

In the present paper it is shown how to combine the best of these two technologies in the same drawing: the high-quality smooth lines created by vector graphics like Illustrator, and greyscale bitmap shading with Photoshop.

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## Material and methods

Pencil drawings, made with a Leica DMLB microscope equipped with a camera lucida, were scanned with a DINA3 scanner (Plustek Optibook A300) as a template. Several scans of a large pencil drawing can be combined into one template within the vector graphics software program Adobe Illustrator CS2, which was used for this paper, subsequently using the "Place" command for combining parts of the scans. Alternatively photos can be used as a master (Coleman 2006). The bitmap program Adobe Photoshop CS2 was used to generate the pixel shadings. The method should work in an analogous way with other similar software products.

A Wacom Bamboo graphics tablet was used for creating line drawings in Illustrator as well as for shading in Photoshop.

### Step-by-step instructions

- The starting point for shading is ideally a plate with a habitus drawing on it, opened in Illustrator. On the arranged plate the habitus drawing is already at the right size and the density of pixels in the shadings can be best controlled in the final format.
- From within Illustrator, export the plate into Photoshop format (.psd). Select "Flat Image" in the Photoshop Export Options (Figure 1A–B). Minimize Illustrator and then continue to work in Photoshop.
- Open the file with the line drawings in Photoshop (File-Open or double click on the file name with a psd-extension).
- Limit the areas which should be shaded using the "magic wand" tool (Figure 2A). If needed, select several areas at the same time by holding the "shift" key while clicking inside the designated areas. If everything works correctly, the inner margins of the outlines then appear fuzzy. If the box margin of the picture becomes fuzzy instead, then the areas are not completely closed by a continuous line.
- The selection of areas has to be done on the layer that contains the outlines of the habitus (the first layer) (Figure 2B).

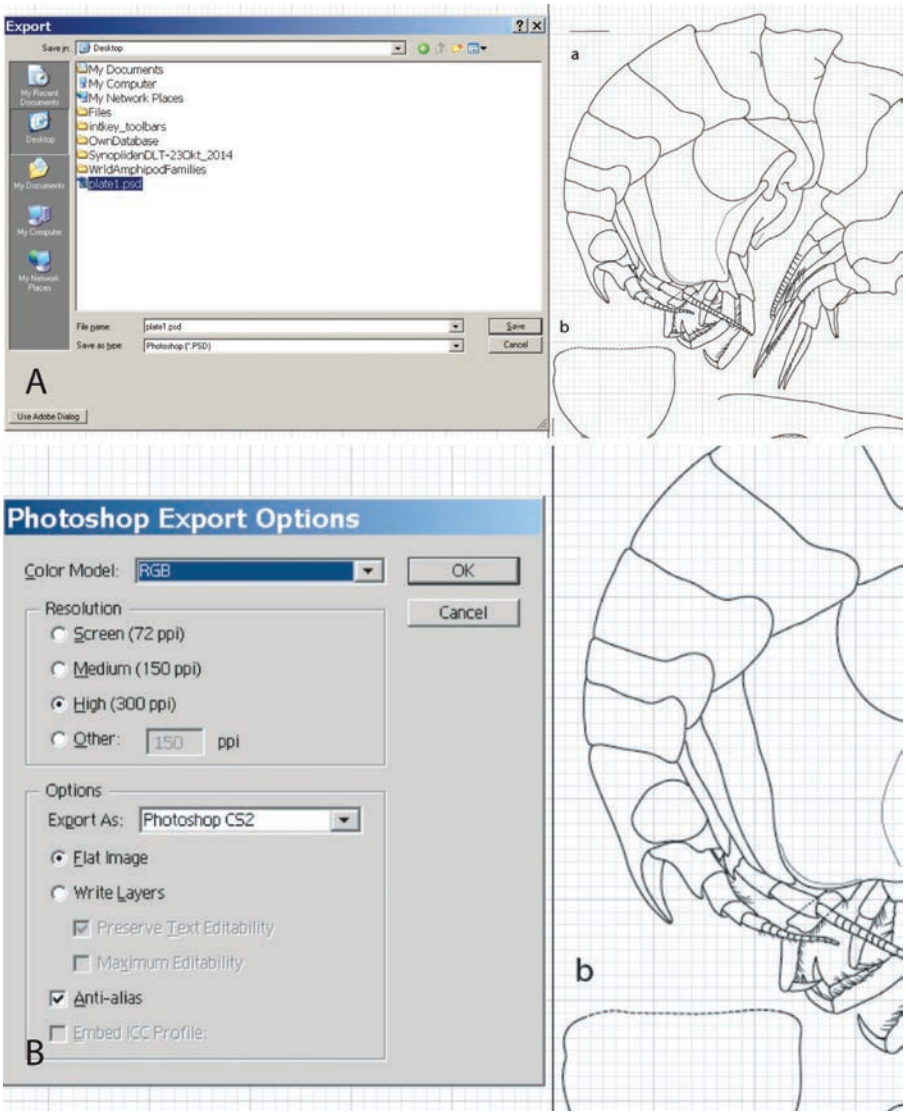


**Figure 1. A)** Export plate from Illustrator into Photoshop format (.psd). **B)** Use "Flat Image" option.

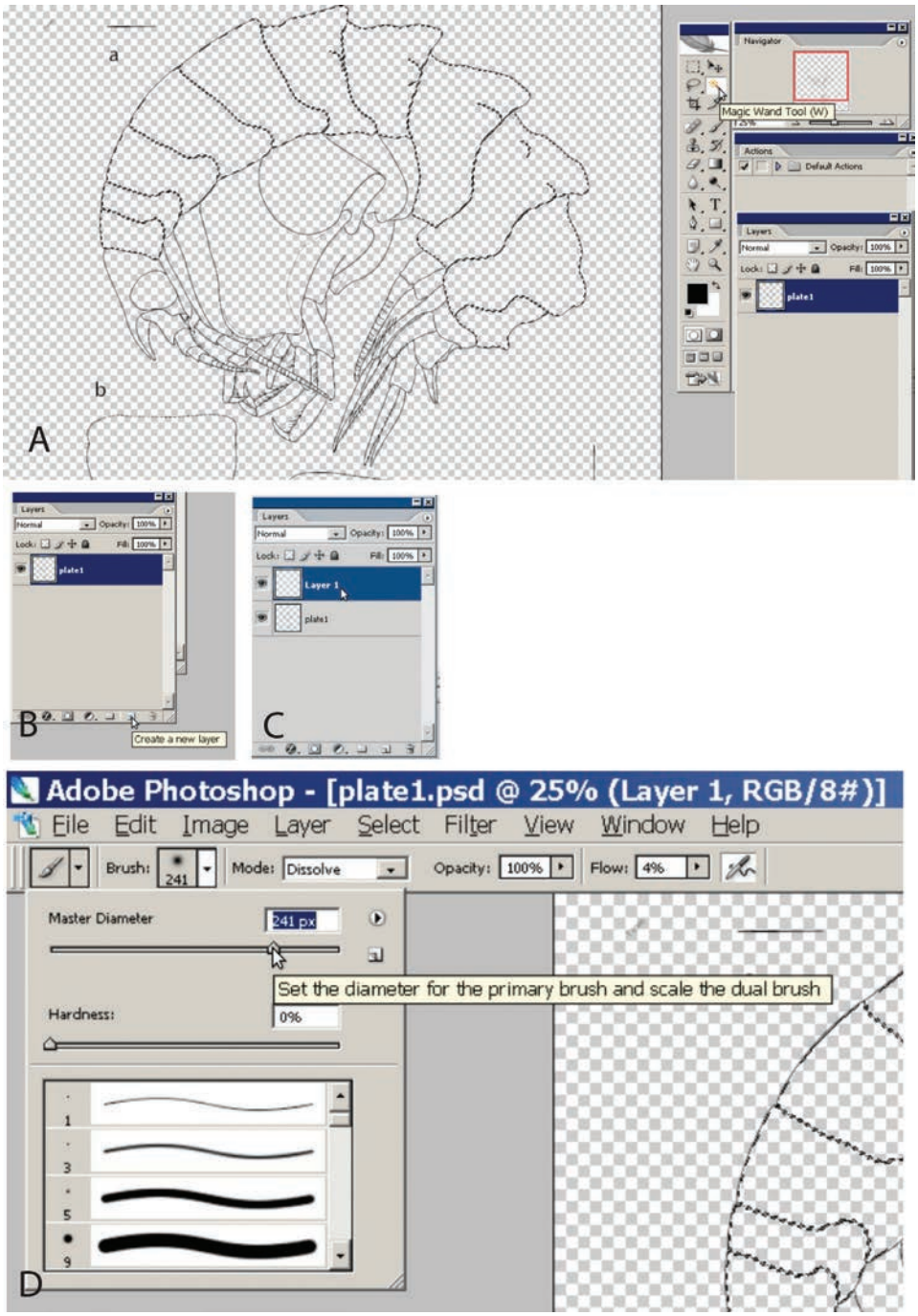


**Figure 2. A)** In Photoshop use "Magic Wand Tool" and the "shift" key to mark areas in the drawing for shading. **B)** Create a new layer, by clicking on second icon from the right in the icon bar at the lower end of the window. **C)** Select the newly created layer before applying the shading. **D)** Select "Brush", modify "Master Diameter" and set "Flow" at about 3–4%.

- Create a new layer (by clicking on the second icon from the right in the icon bar, marked as "Create new layer", below in the "Layers" window, see Figure 2B). It is essential to apply the shadings only to this newly created layer (Figure 2C)!
- Select the "Brush" (B) tool, and then in the Brush dialog (settings bar, upper left corner, below File and Edit pulldown menu) "Airbrush Soft Round".
- Adapt the Brush size by moving the "Master Diameter" lever (Figure 2D).
- There are two options: 1) "Mode: Normal" for greyscale or 2) "Mode: Dissolve" for stippling (creating a pixel cloud).
- Keep "Opacity" at 100%, the setting for "Flow" should only be at 3–4% (Figure 2D), in order to prevent too many pixels being sprayed.
- Foreground colour must be black.
- Spray pixel clouds with the mouse or the Wacom board. If everything was set up correctly the pixels will only appear inside the selected areas (Figure 3A).

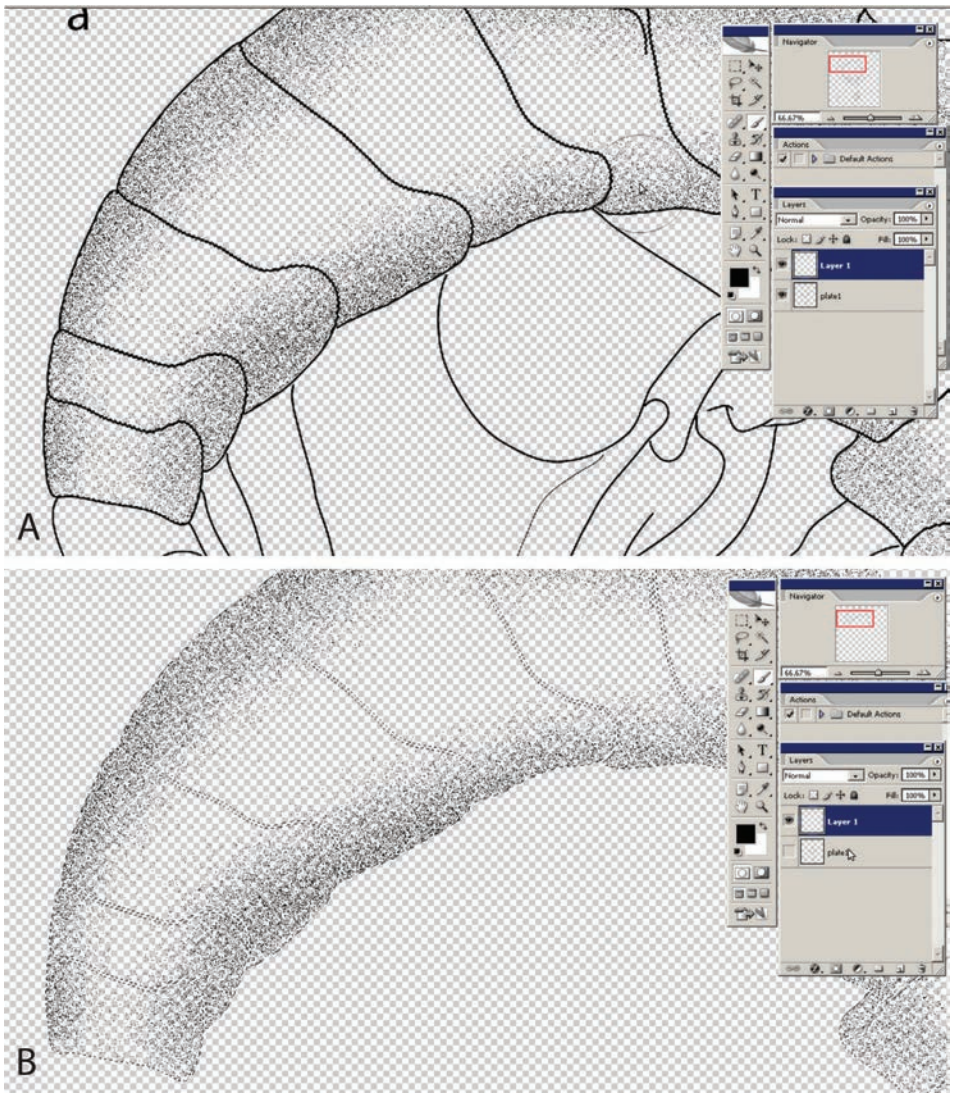


**Figure 3. A)** In Photoshop select newly created layer and apply shading by spraying pixels onto selected areas. **B)** After airbrushing, make first layer, containing the outlines, invisible by clicking on eye icon in "Layers". By this only the pixel clouds are retained.

- If some areas appear too dark, change "Set foreground colour" to white by clicking on the double arrow close to the white and black squares in the Tools. Then spray white pixels on top of the black pixels.
- In order to get an idea about the right density of the shadings, print out the file from time to time. The density of the pixel clouds in the printout may differ from what you see on the screen.
- When you are content with the result, make the layer that contains the outlines (the first layer) invisible by clicking on the eye symbol beside the first layer unselecting it (Figure 3B).
- Then save the Photoshop file, which now only contains the shadings, but not the outlines (Figure 3B).
- Expand the minimized Illustrator drawing, create a new layer and place the Photoshop file with the pixel shadings onto the habitus using the File-Place dialog. Carry out fine adjustments with the cursor keys (or simultaneously hold "shift" and use the cursor keys, for faster movements) (Figure 4A–B).

## Discussion

Coleman described in a series of papers (2003, 2006, 2009) how to use Illustrator for taxonomic drawings. These three papers show how to produce high quality vector drawings both quickly and accurately. The only part that was missing in this series of publications is shading. Shadings in vector graphics work theoretically (Bober and Riehl 2014), but the results are not very convincing. During initial trials with Photoshop (Coleman 2003) a whole plate was exported into bitmap format prior to shading, leading to the loss of the vector information and thus rough lines. In the method proposed here, however, the vector graphics are unchanged and only the bitmap shadings are placed as an overlay on top of the vector graphics. In this way the advantages of vector graphics (perfect, smooth lines) and the greyscale and stippling capabilities of bitmap graphics are combined, leading to a final image of superior quality.

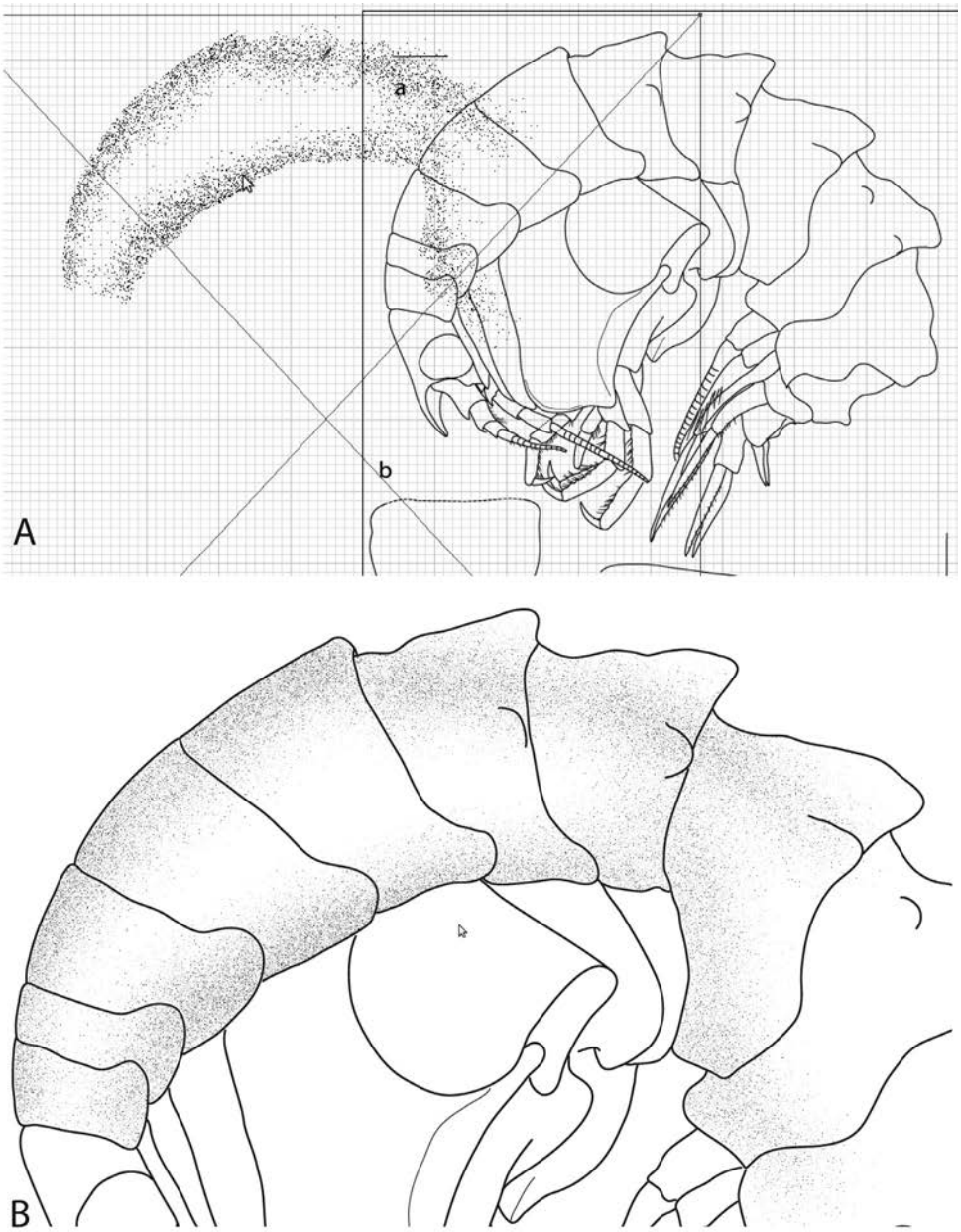


**Figure 4. A)** Place pixel cloud, saved without outlines from Photoshop into Illustrator using the File-Place command. B) Move the pixel cloud in the corresponding areas of the vector graphics in Illustrator.

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## References

Bober S, Riehl T (2014) Adding depth to line artwork by digital stippling – a step-by-step guide to the method. Organisms Diversity & Evolution 14(3): 327–337. https://doi.org/10.1007/s13127-014-0173-7

Coleman CO (2003) "Digital inking": How to make perfect line drawings on computers. Organisms Diversity & Evolution, Electronic Supplement 14: 1–14.

Coleman CO (2006) Substituting time-consuming pencil drawings in arthropod taxonomy using stacks of digital photographs. Zootaxa 1360: 61–68.

Coleman CO (2009) Drawing setae the digital way. Zoosystematics and Evolution 85: 305–310. https://doi.org/10.1002/zoos.200900008

Montesanto G (2015) A fast GNU method to draw accurate scientific illustrations for taxonomy. Zookeys 515: 191–206. https://doi.org/10.3897/zookeys.515.9459

Montesanto G (2016) Drawing setae: a GNU way for digital scientific illustrations. Nauplius 24: 1–6. https://doi.org/10.1590/2358-2936e2016017

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# Redescription and reassignment of *Ondina semicingulata* to the Pyramidellidae, with review of the occurrence of genus *Evalea* in the Western Atlantic (Gastropoda)

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## Abstract

*Acteon semicingulatus* Dall, 1927, previously known only by its original description is reassigned to the Pyramidellidae, in *Ondina*, based on the collecting of several new specimens along the coast of Brazil, in the same bathymetry as the type locality. Its shell shape variation is discussed and *Odostomia* (*Evalea*) *ryclea* Dall, 1927 is considered a synonymy. Other Western Atlantic species, previously allocated to other genera are transferred to *Ondina*: *Aclis striata* Verrill, 1880, *Odostomia* (*Iolaea*) *hendersoni* Bartsch, 1909, *Evalea stocki* De Jong & Coomans, 1988 and *Odostomia* (*Evalea*) *emeryi* Bartsch, 1955 based on conchlogical comparison to the revison by Høisæter (2014), from Northeastern Atlantic. The genus *Evalea* is considered to be absent in the Atlantic Ocean.

## Introduction

The Pyramidellidae Gray, 1840 is a notoriously rich and taxonomically complex gastropod family. It was included in the "big-five" group of the richest mollusks families by Albano et al. (2002) after extensive surveys in New Caledonia. It includes about 3,000 accepted names at the species level which are classified in around 140 accepted genera (MolluscaBase 2018).

Although recent advances and changes in the phylogenetic position of the family within Heterobranchia have been proposed (e.g. Dinapoli and Klussmann-Kolb 2010), the alpha taxonomy of several genera remains to be revised and knowledge of diversity and distribution of species is still far from satisfactory. Taxonomic studies in regions where the Pyramidellidae was poorly studied, generally reveal several new species (e.g. Peñas and Rolán 2010, in the South Pacific region). Knowledge of Pyramidellidae diversity in Brazil grew considerably after several taxonomic works (e.g. Pimenta and Absalão 2001a, b, 2002, 2004a, 2004b, Pimenta et al. 2000, 2008, 2009, 2011, Pimenta 2012), but several genera remain to be revised. Additionally, old published names should be revised in both nomenclature and taxonomy.

This is the case of *Ondina* de Folin, 1870, a genus with 20 valid species (MolluscaBase 2018), mainly from European and west African waters but so far not recorded in the Tropical western Atlantic. The nomenclature of *Ondina* was revised by van Aartsen (1984) and the Eastern Atlantic species (from Europe and Africa) were re-de-

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scribed in a series of papers (e.g. van Aartsen 1987, van Aartsen et al. 1996, Peñas et al. 1996, Peñas and Rolán 1999, Warén 1991, Høisæter 2014).

During a visit to the USNM collection, the type series of *Acteon semicingulatus* was examined, which led to its reassessment in the Pyramidellidae, genus *Ondina*, as well as in the review of the previous records of the Pyramidellidae genera *Ondina* and *Evalea* in the Western Atlantic.

## Material and methods

Taxonomic identification of the new material from Brazil was based on conchological comparison with type specimens and with the recent revision by Høisæter (2014). All available material consists of dry shells; in the "Examined material" the number of shells is indicated between brackets.

For detailed examination, shells were prepared following the standard methods to preparation of micromollusc shells for SEM of Geiger et al. (2007) and observed by scanning electron microscopy at the Centro de Microscopia Eletrônica, Departamento de Invertebrados, Museu Nacional/UFRJ, with a JEOL JSM-6390LV microscope.

Measurements were made with the software ImageJ (Rasband 2012).

Institutional Acronyms: ANSP, Academy of Natural Sciences of Drexel University, Philadelphia, USA; MNRJ, Museu Nacional / Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA; YPM, Yale Peabody Museum–Invertebrate Zoology/Yale University, New Haven, Connecticut, USA; USFC, United States Fish Commission, formally known as the United States Commission of Fish and Fisheries. Shell measurements: H, shell height parallel to coiling axis; D, greatest shell width perpendicular to H; h, aperture height (maximum length parallel to coiling axis); d, greatest width of aperture (maximum width perpendicular to coiling axis).

Due to the fire in the Museu Nacional, in September 2018, the non type material of *Ondina semicingulata* was destroyied.

## Systematics

### Family Pyramidellidae Gray, 1840
### Subfamily Odostomiinae Pelseneer, 1928

### Genus *Ondina* de Folin, 1870

*Ondina* de Folin, 1870: 200.

**Type species.** *Ondina semiornata* de Folin, 1872 [= *Ondina warrenii* (Thompson, 1845)] by subsequent designation (van Aartsen 1984: 134). Atlantic coast of France.

### *Ondina semicingulata* (Dall, 1927), comb. n.

Figures 1a–n, 2a–c

*Acteon semicingulatus*: Dall 1927: 19–20; Poirier 1954: 102; Marcus 1974: 319; Abbott 1974: 311.
*Odostomia* (*Evalea*) *ryclea*: Dall 1927: 85. New synonym.

**Type material.** *Ondina semicingulata* – Syntypes: USNM 107913 [5 shells], from type locality; *Odostomia* (*Evalea*) *ryclea* – Holotype: USNM 108365, R/V Albatross, sta. 2415, off Georgia (30°44' N 79°26' W, 805.2 m depth) depth, on broken coral, coarse sand and broken shell bottom).

**Type locality.** R/V Albatross, sta. 2668, off Fernandina, Florida [Cumberland Island, off Georgia], (30°58'30"N, 79°38'30"W, 538 m depth).

**Material examined.** The syntypes and: Off Barbados: 183 m depth, USNM 87264, [1], Blake Expedition; Upper Pliocene (Lower Pinecrest) of Sarasota Co., Florida, USA: Harry Lee Private Collection [1]; Brazil: Amapá state: off Cape Orange, (4°27'54.0"N, 49°58'05.0"W, 160 m depth), MNRJ 26217, [2], R/V Sartro 25 coll., 13/x/2000; off Maracá Island, (2°21'00.0"N, 48°29'54.0"W, 72 m depth), MNRJ 27809, [2], R/V Columbus Iselin coll., 22/x/1991; Ceará state: off Camocim, (2.089S, 41.084W, 390 m depth): MNRJ 27823, [9]; MNRJ 27830, [1], R/V Natureza coll., 30/x/2001; Rio Grande do Norte state: off Touros, (4.861S, 35.134W, 384 m depth), MNRJ 27821, [7], R/V Natureza coll., 24/xi/2001; off Sibaúma, (6.234S, 34.876W, 510 m depth), MNRJ 27834, [2], R/V Natureza coll., 26/x/2001; Bahia state: off Itacaraé, (14.482S, 38.901W, 278 m depth), MNRJ 28264, [1], R/V Natureza coll., 02/vii/2001; off Salvador, (13.238S, 38.578W, 316 m depth), MNRJ 26258, [1], R/V Astro Garoupa, 25/vi/2002; Santa Catarina state: off Itajaí, (26°38'44.9"S, 46°51'54.2"W, 150 m depth), MNRJ 15318, [3], ii/2004.

**Redescription.** Shell small, thin, up to 3.2 mm, width ~50% of length; oblong ovate to biconical; color translucent white; spire regularly conical, ~30° angled, ~40% of shell length. Protoconch heterostrophic, helicoidal, with about one smooth whorl, intorted, oriented ~180° to teleoconch axis, immersed into first teleoconch whorl, with no visible nucleus; width about 210 µm; transition with teleoconch weak, hardly discernible. Teleoconch up to four stepped whorls, each whorl slightly convex, last whorl somewhat globose; suture deep, forming a narrow furrow. Axial sculptured absent, except for growth lines; spiral sculpture with very narrow spiral grooves of variable distribution and number; usually restricted to the periphery, near the area of implantation of the outer lip, extending anteriorly up to about 1/3 of last whorl and on the base; young shells with three-four very thin furrows; some adult shells with up to 20 furrows covering all base surface and extending anteriorly on last whorl more than half of its length, but not reaching anterior suture; adult shells with visible furrows just above suture; spiral furrows covered by microscopic axial threads. Aperture

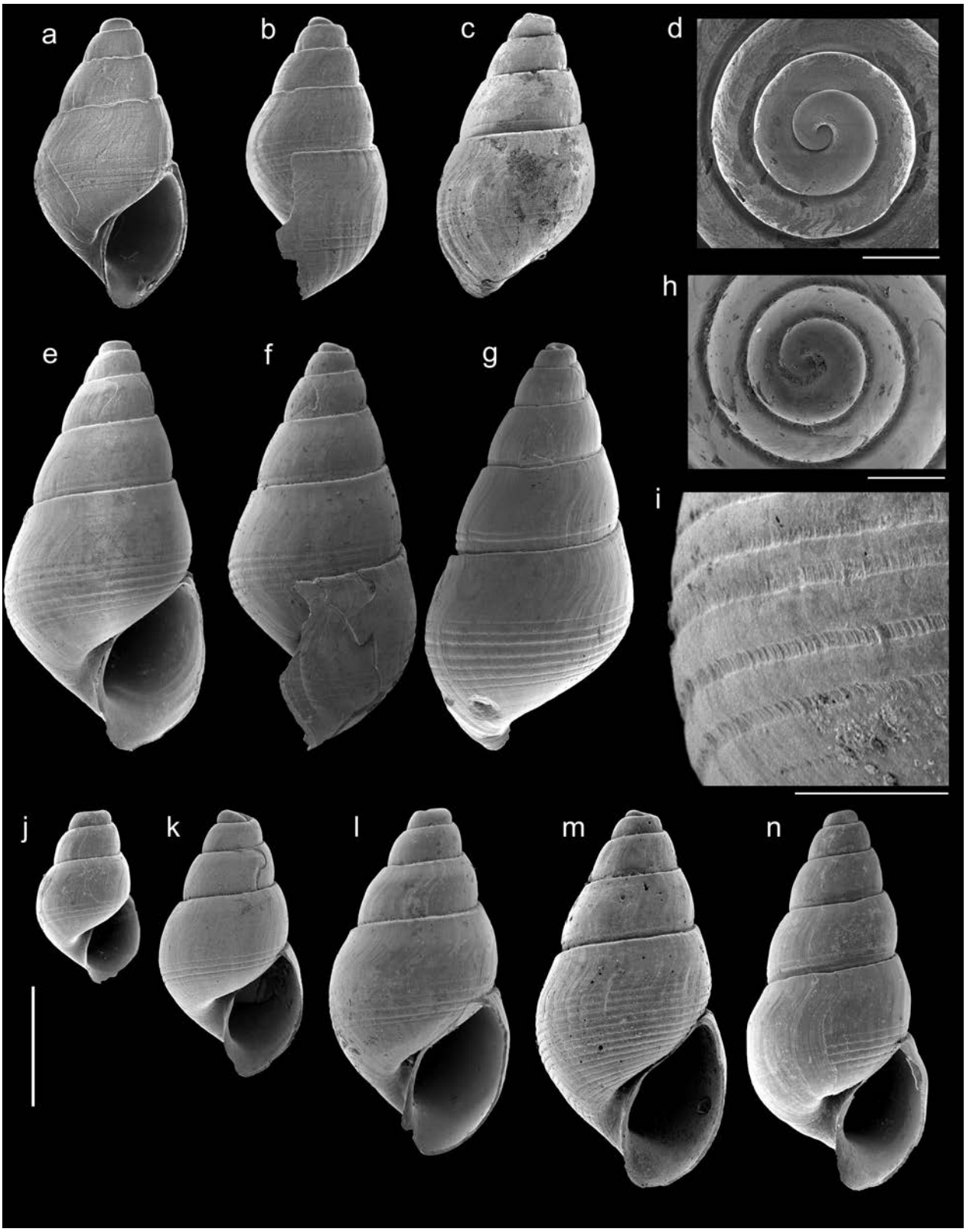


**Figure 1.** Shells of *Ondina semicingulata*. **A.** Syntype (USNM 107913), in apertural view; **B.** same in lateral view; **C.** same in ventral view; **D.** same, detail of protoconch in apical view. **E.** From Rio Grande do Norte state (MNRJ 27821), in apertural view; **F.** same, lateral view; **G.** same, adapertural view; **H.** same, detail of protoconch in apical view; **I.** same, detail of sculpture. **J.** From Brazil, Amapá state (MNRJ 27809), shell #1 in apertural view; **K.** same, shell #2 in ventral view. **L.** From Brazil, Bahia state, MNRJ 28264, in apertural view. **M.** From Brazil, Amapá state (MNRJ 26217), in ventral view. **N.** From Brazil, Rio Grande do Norte state (MNRJ 27834), in apertural view. Scale bars: whole shells (vertical bar): 1 mm; details (horizontal bar): 200 µm.

elliptical-oblong, length about half of last whorl length, anteriorly elongated-rounded, posteriorly narrow and somewhat acute. Columellar margin slightly concave, without tooth. Outer lip thick. Umbilicus deep and wide, ranging from circular to wide chink.

**Geographic distribution.** USA: Florida (type locality); Barbados (present study); Brazil: Amapá, Ceará, Rio Grande do Norte, Bahia and Santa Catarina states (present study).

**Remarks.** Except for the features of soft parts, absent in the type specimens of *Acteon semicingulatus*, all characteristics agree with the diagnose provided by Høisæter (2014) for *Ondina*, including the oblong-ovate shell, oblong aperture and intorted protoconch. In comparison to the Eastern Atlantic species, which usually have more elongate shells, *O. semicingulata* has a wider shell, with biconical general shape. The original allocation in Acteon is rejected since Acteonids have a solid shell, with columellar tooth and rounded protoconch (Valdés 2008).

Høisæter (2014) discussed the high variability of the surface of the shell, being smooth or with variable incised spirals. According to this author, in a single species, the spirals may cover uniformly the whole shell, they may be confined to the lower half of each teleoconch whorl or the shell may be smooth and shiny.

*Ondina semicingulata* (Figures 1a–n, 2a–c) is strongly sculptured with spiral furrows (Figure 1i) restricted to the anterior 1/3 to half of the whorls. On earlier whorls, these lines are visible only above suture (Figure 1e–g), but on the last whorl, they are visible also below the periphery of whorl (Figure 1e), extending to the base (Figure 1g). The amount of lines on the last whorl (including the base) is variable, both ontogenetically and between shells with the same number of whorls.

Figure 1j–n illustrates shells of *O. semicingulata* in a growth series. The amount of spiral lines and strength of the spiral sculpture increase from shells of younger specimens (Fig. 1j–k) to adult ones (Fig. 1l–n). Besides these three adult specimens, with same number of whorls, exhibit variation in the sculpture, covering almost entire last whorl of the shell in Figure 1m.

*Odostomia* (*Evalea*) *ryclea* with type locality off Goergia is here considered a synonymy name of *Ondina semicingulata*, since it has identical shell shape and protoconch. Despite the eroded shell surface of the holotype (Figure 2a–c), the original description (Dall 1927) states the presence of spiral lines in the teleoconch whorls.

### *Ondina striata* (Verrill, 1880), comb. n.

Figure 2d–h

*Aclis striata*: Verrill 1880: 377; 1882: 528, pl. 58, fig. 13; Johnson 1989: 66, pl. 11, fig. 7.
*Odostomia* (*Menestho*) *striata*: Bartsch 1911: 435.

**Type material.** Lectotype (designated by Johnson 1989: 66): YPM 15757, missing; paralecotype: USNM 44820, USFC sta. 873, 183 m depth, off Newport, Rhode Island.

**Type locality.** Bay of Fundy, near Eastport, Maine, Verrill coll. 1868.

**Material examined.** Photographs of the paralectotype and of: USFC sta. 863, 33 m, Vineyard Sound, Rhode Island: YPM 15704 [1]; ANSP 102517 [1].

**Geographic distribution.** USA: Rhode Island (type locality), Maine.

**Remarks.** *Aclis striata* was described (Verrill 1880) based on two shells: from shallow water in the Bay of Fundy; and from deep-water off Newport, Rhode Island, by the USFC. Two years later, Verrill (1882) referred to the same two shells, adding station number information (USFC sta. 873) to the the deep-water shell. In this work, Verrill (1882: pl. 58, fig. 13) presented the drawing of a shell without indicating which one of the two syntypes.

The type material of *Aclis striata* was studied by Johnson (1989: 66, pl. 11, fig. 7), who designated and figured the lectotype (YPM 15757, from the Bay of Fundy), and listed YPM 15704, from USFC sta. 873, as a paralectotype (but see comments below about its type status). After searching the YPM Molluscan Collection (E. Lazo-Wasen pers. comm.), it was noticed that the lectotype was missing (the vial was empty) and the label of the supposed paralectotype YPM 15704 (Figure 2h) mentions 'USFC sta. 863' (which is a shallow water station in the Vineyards Sounds, 33 m depth).

According to Johnson (1989: 15), Verrill sent the samples (types and other specimens) of the species described by himself to the National Museum of Natural History (USNM), keeping nevertheless, some duplicate specimens with him, which he later sold to YPM (Lazo-Wasen pers. comm.). Thus, it seems that the two original syntypes of *Aclis striata* were split by Verrill between the USNM and the YPM collections.

This is corroborated by the original label of USNM 44820 (Figure 2g) that states: "*Aclis striata* V. Off Newport, R. I. U.S.F.C. 1880". This label has a hand-written indication of "figd. type" that corresponds to Verrill´s calligraphy (E. Lazo-Wasen pers. comm.); such "figd. type" (Figure 2d–e) matches perfectly the figured syntype by Verrill (1882: pl. 58, fig. 13), and thus, it must be considered the paralectotype from deep-water (USFC sta. 873), while the missing lectotype (figure in Johnson 1989: pl. 11, fig. 7), by the other hand, is a smaller shell.

Thus, the shell YPM 15704 (Figure 2h), considered by Johnson to be a paralectotype has no type status. As indicated by its label, it was dredged by USFC is sta. 863, in which an additional shell (ANSP 102517) was also collected.

The only remaining question is the reference to USFC sta. 863 in the original label of specimen USNM 44820 (Figure 2g). We believe that such information was mistakenly inserted by someone later since it has a different

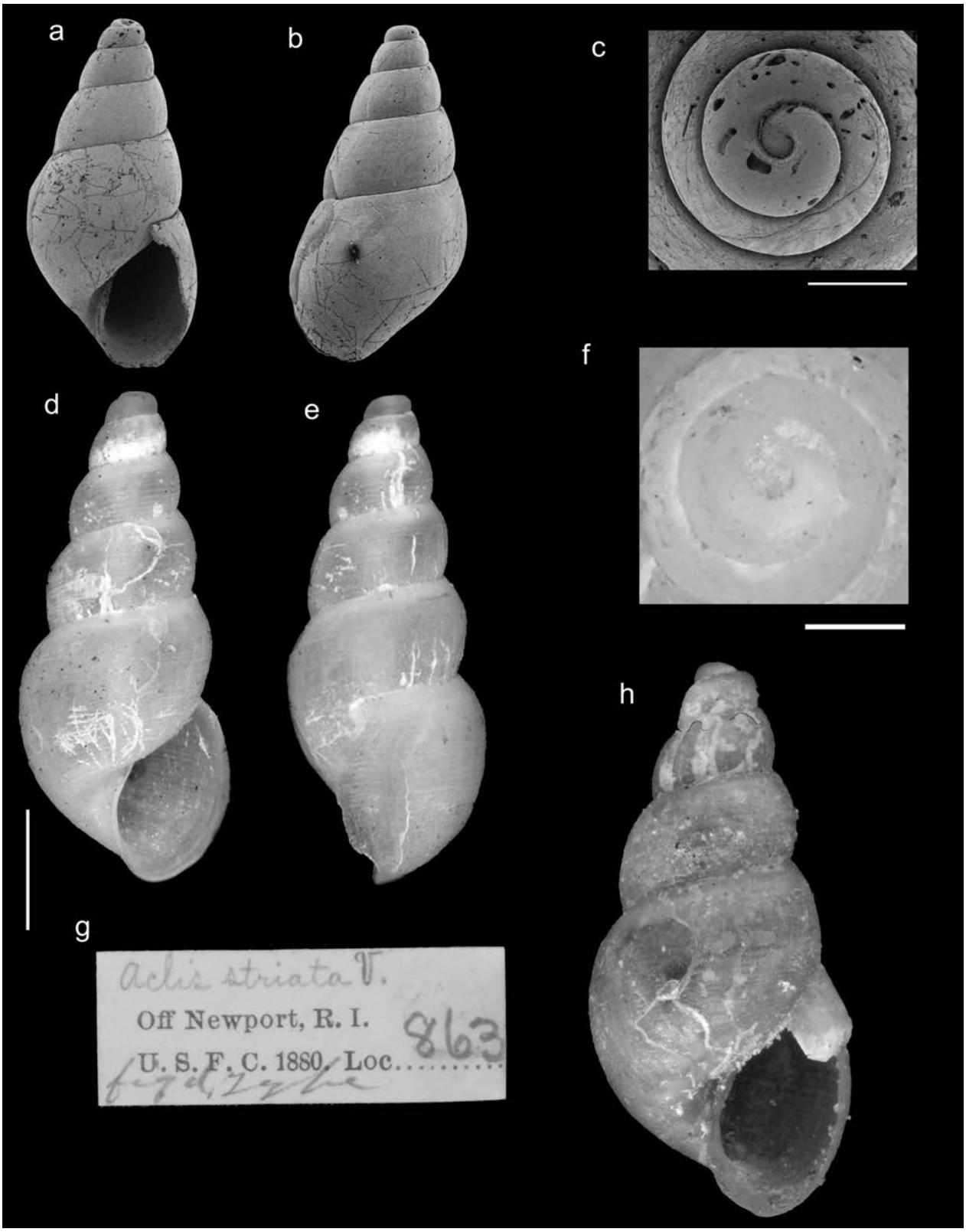


**Figure 2. A.** Shell of *Odostomia* (*Evalea*) *ryclea*, holotype (USNM 108365) in ventral view; **B.** same, in adapertural view; **C**. same, detail of protoconch in apical view. **D.** Shell of *Ondina striata*, paralectotype (USNM 44820), in apertural view; **E.** same, in adapertural view; **F.** same, detail of protoconch in apical view. **G.** Original label of USNM 44820, showing a hand-written indication of "figd. type" by A. E. Verrill.; **H.** *Ondina striata*, a non-type shell (YPM 15704) erroneously indicated as lectotype by Johnson (1989). Scale bars: whole shells (vertical bar): 1 mm; detail (horizontal bar): 200 µm.

handwriting and Verrill (1880, 1882) did not list material from that station.

*Ondina striata* was originally described as belonging to *Aclis* due to its spiral striae and was later transferred to the Pyramidellidae genus *Odostomia* (*Menestho*) by Bartsch (1911: 435) without any comments on this taxonomic rearrangement. The species has all features that characterize the genus *Ondina* (Høisæter 2014) and is very similar to the type species of the genus.

### *Ondina hendersoni* (Bartsch, 1909), comb. n.

Figure 3a, b

*Odostomia* (*Iolaea*) *hendersoni*: Bartsch 1909: 101, pl. 13, fig. 43.

**Type material.** Holotype: USNM 203813.

**Type locality.** Woods Hole, Massachusetts.

**Material examined.** Photographs of the holotype.

**Geographic distribution.** USA: Massachusetts (type locality).

**Remarks.** The holotype of *Ondina hendersoni* (Figure 3a, b) matches the general features of the genus *Ondina*, being however, larger with more globose whorls. The typical spiral lines of the genus are very numerous in the figured holotype and especially evident on the lower half of the teleoconch whorls.

This species was recorded from Texas by Odé (1994) as *Amoura* cf. *hendersoni* but the drawing of the shell (Odé 1994: 48, fig. 4) exhibits a slender shell, with few spiral lines above suture, which resembles *O. stocki* (see below).

### *Ondina stocki* (De Jong & Coomans, 1988), comb. n.

Figure 3c–e

*Evalea stocki*: De Jong and Coomans 1988: 124, pl. 6, fig. 655.
?*Amoura hendersoni* auct. non Bartsch 1909: Gundersen (1998: 13, figured).
?*Amoura* cf. *hendersoni* auct. non Bartsch 1909: Odé (1994: 39, fig. 4).

**Type material.** Holotype: ZMA.MOLL. 138319 [original publication indicates ZMA 3.87.105]; two paratypes: ZMA.MOLL. 138320.

**Type locality.** Curaçao/Aruba [according to Jong and Coomans (1988: 124), the holotype was selected from mixed material from those two localities].

**Material examined.** Photographs of the types.

**Geographic distribution.** Curaçao/Aruba (type locality).

**Remarks.** *Ondina stocki* (Figure 3c–e) was originally described in *Evalea*, based on a sample of shells from Aruba/Curaçao; the original illustration is a simple drawning that precludes a precise generic allocation, but the photographs of the holotype (Figure 3c) and of a young and an adult paratypes (Figure 3d–e) clearly shows that this species belong in the genus *Ondina*. The shell shape is similar to *Ondina hendersoni*, but the shell is narrower, and the spiral grooves are less numerous.

Lee (2009) recorded *Evalea stocki* from Jacksonville Beach, Florida, (USA) based on a young specimen. The author, in the same work, referred to a color illustration of that species, published by Gundersen (1998), but named by Gundersen (1998) as *Amoura hendersoni*. The figured shells from Sanibel Island in Gundersen (1998) and that from Jacksonville in Lee (2009) clearly can be ascribed to the genus *Ondina*, but the images provided by the authors as well as the drawing of *Amoura* cf. *hendersoni* by Odé (1994), do not allow a precise separation between *O. stocki* or *O. hendersoni*. Also, the simple drawing of *Amoura* cf. *hendersoni* from Texas in Odé (1994) does not allow a conclusive identification, in spite of the pattern of spiral grooves resembling that found in *O. stocki*.

Therefore, we consider that the only confirmed occurrence of *O. stocki* is that restricted to the type locality area.

### *Ondina emeryi* (Bartsch, 1955), comb. n.

*Odostomia* (*Evalea*) *emeryi* Bartsch, 1955: 84, pl. 17, fig. 1.
*Evalea emeryi*: Odé and Speers (1972: 11, figs 13–14); Tunnel Jr. et al. (2010: 364, figured); Rosenberg et al. (2009: 672).
*Amoura emeryi*: Odé (1994: 40, fig. 5).

**Type material.** Holotype: USNM 561672 (Bartsch 1955: pl. 17, fig. 1); paratype: ANSP IP 31335. All from type locality.

**Type locality.** Pliocene of North St. Petersburg, Florida (type locality); Texas (Odé and Speers (1972), Odé (1994), Tunnel Jr. et al. (2010), Rosenberg et al. (2009).

**Remarks.** This species was originally described from the Pliocene of Florida and later recorded from the Gulf of Mexico by Odé and Speers (1972), Odé (1994), Tunnel Jr. et al. (2010) and Rosenberg et al. (2009).

**Geographic distribution.** USA: Pliocene of Florida (type locality);

## Discussion

Knowledge of species richness, geographic distribution and accurate taxonomic status of marine molluscan fauna from Brazil is still far from satisfactory. Traditionally, the marine molluscan diversity was compiled in catalogues (e.g. Rios 1994, 2009), with the main purpose of working as identification guides, with a brief description of each taxa, and an image. Despite the recent contributions to the Brazilian molluscan diversity (e.g. Pimenta et al. 2004a), several taxa remain poorly known, and the revision of

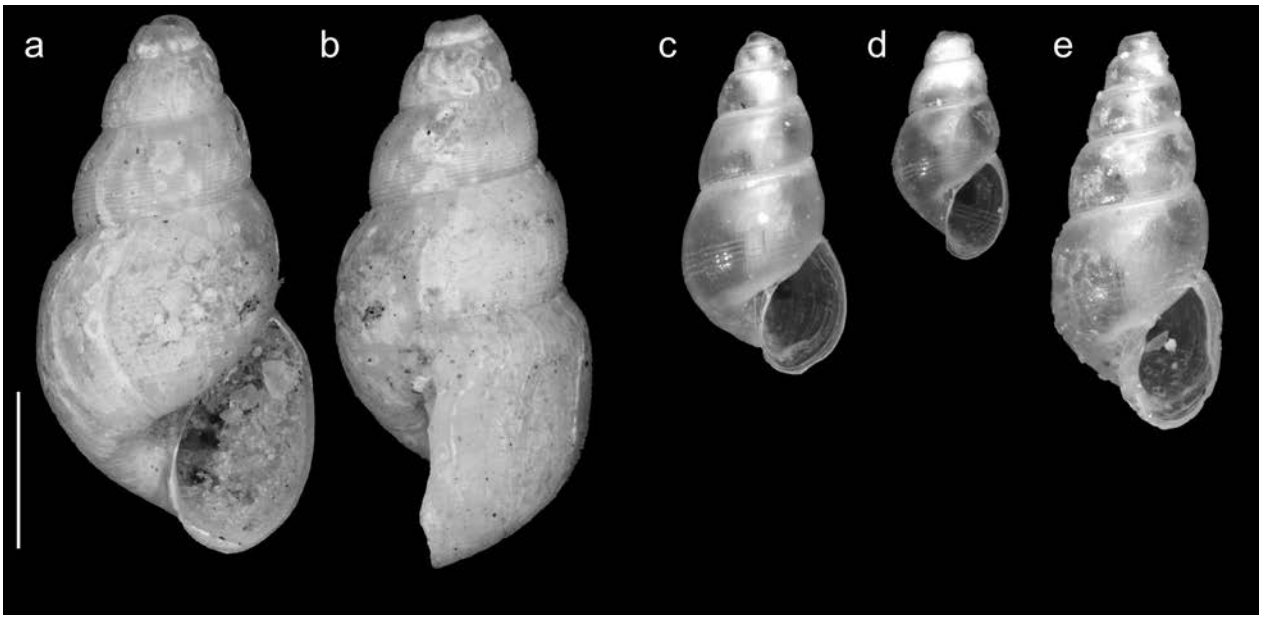


**Figure 3. A.** Shell of *Ondina hendersoni*, holotype (USNM 203813) in apertural view; **B.** same, in lateral view. **C.** Shell of *Ondina stocki*, holotype (ZMA.MOLL. 138319) in apertural view [photo: Naturalis, Jeroen Goud. **D.** *Ondina stocki*, paratype (ZMA. MOLL. 138320), smaller shell in apertural view; **E.** same, bigger shell in apertural view. Scale bar: 1 mm.

genera usually gives rise to the discover of new records and/or new species (e.g. Fernandes and Pimenta 2011). This is especially true to the marine micromollusks, as discussed by Pimenta and Geiger (2015).

In the last two decades, the Pyramidellidae species richness from Brazil raised from 35 previously recorded species (Rios 1994) to 94 species, including several new species, after the works by Pimenta (2012), Pimenta and Absalão (2001, 2002, 2004a, 2004b), Pimenta et al. (2008, 2009, 2011). Nevertheless, there is still many species of this family to be described, especially from deep-water (Pimenta pers. obs.).

On the other hand, acteonids also lack dedicated studies in the Western Atlantic, where few works have been published (Rehder 1939, Marcus 1972, 1974, Cunha 2011, Zelaya et al. 2011). Interestingly, recent studies have revealed new genera (Bouchet 1975, Salvador and Cunha 2016) and species (Smriglio and Mariottini 1996, Valdés 2008, Cunha and Simone 2018) from around the world.

For both families, re-examination of type material is imperative and in the case of *Acteon semicingulatus*, its reassignment to the Pyramidellidae revealed the first record of the genus *Ondina* in the Western Atlantic.

## On the occurrence of *Ondina* in the Western Atlantic

The most complete and recent account on the taxonomy of *Ondina* was provided by van Aartsen (1987), Waren (1991) and Høisæter (2014), based on European species. Høisæter (2014) figured the type species *Ondina semiornata* de Folin, 1870 (= *Ondina warrenii*) and discussed the intraspecific variation found in the genus.

According to the works by van Aartsen (1987), van Aartsen et al. (1998), Peñas et al. (1996), Peñas and Rolán (1999), Warén (1991), and Høisæter (2014), *Ondina* is mainly distributed in the Eastern Atlantic, both in temperate and tropical latitudes, including the Northern European Seas, the Lusitanian, the Mediterranean Sea and West African Transition Provinces, while a single species is known from the temperate northern Pacific, in the coast of Japan (Hori and Fukuda 1999).

The present records of *Ondina semicingulata*, *O. emeryi*, *O. stocki* and *O. hendersoni* in the western Atlantic broadens the distribution of the genus including geographical areas that go from Georgia (north-western Atlantic) and south Brazil (south western Atlantic), which gives the genus a wider latitudinal range in the western Atlantic when compared to its distribution in the eastern part of the Atlantic. *Ondina semicingulata*, besides presenting a wider distribution in the western Atlantic, also presents a larger bathymetric range from 72–500 m depth. *Ondina mosti* van Aartsen, Gittenberger & Goud, 1998 is the only eastern Atlantic species with similar bathymetry (119–405 m), while the other species are restricted to littoral and continental shelf depths (Aartsen et al. 1998).

## Evaluation of the occurrence of *Evalea* in the Western Atlantic

Aartsen (1987) evaluated the generic allocation of several European odostomids species previously included in *Evalea* and based on the absence of a well-developed columellar tooth, trasnferred them to *Ondina*. This criterion was followed by Høisæter (2014).

According to Aartsen and Menkhorst (1996: 51–52), *Evalea* was confused by Nordsieck (1972), most likely because of the lack of an illustration of the type species *Odostomia* (*Evalea*) *elegans* A. Adams, 1860. Aartsen and Menkhorst (1996: fig. 11) designated a neotype for the type species of *Evalea* which shows a shell with

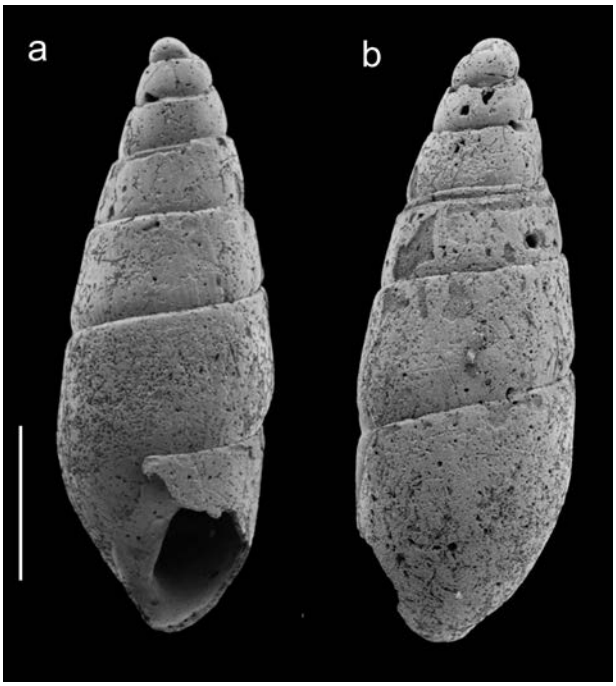


**Figure 4. A.** Shell of *Odostomia fernandina*, holotype (USNM 108053) in apertural view; **B.** same, in adapertural view. Scale bar: 1 mm.

coarse spiral striae covering all whorls and a visible columellar tooth.

According to MolluscaBase (2018), *Evalea* has 18 species and it is not present in European waters (e.g. East Atlantic and Mediterranean), being restricted to the Pacific coast of Japan, New Zealand, Indian South Africa, and to the western Atlantic.

In the present work, an attempt was made to evaluate the presence of *Evalea* in the western Atlantic by checking the previous species recorded in that genus and comparing them to the type species as illustrated by Aartsen (1996). According MolluscaBase (2018), the following species are recorded from the western Atlantic: *Evalea fernandina* (Bartsch, 1927), *E. ryclea* (Bartsch, 1927), *E. emeryi* (Bartsch, 1955), and *E. stocki* De Jong & Coomans, 1988; besides that, Odé (1994) recorded an aditional taxon as "*Evalea* sp. indet. A".

As demonstrated above, *Evalea stocki*, *E. ryclea* (= *O. semicincgulata*) and *E. emeryi* belong in fact to the genus *Ondina*. As for the other species we do not have at present enough evidence to critically discuss their generic placement.

*Odostomia* (*Evalea*) *fernandina* (Dall, 1927: 85) was originally described in *Odostomia* and it has an elongate shell with whorls with an almost rectilinear outline. Although the holotype, USNM 108053, (Figure 4a, b) is eroded and has a partially broken outer lip, it is possible to distinguish from *Evalea* by the absence of spiral sculpture and columellar tooth. However, it does not seem to belong to *Ondina*, because of the different protoconch which is not fully immersed. Thus, until further evidence is available we suggest this species to be kept in the genus *Odostomia*. The record of *Evalea* sp. A by Odé (1994: 46, fig. 8) is considered doubtful because in the drawing provided by the author, characters that could relate the specimen to *Evalea* such as the spiral sculpture throughout the shell and the presence of a columellar fold, are difficult to interpret.

Therefore, based on previous studies (e.g Høisæter 2014) and our own results it is here suggested that the genus *Evalea* is absent in the Atlantic Ocean.

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## References

Abbott RT (1974) American Seashells. The Marine Mollusca of the Atlantic and Pacific coasts of North America. Van Nostrand Reinhold Company, New York.

Adams A (1860) Mollusca Japonica: New species of *Odostomia*. The Annals and magazine of natural history 4 série 6(31–36): 20–22. https://biodiversitylibrary.org/page/2267089

Albano PG, Sabelli B, Bouchet P (2011) The challenge of small and rare species in marine biodiversity surveys: microgastropod diversity in a complex tropical coastal environment. Biodiversity and Conservation 20: 3223–3237. https://doi.org/10.1007/s10531-011-0117-x

Bartsch P (1909) Pyramidellidae of New England and the adjacent region. Proceedings of the Boston Society of Natural History 34(4): 67–113.

Bartsch P (1911) New mollusks of the genus *Aclis* from the North Atlantic. Proceedings of the United States National Museum 40(1829): 435–438. [pl. 59]

Bartsch P (1955) The pyramidellid mollusks of the Pliocene deposits of North St. Petersburg, Florida. Smithsonian miscellaneous collections 125(2): 1–102.

Bouchet P (2011) Pyramidellidae. MolluscaBase (2018). [accessed through: World Register of Marine Species. http://www.marinespecies.org/aphia.php?p=taxdetails&id=162 on 2018–01–23]

Bouchet P (1975) Opisthobranches de profondeur de l'Océan Atlantique: 1. Cephalaspidea = Deep-sea opisthobranches from the Atlantic Ocean: 1. Cephalaspidea. Cahiers de Biologie Marine 16(3): 317–365.

Cunha CM (2011) A new species of *Acteon* (Opisthobranchia: Acteonidae) from Northeast Brazil. Zoologia 28: 229–232. https://doi.org/10.1590/S1984-46702011000200012

Cunha CM, Simone LRL (2018) A new species of the genus *Rapturella* (Gastropoda: Acteonidae) from southeast Brazil. Zootaxa. [in press]

Dall WH (1927) Small shells from dredgings off the southeast coast of the United States by the United States Fisheries Steamer 'Albatross' in 1885 and 1886. Proceedings of the United States National Museum 70(2667): 1–134. https://doi.org/10.5479/si.00963801.70-2667.1

de Folin L (1870) D'une méthode de classification pour les coquilles de la famille des Chemnitzidae. Annales de la Société Linneenne de Maine-et-Loire 12: 191–202. http://www.biodiversitylibrary.org/page/34137448

Dinapoli A, Klussmann-Kolb A (2010) The long way to diversity – phylogeny and evolution of the Heterobranchia (Mollusca: Gastropoda). Molecular Phylogenetics and Evolution 55: 60–76. https://doi.org/10.1016/j.ympev.2009.09.019

Fernandes MR, Pimenta AD (2011) Taxonomic review of *Metaxia* (Gastropoda: Triphoridae) from Brazil, with description of a new species. Zoologia 28(6): 819–830. https://doi.org/10.1590/S1984-46702011000600016

Geiger DL, Marshall BA, Ponder WF, Sasaki T, Warén A (2007) Techniques for collecting, handling, preparing, storing and examining small molluscan specimens. Molluscan Research 27(1) :1–50.

Gundersen R (1998) The seashells of Sanibel and Captiva Islands. Published privately, Racine, Wisconsin, 1–32.

Høisæter T (2014) The Pyramidellidae (Gastropoda, Heterobranchia) of Norway and adjacent waters. A taxonomic review. Fauna Norv 34: 7–78. https://doi.org/10.5324/fn.v34i0.1672

Hori S, Fukuda H (1999) New species of the Pyramidellidae (Orthogastropoda: Heterobranchia) from the collections of the Yamaguchi Museum and the Hagi City Museum. Venus 58(4): 175–190.

Johnson RI (1989) Molluscan taxa of Addison Emery Verrill and Katharine Jeannette Bush, including those introduced by Sanderson Smith and Alpheus Hyatt Verrill. Occasional papers on mollusks 5(67): 1–144.

Jong KM de, Coomans HE (1988) Marine Gastropods from Curacao, Aruba and Bonaire. E. J. Brill, Leiden, 261 pp. [47 pls]

Lee HG (2009) Marine shells of Northeast Florida. Shell Club, Jacksonville, 204 pp. [19 pls]

Marcus E (1972) On some Acteonidae (Gastropoda, Opisthobranchia). Papéis Avulsos de Zoologia 25(19): 167–188.

Marcus E (1974) On some Cephalaspidea (Gastropoda: Opisthobranchia) from the Western and middle Atlantic warm waters. Bulletin of Marine Science 24(2): 300–371.

MolluscaBase (2018) MolluscaBase. http://www.molluscabase.org [2018-09-18]

Nordsieck F (1972) Die Europäischen Meeresschnecken Opisthobranchia mit Pyramidellidae; Rissoacea. Gustav Fischer, Stuttgart, 327 pp.

Odé H, Speers AB (1972) Notes concerning Texas beach shells – Superfamily Pyramidellacea, Part II. The odostomiid genera and the genus *Longchaeus*. Texas conchologist 9(1): 11–17.

Odé H (1994) Distribution and records of the marine Mollusca in the Northwest Gulf of Mexico (A continuing monograph: Superfamily Pyramidelloidea, Family Pyramidellidae Part III). Texas conchologist 30(2): 38–50.

Peñas A, Templado J, Martínez JL (1996) Contribucíon al conocimiento de los Pyramidelloidea (Gastropoda: Heterostropha) del Mediterráneo español. Iberus 14: 1−82.

Peñas A, Rolán E (1999) La familia Pyramidellidae Gray, 1840 (Mollusca, Gastropoda, Heterostropha) en Africa Occidental. 4. los géneros *Megastomia, Odostomia, Noemiamea* y *Syrnola*. Iberus 5(suppl): 1–150.

Peñas A, Rolán E (2010) Deep water Pyramidelloidea of the tropical South Pacific: *Turbonilla* and related genera. In: Gofas S (Ed.) Tropical Deep Sea Benthos 26. Mémoires du Muséum National d'Histoire Naturelle 200: 1–436.

Pimenta AD (2012) Four new species and two new records of Odostomiinae (Gastropoda: Pyramidellidae) from Brazil. Zoologia 29(5): 439–450. https://doi.org/10.1590/S1984-46702012000500007

Pimenta AD, Absalão RS (2001a) Taxonomic revision of the species of *Turbonilla* Risso, 1826 (Gastropoda, Heterobranchia, Pyramidellidae) with type localities in Brazil, and description of a new species. Basteria 65(1–3): 69–88.

Pimenta AD, Absalão RS (2001b) The genera *Bacteridium* Thiele, 1929 and *Careliopsis* Mörch, 1875 (Gastropoda: Pyramidellidae) from the east coast of South America. Bollettino malacologico 37(1–4): 41–48.

Pimenta AD, Absalão RS (2002) On the taxonomy of *Turbonilla puncta* (C. B. Adams, 1850) (Gastropoda, Pyramidellidae), with the description of a new species from Brazil and remarks on other western Atlantic species. Zootaxa 78: 1–16. https://doi.org/10.11646/zootaxa.78.1.1

Pimenta AD, Absalão RS (2004a) Fifteen new species and ten new records of *Turbonilla* Risso, 1826 (Gastropoda, Heterobranchia, Pyramidellidae) from Brazil. Bollettino malacologico 39(5–8): 113–140.

Pimenta AD, Absalão RS (2004b) Review of the genera *Eulimastoma* Bartsch, 1916 and *Egila* Dall & Bartsch, 1904 (Mollusca, Gastropoda, Pyramidellidae) from Brazil. Zoosystema 26(2): 157–17.

Pimenta AD, Absalão RS, Alencar AS (2000) *Odostomella carceralis* spec. nov. from Ilha Grande, SE Brazil (Gastropoda: Heterobranchia, Pyramidellidae). Basteria 64: 65–70.

Pimenta AD, Santos FN, Absalão RS (2008) Review of the genera *Ividia, Folinella, Oscilla, Pseudoseilla, Tryptichus* and *Peristichia* (Gastropoda, Pyramidellidae) from Brazil, with descriptions of four new species. Veliger 50(3): 171–184.

Pimenta AD, Absalão RS, Miyaji C (2009) A taxonomic review of the genera *Boonea, Chrysallida, Parthenina, Ivara, Fargoa, Mumiola, Odostomella* and *Trabecula* (Gastropoda, Pyramidellidae, Odostomiinae) from Brazil. Zootaxa 2049: 39–66.

Pimenta AD, Geiger DL (2015) Taxonomic revision of the Anatomidae (Mollusca: Gastropoda: Vetigastropoda) from Brazil, with description of four new species. Malacologia 59(1): 135–175. https://doi.org/10.4002/040.059.0109

Pimenta AD, Santos FN, Absalão RS (2011) Taxonomic revision of the genus *Eulimella* (Gastropoda, Pyramidellidae) from Brazil, with description of three new species. Zootaxa 3063: 22–38.

Poirier HP (1954) An up-to-date systematic list of 3200 seashells from Greenland to Texas: translation, explanation, and gender of their names. Brooklyn[?], 434 pp.

Rasband WS (2012) ImageJ. U. S. National Institutes of Health, Bethesda.

Rehder HA (1939) New marine mollusks from the Western Atlantic. Nautilus 53(1):16–21

Rios E (1994) Seashells of Brazil. Second edition. Editora da Fundação Universidade Rio Grande, Rio Grande, 368 pp.

Rios E (2009) Compendium of Brazilian sea shells. Evangraf, Rio Grande, 668 pp.

Rosenberg G, Moretzsohn F, García EF (2009) Gastropoda (Mollusca) of the Gulf of Mexico. In: Felder DL, Camp DK (Eds) Gulf of Mexico-Origins, Waters, and Biota. Biodiversity. Texas A&M University Press, College Station, 579–699.

Salvador R, Cunha CM (2016) Taxonomic revision of the fossil genera *Bulimactaeon, Hemiauricula* (= *Liocarenus*) and *Nucleopsis*, with description of a new Recent genus and species (Gastropoda: Heterobranchia: Acteonidae). Journal of Molluscan Studies 82(3): 472–483. https://doi.org/10.1093/mollus/eyw010

Smriglio C, Mariottini P (1996) Central Tyrrhenian Sea Mollusca: XI. Description of *Callostracon tyrrhenicum* sp. nov. (Gastropoda, Acteonidae) and remarks on other Mediterranean species of the family. Basteria 60: 183–193.

Tunnell Jr JW, Andrews J, Barrera NC, Moretzsohn F (2010) Encyclopedia of Texas seashells. Identification, ecology, distribution & history. Texas A&M University Press, College Station, 512 pp.

Valdés Á (2008) Deep-sea "cephalaspidean" heterobranchs (Gastropoda) from the tropical southwest Pacific. In: Héros V, Cowie RH, Bouchet P (Eds) Tropical Deep-Sea Benthos, Volume 25. Annales du Muséum d'histoire naturelle 196, 587–792.

van Aartsen JJ (1984) The Pyramidellid genera described by the Marquis L. de Folin. Bollettino malacologico 20: 5–8.

van Aartsen JJ (1987) European Pyramidellidae: III. *Odostomia* and *Ondina*. Bollettino malacologico 23(1–4): 1–34.

Van Aartsen JJ, Menkhorst HPMG (1996) Nordsieck's Pyramidellidae (Gastropoda Prosobranchia): a revision of his types. Part 1: The genera *Chrysallida, Ondina* (s.n. *Evalea*) and *Menestho*. Basteria 60: 45–56.

Van Aartsen JJ, Gittenberger E, Goud J (1998) Pyramidellidae (Mollusca, Gastropoda, Heterobranchia) collected during the Dutch CANCAP and MAURITANIA expeditions in the south-eastern part of the North Atlantic Ocean (part 1). Zoologische Verhandelingen 321: 1–57.

Verrill AE (1880–1881) Notice of recent addition to the marine Invertebrata of the northeastern coast of America, with descriptions of new genera and species and critical remarks on others. Part II - Mollusca, with notes on Annelida, Echinodermata, etc, collected by the United States Fish Commission. Proceedings of the United States National Museum 3: 356–409. [pp. 356–400 issued December 1880, 401–409 January 1881]

Verrill AE (1882) Catalogue of marine Mollusca added to the fauna of the New England region, during the past ten years. Transactions Connecticut Academy of Arts and Sciences 5(2): 447–587. [pl. 57–58]

Zelaya DG, Schejter L, Ituarte C (2011) *Neactaeonina argentina*, new species, and family placement of the genus *Neactaeonina* Thiele, 1912 (Mollusca: Gastropoda). Malacologia 53(2): 251–263. https://doi.org/10.4002/040.053.0204

Warén A (1991) New and little known Mollusca from Iceland and Scandinavia. Sarsia 76(1–2): 53–124. https://doi.org/10.1080/00364827.1991.10413466

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# *Neotenorchestia* Wildish, 2014 is a junior synonym of *Orchestia* Leach, 1814

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## Abstract

*Neotenorchestia* Wildish, 2014 and *N. kenwildishi* Wildish, 2014 (Crustacea, Amphipoda, Talitridae) are junior synonyms of *Orchestia* Leach, 1814 and *Orchestia mediterranea* A. Costa, 1853 respectively, based on revised genetic evidence.

## Key Words

*Neotenorchestia kenwildishi* Wildish, 2014
junior synonym

## Introduction

In 2011 a talitrid amphipod living in floating driftwood was discovered in the Swale, U.K., (Wildish et al. 2012). Considerable difficulty was experienced in determining the taxonomic and genetic identity of the specimens in driftwood ("unknown" taxon), although they were genetically closely related to *Orchestia mediterranea* A. Costa, 1853 (Wildish et al. 2012; Pavesi et al. 2014).

Detailed genetic data are routinely submitted to GenBank, including ours (Wildish et al. 2012, Pavesi et al. 2014), to be freely available to other researchers. After one such enquiry it was clear that a calculation error had been made in the CO1 divergence estimate between *O. mediterranea* and the "unknown" taxon, based on Kimura two-parameter distance (Table 6 in Pavesi et al. 2014). The incorrect value given in the latter of K2P = 2% was due to a calculation error and should have been K2P = 0.2%. For the same pairwise comparison both nuclear genes studied (18S and 28S) showed no difference (Pavesi et al. 2014).

Considering the recognized error in Pavesi et al. (2014) I have re-evaluated the status of the "unknown" taxon from the Swale. U.K.

## Review of literature related to *Neotenorchestia*

The "unknown" taxon was described as a new monotypic genus and species: *Neotenorchestia kenwildishi* Wildish, 2014, based on:

1. Detailed relative growth data, inclusive of slower growth, fewer moult stages per life history and sexualization beginning at an earlier moult stage (Wildish et al. 2012; Pavesi et al. 2014)
2. Genetic differences, based on the mitochondrial gene CO1 with a K2P divergence difference between *Orchestia mediterranea* A. Costa, 1853 and the "unknown taxon" from the Swale, U.K., of 2% (Pavesi et al. 2014).

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The corrected interpretation of the genetic data establishes that there is no significant genetic difference between *O. mediterranea* and the "unknown taxon". Thus, only the growth differences listed under 1 remain.

A possible explanation for the growth differences is that *O. mediterranea* can acclimate to living on, and in, driftwood. Acclimation to driftwood in *O. mediterranea* may involve all the relative growth changes listed under 1.

A precedent for driftwood acclimation among Talitridae has been established in another wrack generalist: *Platorchestia platensis* (Krøyer, 1845). This species can live indefinitely in driftwood, but it involves slower growth and reduced resting metabolic rate (Wildish and Robinson 2016; Wildish et al. 2018). Acclimation to driftwood in a secondary ecotope, involving reduced metabolic rate and dwarfism, may be a general phenomenon among wrack generalist talitrids, such as *O. mediterranea*.

## Conclusions

Given the corrected interpretation of the genetic data in Pavesi et al. (2014), as outlined above, *Neotenorchestia kenwildishi* Wildish, 2014, becomes a junior synonym of *O. mediterranea* A. Costa, 1853.

For the same reason the the monotypic genus *Neotenorchestia* Wildish, 2014 becomes a junior synonym of *Orchestia* leach, 1853.

The immature male (NHMUK 2014.397) and the 9 juvenile specimens (NHMUK 2014 398-406) submitted as type specimens of *Neotenorchestia kenwildishi* Wildish, 2014, to the Natural History Museum, London, U.K., in support of *Neotenorchestia* Wildish, 2014, are re-labelled as *O. mediterranea* A. Costa 1853, driftwood morphological variant.

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## References

Pavesi L, Wildish DJ, Gasson P, Lowe M, Ketmaier V (2014) Further morphological and molecular studies of driftwood hoppers (Crustacea: Amphipoda: Talitridae) from Mediterranean / north-east Atlantic coastlines. Journal of Natural History 49: 1047–1071. https://doi.org/10.1080/00222933.2014.974708

Wildish DJ (2014) New genus and two new species of driftwood hoppers (Crustacea, Amphipoda, Talitridae) from the northeast Atlantic and Mediterranean coastal regions. Zoosystematics and Evolution 90: 133–146. https://doi.org/10.3897/zse.90.8410

Wildish DJ, Pavesi L, Ketmaier V (2012) Talitrid amphipods (Crustacea: Amphipoda: Talitridae) and the driftwood ecological niche: a morphological and molecular study. Journal of Natural History 46: 2677–2700. https://doi.org/10.1080/00222933.2012.717971

Wildish DJ, Robinson SMC (2016) Ultimate cause(s) of dwarfism in invertebrates: the case of driftwood talitrids. Evolutionary Ecology Research 17: 685–698.

Wildish DJ, Pavesi L, Robinson SMC (2018) Comparing oxygen uptake rates of driftwood and wrack generalist talitrid amphipods. Marine and Freshwater Behaviour and Physiology.

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# *Austrolebias queguay* (Cyprinodontiformes, Rivulidae), a new species of annual killifish endemic to the lower Uruguay river basin

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## Key Words

*Austrolebias bellottii* species group
Systematics
La Plata basin

## Abstract

In this article we describe a new species of the annual fish genus *Austrolebias* from the lower Uruguay river basin. The fusion of the urogenital papilla to the first anal fin ray in males and the pigmentation pattern, indicates a close relationship with the clade formed by *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*. The new species can be differentiated from those by the following combination of characters: presence of well-defined light bands contrasting with the sides of the body, the distal portion of the anal fin dark gray, pelvic fins dark bluish green and bases united at about 50–80% on their medial margins, pectoral fins with iridescent blue sub-marginal band, and general coloration of body bluish green. The new species can only be found in wetlands of the Queguay river, an area included in the Uruguayan protected areas system and represents so far the only annual fish species endemic to the lower Uruguay river basin.

## Introduction

*Austrolebias*, one of the most specious annual fish genus in the family Rivulidae, is composed of 47 valid species and is distributed in Bolivia, Paraguay, southern Brazil, northeast Argentina, and Uruguay, in the La Plata, Patos-Merin, and southwestern Amazon basins (Alonso et al. 2018, Calviño et al. 2016, Costa, 2006, 2014, Costa et al. 2017, García et al. 2012, Loureiro et al. 2011, Nielsen and Pillet 2015, Volcan et al. 2014, 2017). In a systematic revision of the genus, Costa (2006) provided three exclusive synapomorphies and three synapomorphies independently arisen in other cynolebiatines.

Costa (2006) defined five species groups within the genus. One of them, the "*Austrolebias bellottii*" species group (clade A4; fig. 1 in Costa 2006), diagnosed by the presence of anteromedian rays of the anal fin elongated in females (triangular fin shape) is composed of two subclades: the "*Austrolebias adloffi*" species group (clade A5, fig. 1 in Costa 2006), diagnosed by chromatic characters; and an unnamed clade that lacks morphological diagnostic characters. This clade is composed of *A. bellottii* (Steindachner), *A. vandenbergi* (Huber), *A. apaii* Costa (all from La Plata basin), *A. melanoorus* (Amato) (from La Plata and Patos Merin basins), and *A. univentripinnis* Costa and Cheffe (from Patos Merin basin).

Afterwards, based on another morphological phylogeny, Costa (2010) added *A. patriciae* to the "*A. bellottii*" species group. In addition, Nielsen and Pillet (2015), described a species from the upper Mamoré river basin (Amazon basin), *A. accorsii*, morphologically very similar to *A. vandenbergi*. However, Alonso et al. (2016) questioned some of the diagnostic characters for *A. acorsii*.

In spite of the low statistical support of A5 and the unnamed clade in morphological analyses (Costa 2006, 2010), mitochondrial and total evidence phylogenetic reconstructions, support the monophyly of both clades (García et al. 2002, 2012, 2014, Loureiro and García 2008; Loureiro et al. 2018). However, these analyses differ in the relationships between and within them, and with other *Austrolebias* species groups. According to Garcia et al. (2014) and Loureiro et al. (2018), *A. patriciae* is

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not closely related to the "*A. bellottii*" species group, and according to Garcia et al. (2012), *A. apaii* should be considered a synonym of *A. bellottii*, a proposal that was followed by Calviño et al. (2016).

In this article we describe a new species of *Austrolebias* from wetlands of the Queguay river (lower Uruguay river basin), that shares similar morphological traits to the clade formed by *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*.

## Materials and methods

Specimens analyzed and comparative material are deposited in the Fish Collections of Facultad de Ciencias, Universidad de la República (ZVC-P), and Museo Nacional de Historia Natura (MHNM), Montevideo, Uruguay, Universidade Federal de Rio Grande do Sul (UFRGS), Rio Grande do Sul, Brazil, and The National Academy of Sciences, Philadelphia, USA (ANSP). Alcohol fixed individuals belong to the tissue collections of Genetica Evolutiva Section (GP) and Zoologia Vertebrados Laboratory (CAP) (Facultad de Ciencias, UdelaR), and Departamento de Zoologia (TEC) da Universidade Federal do Rio Grande do Sul. Measurements and meristic counts were taken under dissecting microscope, according to Costa (2006) and Loureiro and García (2008). Morphological variation was also assessed using a geometric morphometric analyses of landmark configurations using a thin plate spline approach (Bookstein 1991). Landmark positions were modified from D'Anatro and Loureiro (2005) (adding the lower insertion of pectoral fin as a new landmark), acquired from scanned fish images (MicroTek Scanner, Hsinchu, Taiwan), and digitized using the TPSdig program (Rohlf 2003). Weight matrices of partial warps were generated using MorphoJ 1.05f (Klingenberg 2011). Visualization of specimen groupings and the corresponding shape variation were obtained by canonical variate analyses. Cephalic neuromast series nomenclature follows Costa (2006). Cleared and double stained specimens (c&s) were prepared following Dingerkus and Uhler (1977).

## Taxonomy

### ***Austrolebias queguay* sp. n.**

http://zoobank.org/ A53F68BB-4612-4D24-A13B-C4CC609FFA3C
Figs 1, 2A, B, G, 3A

*Austrolebias* sp. in Loureiro et al. (2018)

**Holotype.** ZVC-P 13576, male, 39.4 mm SL, Uruguay, Paysandú, wetlands of Río Queguay Grande, Estancia La Beba, 32°11'08"S, 57°26'08"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, Sep. 2011.

**Paratypes.** Uruguay: Paysandú: MHNM 3728, 2 males 29.9–33.6 mm SL, 2 females 27.4–33.3 mm SL, Río Queguay, 32°08'21"S, 57°26'19"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, Sep. 2011. MHNM 3729, 1 male 34.0 mm SL, 1 female 31.0 mm SL, same data of the holotype. ZVC-P 8657, 10 males 16.1–27.2 mm SL, 12 females 16.9–30.1 mm SL, Río Queguay, Rincón de Pérez, 32°08'23"S, 57°25'49"W, M. Loureiro and S. Clavijo, Aug. 2006. ZVC-P 11620, 37 males 22.8–39.4 mm SL (8 c&s 26.6–38.1 mm SL; 10 fixed in alcohol 95°, 21.9–28.4 mm SL, CAP 1193, GP 3353–3359 and GP 3364–3366), 60 females 24.2–40.1 mm SL (8 c&s 22.7-37.4 mm SL; 4 fixed in alcohol 95°, 22.7–28.7 mm SL, CAP 1193, GP 3360–3363), Río Queguay, 32°08'21"S, 57°26'19"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, Sep. 2011. ZVC-P 11621, 28 males 22.1–34.9 mm SL (5 fixed in alcohol 95°, 22.1–27.7 mm SL, CAP 1181, GP 3367–3371), 33 females 21.2–29.9 mm SL (5 fixed en alcohol 95°, 23.4–27.8 mm SL, CAP 1181, GP 3372–3376), Río Queguay, 32°07'26"S, 57°30'45"W, M. Loureiro, A. Duarte, M. Zarucki, J. Bessonart and D. Hernández, 8 Sep. 2011. ZVC-P 12460, 33 males 25.7–39.1 mm SL (2 c&s 32.4–39.1 mm SL; 4 fixed in alcohol 95°, 27.1–29.0 mm SL, CAP 1194, GP 3377–3380), 25 females 24.9–37.4 mm SL (2 c&s 27.2- 33.1 mm SL; 3 fixed in alcohol 95°, 24.9–26.5 mm SL, CAP 1194, GP 3381–3383), same data of the holotype.

**Figure 1.** *Austrolebias queguay* sp. n., ZVC-P 13576, 39.4 mm SL, holotype, male, Estancia La Beba (32°11'08"S, 57°26'08"W), wetlands of Río Queguay Grande, Paysandú Department, Uruguay.

**Diagnosis.** The new species differs from all the other species of the genus except *Austrolebias bellottii*, *A. univentripinnis* and *A. melanoorus*, by the presence of the urogenital papilla attached to the anal fin in males (vs. free from the anal fin). It differs from *A. bellottii* and *A. univentripinnis* by the presence of well-defined light blue bands contrasting with the sides of the body in adult males (vs. vertical rows of light blue dots) (Fig. 2); from *A. melanoorus*, by the presence of dark gray coloration of the distal portion of the anal fin in males (vs. distal portion of anal-fin black), pelvic-fins dark bluish green (observed in ventral view) and bases united at about 50–80% on their medial margins (vs. dark gray and united about 50% or less), pectoral-fins with iridescent blue sub-marginal band (vs. sub-marginal band absent), and general coloration of the body bluish green (vs. grayish sky blue).

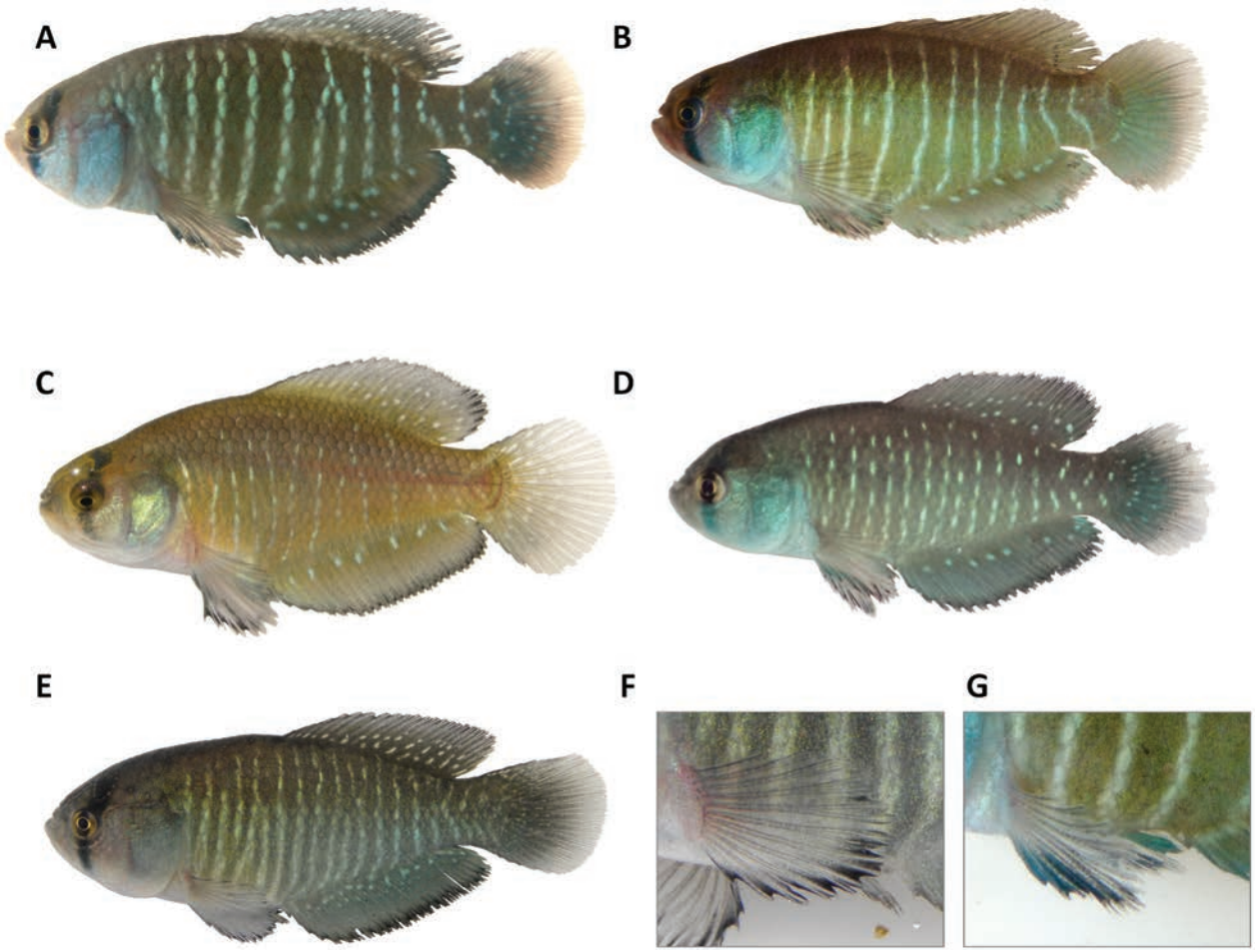


**Figure 2. A.** *Austrolebias queguay* sp. n. paratype male (ZVCP 11620); **B.** *A. queguay* non type male (32°07'26"S, 57°30'45"W), not preserved (right side, photo flipped); **C**. *A. bellottii* non preserved male; **D.** *A. univentripinnis* male (UFRGS 18064, right side photo flipped); **E.** *A. melanoorus* topotype male (ZVCP13651); **F.** Detail of pectoral and pelvic fins of *A. melanoorus*; **G.** Detail of pectoral and pelvic fins of *A. queguay*.

**Figure 3. A.** *Austrolebias queguay* sp. n. female: paratype ZVCP 11620; **B.** *A. bellottii* female (ZVCP 11560); **C.** *A. univentripinnis* female (UFRGS 18066); **D.** *A. melanoorus* topotype female (ZVCP 13651).

**Description.** Morphometric data in Table 1. Largest examined male 39.4 mm SL; largest examined female 40.1 mm SL. Body orbicular and compressed. Maximum body depth between pelvic-fin origin and anal-fin origin in both sexes. Dorsal profile of head straight to slightly concave. Dorsal profile of body convex between head and posterior insertion of dorsal fin. Ventral profile of body convex between anterior margin of mandible and the origin of anal fin; base of anal fin straight in males and straight to concave in females. Upper and inner margin of caudal peduncle usually straight. Snout short and rounded.

Posterior end of anal and dorsal fins rounded; presence of short filaments in distal margin of anal-fin in males. Anal fin in females triangular shaped (anteromedian rays prolonged forming anterior lobe). Caudal fin rounded. Pectoral fin elliptical, posterior margin on vertical between 2$^{nd}$ to 5$^{th}$ anal-fin ray bases in males, and between pelvic-fin origin and urogenital papilla in females. Pelvic fins medially united between 50–80%, with posterior tip reaching between urogenital papilla and base of 4$^{rd}$ anal-fin ray in both sexes. Urogenital papilla in males partially attached (only tip of papillae free) to anal fin. Base of dorsal-fin origin anterior to the anal-fin origin in males, between 8$^{th}$ to 11$^{th}$ vertebrae and 7$^{th}$ to 10$^{th}$ neural spine; in females usually vertical to posterior to the anal fin origin, between 12$^{th}$ to 14$^{th}$ vertebrae and 10$^{th}$ to 13$^{th}$ neural spine. Origin of anal fin between pleural ribs 8$^{th}$ to 9$^{th}$ and vertebrae 10$^{th}$ and 12$^{th}$ in males: between pleural ribs 9$^{th}$ and 12$^{th}$ and vertebrae 12$^{th}$ and 15$^{th}$ in females. Dorsal fin rays 22–25 in males and 17–20 in females; anal-fin rays 24–27 in males and 21–24 in females. Caudal fin rays 20–25; pectoral fin rays 11–13; pelvic fin rays 5.

Scales cycloid. Trunk and head scaled, except ventral surface of head. Longitudinal series of scales 28–33, regularly arranged; transversal series 11–16 (N=29 and one specimen with 21 scales); circumpeduncular series 13–20. Anal-fin base without scales; caudal fin with three rows of irregularly arranged scales. Contact organs present in all analyzed males, 1 to 8 contact organs per scale (usually 1 or 2); contact organs present in first 6 upper rays of pectoral fins; no contact organs on unpaired and pelvic fins.

Cephalic neuromasts: supraorbital 13–23, parietal 0–4, anterior rostral 1–2 (usually 1), posterior rostral 0–2 (usually 1), infraorbital 1–3 + 18–27, preorbital 2–4, otic 2–5, post-otic 1–5, supratemporal 1–3 (usually 1), median opercular 1–2 (usually 1), ventral opercular 1–3, preopercular 19–29, mandibular 11–15, lateral mandibular 3–7.

Basihyal cartilage anteriorly widened, about 50–60% of total length of basihyal; anterior margin of cartilage usually concave or with little commissures. Second pharyngobranchial with 3–8 teeth and 3$^{rd}$ with 17–37. First branchial arch with 3–4 epibranchial spines and 10–12 hypobranchial. Dermosphenotic ossifications present only in 5 % of specimens analyzed; proximal radials 3–5 (usually 4); ventral process posttemporal well-developed. Total vertebrae 27–30.

**Color in life.** Males (Fig. 2A–B). Ground color of body bluish green, darker in dorsal region, with 8–15 light sky blue vertical bands. Some specimens with dark green spots in the middle of the flank. Pectoral and ventral region whitish. Opercle and preopercle intense sky blue. Iris yellow; dark vertical band crossing the eye. Pectoral fins hyaline with black margin and iridescent blue sub-marginal band; pelvic fins blue to green. Dorsal and anal fins greenish blue with light sky blue dots on base; distal margin of anal fin darker. Caudal fin greenish blue with disperse light sky blue dots present or not, distal margin hyaline.

**Table 1.** Morphometric data of *Austrolebias queguay* sp. n., Standard length is expressed in mm; measurements numbered 2–13 are percentage of standard length; subunits of head measurements (numbered 14–18) are percentage of Head length. Ranges presented for males include the holotype.

| Character | Holotype | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | Low | High | Mean | SD | N | Low | High | Mean | SD |
| **1. Standard length (mm)** | 39.4 | 54 | 22.8 | 39.4 | 31.0 | – | 88 | 21.2 | 40.1 | 29.2 | – |
| **2. Body depth** | 37.4 | 54 | 32.8 | 39.5 | 36.5 | 1.75 | 88 | 30.0 | 40.0 | 34.7 | 2.09 |
| **3. Head lenght** | 27.2 | 54 | 23.9 | 29.6 | 27.1 | 1.28 | 88 | 22.6 | 30.2 | 27.7 | 1.36 |
| **4. Caudal peduncle depth** | 14.6 | 54 | 11.6 | 15.6 | 13.7 | 0.77 | 88 | 11.3 | 15.8 | 13.1 | 0.83 |
| **5. Caudal peduncle length** | 11.5 | 54 | 6.5 | 17.3 | 11.5 | 2.16 | 88 | 12.1 | 26.4 | 16.0 | 2.25 |
| **6. Pre dorsal length** | 50.6 | 54 | 43.1 | 54.3 | 50.3 | 2.37 | 88 | 43.1 | 64.8 | 59.9 | 2.81 |
| **7. Dorsal-fin base length** | 42.3 | 54 | 34.8 | 47.7 | 40.6 | 2.79 | 88 | 22.4 | 32.6 | 27.3 | 2.42 |
| **8. Pre anal-fin length** | 50.5 | 54 | 46.7 | 60.2 | 51.9 | 2.39 | 88 | 52.6 | 67.1 | 60.4 | 2.65 |
| **9. Anal-fin base length** | 44.8 | 54 | 35.5 | 46.1 | 42.4 | 2.19 | 88 | 21.8 | 30.9 | 26.3 | 2.07 |
| **10. Pre pelvic-fin length** | 45.9 | 54 | 41.3 | 48.8 | 45.3 | 1.51 | 88 | 46.3 | 61.1 | 51.9 | 2.38 |
| **11. Pectoral fin length** | 20.2 | 54 | 18.8 | 27.7 | 23.5 | 2.10 | 88 | 20.2 | 30.4 | 24.4 | 1.74 |
| **12. Pelvic fin length** | 9.6 | 54 | 6.7 | 11.9 | 9.2 | 1.01 | 88 | 9.2 | 14.9 | 12.3 | 1.11 |
| **13. Caudal fin length** | 20.5 | 54 | 15.3 | 28.5 | 24.5 | 2.39 | 88 | 22.6 | 30.8 | 26.7 | 1.88 |
| **14. Head width** | 65.1 | 54 | 59.9 | 77.2 | 67.7 | 3.95 | 88 | 60.8 | 90.3 | 75.3 | 6.28 |
| **15. Head depth** | 112.3 | 54 | 92.1 | 125.7 | 110.3 | 6.70 | 88 | 88.5 | 133.7 | 104.7 | 7.33 |
| **16. Interorbital width** | 51.7 | 54 | 39.8 | 58.0 | 47.6 | 4.50 | 88 | 39.5 | 54.1 | 45.9 | 3.17 |
| **17. Eye diameter** | 30.9 | 54 | 25.0 | 37.7 | 30.3 | 2.83 | 88 | 25.7 | 35.7 | 30.9 | 2.12 |
| **18. Snout length** | 27.6 | 54 | 16.7 | 29.5 | 21.9 | 2.10 | 88 | 16.4 | 25.5 | 20.2 | 1.98 |

Females (Fig. 3A). Ground color light brown, darker in dorsal region; sides of abdominal area yellow; pectoral and ventral region whitish. Black spots in central area of flanks present or absent, when present usually horizontally aligned; rest of the body with diffuse brown spots or vertical bands. Iris yellow; diffuse gray vertical band crossing the eye. Opercle and preopercle with sky blue reflections. Paired fins hyaline; unpaired fins yellowish at base to hyaline on distal margin, sometimes with diffuse brown spots or bars in the space between rays.

**Geometrical morphometric analyses.** Canonical variate analyses discriminated *A. bellotti* specimens from the other species along Root 1 (84.7 and 83.7% of total variation in males and females respectively, Figs 4–5), and partially discriminated *A. queguay* specimens from the other species along Root 2 (10.2 and 11.8 % of total variation in males and females respectively, Figs 4–5). In both cases, but especially in females, shape changes along Root 1 were associated to a deeper body in *A. bellotti* in relation to the other species.

**Etymology.** The specific name, *queguay*, is in reference to Queguay river basin, the type locality of the new species, treated as a noun in apposition to the generic name.

**Distribution.** *Austrolebias queguay* is endemic to the wetlands of middle Queguay river basin (30 meters above sea level), Paysandú Department, Uruguay, which flows to the lower Uruguay river (Fig. 6).

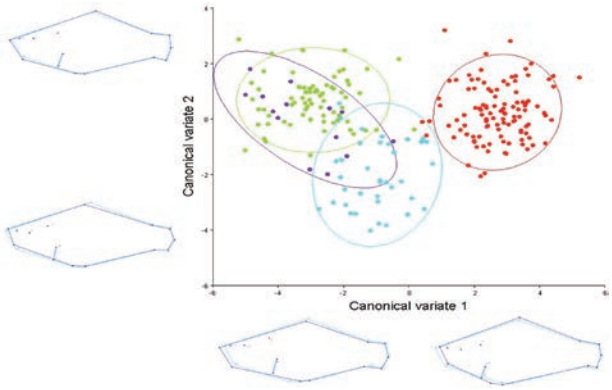


**Figure 4.** Canonical variate analysis of the morphometry of males. Red dots = *A. bellottii*, Purple dots *A. univentripinnis*, Green dots = *A. melanoorus*, Skyblue dots = *A. queguay*. Deformation (dark blue line) from consensus configuration (sky blue line) associated to each canonical axis.

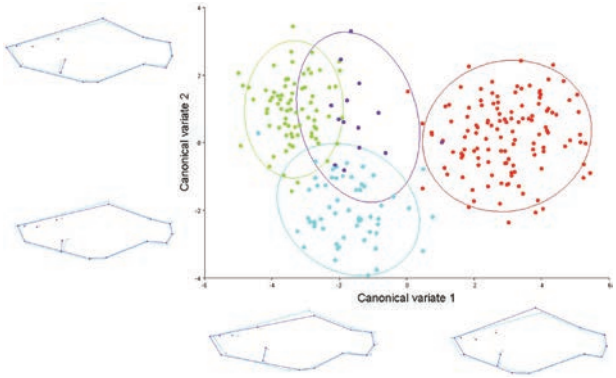


**Figure 5.** Canonical variate analysis of the morphometry of females. Red dots = *A. bellottii*, Purple dots *A. univentripinnis*, Green dots = *A. melanoorus*, Skyblue dots = *A. queguay*. Deformation (dark blue line) from consensus configuration (sky blue line) associated to each canonical axis.

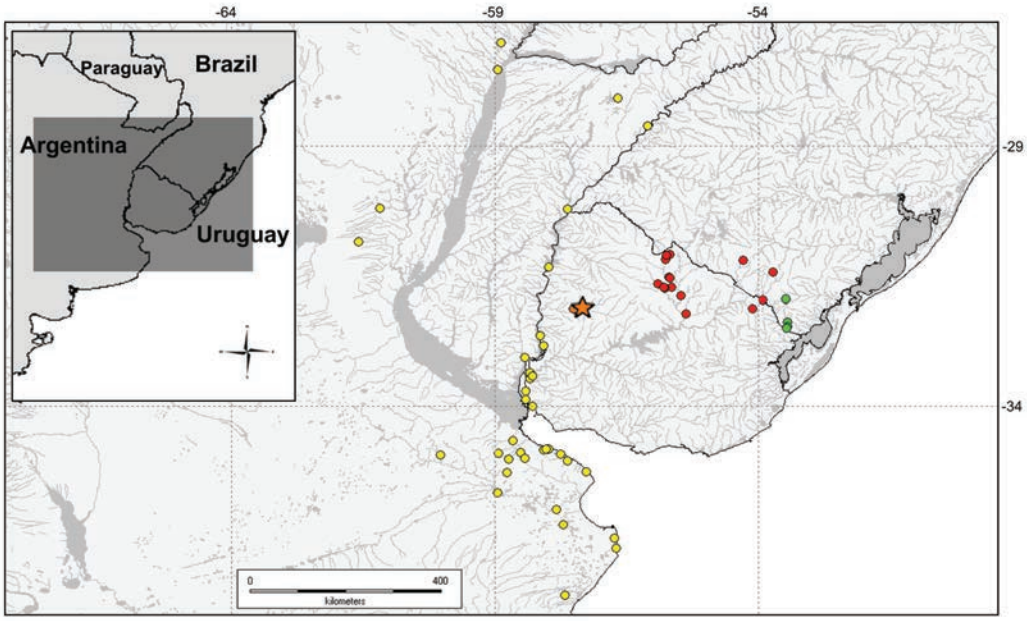


**Figure 6.** Geographic distribution of *Austrolebias queguay* sp. n. (orange dots, Uruguay river basin), *A. bellottii* (yellow dots, Paraná and Uruguay river basins), *A. melanoorus* (red dots, Negro and Yaguarón river basins), and *A. univentripinnis* (green dots, Yaguaron river basin). Orange star indicates type locality.

**Conservation.** All its known locations are within "Montes del Queguay", a permanent protection reserve under the control and regulation of the Uruguayan government, with an area of about 200 km2, but without a management plan (MVOTMA 2018; http://www.mvotma.gub.uy/portal/areas-protegidas/item/10006542-area-protegida-con-recursos-manejados-montes-del-queguay-paysandu.html). Furthermore, projected land-use changes for surrounding areas for the next decade may result in increases in forestry for cellulose production purpose (Brazeiro 2015), and wetlands are potentially vulnerable to hydric changes in the region. For these reasons, considering UICN criteria (IUCN 2012), and until a formal assessment is done, *A. queguay*, could be considered preliminarily as an 'Endangered Species'.

**Ecology.** As many species of the family Rivulidae, *A. queguay* presents an annual life cycle which includes drought resistant eggs and diapausing embryos. All species of *Austrolebias* are obligate annuals (Berois et al. 2016). In the Pampa biome there is not a defined dry season, so dried environments can be found between mid spring to early fall (depending on the year), when evaporation is higher than precipitations (Williams 2006; García et al. 2017). *Austrolebias* species can be found in small grassland ponds and seasonal floodplain wetlands; however the new species has been found so far only in the latter environments (Fig. 7).

**Figure 7.** Type locality of *Austrolebias queguay* sp. n., wetlands of middle Queguay river basin.

## Discussion

The new species described in this article presents all diagnostic characters for the genus *Austrolebias* proposed by Costa (2006): absence of scales between corner of mouth and anterior portion of preopercular region and ventral portion of opercular region, deep urohyal, dark gray to black infraorbital bar and supraorbital spot, dorsal and anal fins rounded in males, long urogenital papilla in male, and reduced ventral process of angulo-articular. The partial fusion of the urogenital papilla to the first anal fin ray in males, places *A. queguay* in the clade composed by *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*.

Differences with the other species of the clade concern pigmentation pattern in males. This kind of variation is common among species of other *Austrolebias* clades, such as the "*A. affinis*" species group, where *A. juanlangi* and *A. paucisquama* present well-defined clear bands (in different degree) over a darker background coloration, vs. vertical rows of light blue dots in the other species (Costa 2006; Ferrer et al. 2006); or in the "*A. adloffi*" species group, where background pigmentation can be concentrated enough to form black bars over a clear background (Costa 2006; Loureiro and García, 2008). Difference in pigmentation pattern among males in different species of animals has been hypothesized to have resulted from sexual selection and reinforcement (Panhuis et al. 2001). However, the allopatric distribution of species of the same clade in most *Austrolebias* (Loureiro et al. 2016), suggests that variation in these characters may be due to other forces, such as local adaptation, sensory drive (Boughman 2002), or just random variation caused by genetic drift.

Additionally, *A. queguay* differs from of *A. bellottii*, *A. melanoorus*, and *A. univentripinnis*, by the disposition of black spots on the central area of the flanks in females, when present horizontally aligned vs. when present, usually not aligned, from *A. bellottii* by dermosphenotic ossifications usually absent (95% of the *A. queguay* specimens analyzed) vs. usually present in *A. bellottii* (91% of specimens analyzed), and from *A. melanoorus* by the number of anterior rostral neuromasts, usually one pair (85 % of specimens analyzed) vs. usually two pairs in *A. melanoorus* (90% of specimens). Although these characters cannot be used as diagnostic, they support the idea of genetic

isolation of the new species from the others, particularly from the nearby located populations of *A. bellottii*.

According to a recent morphological and molecular phylogenetic analysis the new species (*Austrolebias* sp; figs 9–12, Loureiro et al. 2018), is closely related to *A. bellottii*, and both represent the sister clade of *A. melanoorus* + *A. univentripinnis*. Interestingly, body shape of *A. queguay* is more similar to the phylogenetically and distributional distant species of the group, in the Negro and Yaguarón river basins (*A. melanoorus* and *A. univentripinnis*) than to *A. bellottii*, whose nearest localities are just 50 km away downstream and 25 five meters below the new species range.

The lower Uruguay and Paraná rivers have suffered the effect of sea level changes associated to the glacial cycles since the Pleistocene. The last transgression is hypothesized to have occurred around 6 thousand years ago where the sea level rose five meters above actual level (Bracco et al. 2011). During marine transgressions, wetlands and lowlands of the ancient lower Uruguay and Paraná rivers became estuarine or even marine environments (Martínez and Rojas 2011) and tributaries flowed directly into them, generating a barrier that may have isolated *A. queguay* and *A. bellottii* populations. The altitude of the inhabited wetlands and the fact that the highest sea level reached in the area in the last 100 thousand years has been only 5 meters above the current level (Lambeck et al. 2002; Bracco et al. 2011), may also suggest that its isolation from the other species of the group could have occurred before that.

Four species of *Austrolebias* had been recorded in the lower Uruguay river: *A. bellottii*, *A. nigripinnis*, *A. alexandri*, and *A. elongatus* (Costa 2006; Loureiro et al. 2016). The new species could be considered the only annual fish species endemic to this section of the basin, since the other *Austrolebias* species of the region present wider ranges: *A. bellottii* and *A. nigripinnis* in the lower Paraná and middle Uruguay river basins, *A. elongatus* in the lower Paraná basin, and *A. alexandri* in the middle Uruguay river basin. These species have been found living in syntopy in different combinations. Interestingly, *A. queguay* is the only *Austrolebias* inhabitant of the wetlands of the middle Queguay river. The extreme endemism of *Austrolebias queguay* urges National Authorities to elaborate management plans to secure the conservation of its populations. These plans need to address not only direct actions concerning the fish populations, but also oversight the productive activities in the surrounding basin.

## Comparative material examined

***Austrolebias bellottii:*** **ARGENTINA. Buenos Aires. MHNM 2425**, 13 males 18.6–41.6 mm SL, 52 females 17.5–40.1 mm SL, Santa Teresita, 36°32'S, 56°43'W, R. Taberner, 31 Oct. 1975. **MHNM 2801**, 5 males 27.8–35.8 mm SL, 18 females 30.1–39.8 mm SL, road between La Plata and Magdalena, 35°03'S, 57°37'W, J.R. Casciotta, Nov. 1986. **ZVC-P 517**, 1 male 38.3 mm SL, 1 female 37.0 mm SL, road between Villa Elisa and Punta Lara, 34°50'S, 58°01'W, R. Vaz-Ferreira, B. and J. Soriano, 2 Nov. 1962. **ZVC-P 707**, 9 males 30.7–48.6 mm SL, 2 females 38.9–39.4 mm SL, Camino de la Costa, 13km S of La Plata, 34°55'S, 57°45'W, R. López, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 708**, 3 males 41.8–47.4 mm SL, 8 females 31.9–44.1 mm SL, Camino de la Costa, 13km S of La Plata, 34°55'S, 57°45'W, R. López, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 711**, 42 males 30.0–48.4 mm SL, 51 females 25.8–49.3 mm SL, Camino de la Costa, 13km S of de La Plata, 34°55'S, 57°45'W, R. López, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 714**, 1 female 33.1 mm SL, Punta Lara, 34°49'S, 57°57'W, R. Vaz-Ferreira, B. Sierra de Soriano and J. Soriano, 3 Nov. 1962. **ZVC-P 954**, 7 males 55.2–63.7 mm SL, 3 females 44.6–50.8 mm SL, road between Villa Elisa and Punta Lara, 34°50'S, 58°01'W, López-Grancelli, 21 Oct. 1963. **Chaco. MHNM 2566**, 4 males 23.1–36.0 mm SL, 40 females 27.1–39.9 mm SL, Puerto Vilelas, 27°30'S, 58°57'W, R. Taberner, 7 Apr. 1974. **ZVC-P 10448**, 2 males 34.7–34.8 mm SL, 20 km S of Río Oro, 27°00'S, 58°53'W, P. Calviño, 30 Jun. 2005. **Entre Ríos. ZVC-P 10451**, 15 males 14.3–32.7 mm SL, 2 females 19.5–21.5 mm SL, Club de Pesca, Gualeguaychú, 33°03'38''S, 58°25'32''W, 21 Sep. 2008. **Santa Fe. ZVC-P 6409**, 6 males 33.2–41.2 mm SL, 1 female 31.2 mm SL, Río Salado, 18 km N of San Cristobal, 30°11'S, 61°11'W, Lic. J.M. Gallardo, 17 Dec. 1966. **ZVC-P 10449**, 2 males 34.7–34.8 mm SL, 7 females 24.2–29.8 mm SL, Tacural, 30°50'S, 61°35'W, P. Calviño, 22 Aug. 2008. **URUGUAY. Artigas. ZVC-P 5346**, 12 males 24.5–48.2 mm SL (5 c&s 26.5–30.8 mm SL), 11 females 23.1–41.1 mm SL (5 c&s 23.1–27.7 mm SL), Franquia, Bella Unión, 30°13'09"S, 57°37'20"W, M. Loureiro, Jul. 2002. **Colonia. ZVC-P 876**, 17 males 24.6–47.2 mm SL, 11 females 21.6–44.7 mm SL, Radio of Carmelo, 34°00'03"S, 58°17'43"W, R. Vaz-Ferreira, 5 Nov. 1964. **ZVC-P 2086**, 6 males 38.2–48.2 mm SL, 11 females 32.5–43.2 mm SL, Radio of Carmelo, 34°00'03"S, 58°17'43"W, R. Vaz-Ferreira and G. Gannella, 23 Sep. 1973. **Río Negro. ZVC-P 11635**, 1 male 37.1 mm SL, Vizcaíno, 33°21'42"S, 58°20'34"W, D. García, D. Díaz, W.S. Serra and M. Loureiro, 11 Sep. 2012. **Salto. ZVC-P 5343**, 9 males 29.7–39.8 mm SL (4 c&s 29.7–34.0 mm SL), 6 females 26.2–34.4 mm SL (4 c&s 26.2–34.4 mm SL), City of Salto, 31°19'40"S, 57°58'28"W, M. Loureiro, F. Teixeira de Mello and E. Charbonier, May 2002. **Soriano. ZVC-P 7712**, 1 male 37.1 mm SL, 7 females 27.6–37.7 mm SL, W of Villa Soriano, 33°23'33"S, 58°20'41"W, M. Loureiro and S. Clavijo, 31 Jul. 2007. **ZVC-P 7826**, 4 males 44.6–49.6 mm SL, 7 females 37.2–46.4 mm SL, Estancia Curupí, 33°28'27"S, 58°20'03"W, M. Loureiro and S. Clavijo, 13 Jul. 2007. **ZVC-P 11560**, 4 males 29.0–39.4 mm SL, 5 females 27.9–37.9 mm SL, Agraciada, 33°42'42"S, 58°25'11"W, 8 Sep. 2010. **ZVC-P 12475**, 3 males 42.5–62.6 mm SL, 1 female 52.2

mm SL, camino de la Escuela N°1, Villa Soriano, 29 Aug. 2011. **ZVC-P 12480**, 7 males 34.5–62.2 mm SL, 6 females 31.1–50.6 mm SL, Route 96 km 8, M. Loureiro, A. Duarte and I. Berro, 29 Aug. 2011.

***Austrolebias melanoorus*: BRAZIL. Rio Grande do Sul**. **UFRGS 18050**, 16 males 22.0–36.8 mm SL (3 c&s 30.9–33.9 mm SL; 3 fixed in alcohol 95°, 26.4–31.5 mm SL, TEC 3693, GP 3777–3779), 27 females 22.4–30.7 mm SL (3 c&s 25.9–28.1 mm SL; 7 fixed in alcohol 95°, 22.4–26.5 mm SL, TEC 3693, GP 3780–3786), Bagé, temporary pool close to BR-293 highway, 31°12'32"S, 54°17'24"W), L. Malabarba and J. Ferrer, 8 Sep. 2013. **UFRGS 18067**, 20 males 19.3–44.2 mm SL (3 c&s 28.1–40.2 mm SL), 18 females 24.1–37.9 mm SL (3 c&s 24.1–36.2 mm SL), Candiota, Seival, 31°25'57"S, 53°43'05"W, L. Malabarba and J. Ferrer, 8 Sep. 2013. **URUGUAY. Cerro Largo. MHNM 3730**, 1 male 34.1 mm SL, 1 female 28.2 mm SL, Cañada de las Pajas, 32°07'10"S, 54°06'41"W, W.S. Serra, J. Bessonart and M. Loureiro, 2 Sep. 2015. **ZVC-P 7757**, 2 males 41.3–44.5 mm SL, 1 female 40.0 mm SL, Paso San Diego, 31°57'57"S, 53°54'52"W, M. Loureiro, M. Zarucki, S. Clavijo and F. Teixeira, 1 Sep. 2007. **ZVC-P 7780**, 1 female 36.4 mm SL, Paso San Diego, 31°57'57"S, 53°54'52"W, M. Loureiro, M. Zarucki, S. Clavijo and F. Teixeira, Sep. 2007**. ZVC-P 8732**, 28 males 23.6–33.5 mm SL (3 c&s 26.5–33.5 mm SL; 1 fixed in alcohol 95° 26.1 mm SL, CAP 339, GP 3318), 29 females 21.6–28.4 mm SL (3 c&s 22.8–26.1 mm SL), Paso San Diego, 31°57'57"S 53°54'52"W, M. Loureiro, M. Zarucki and A. Duarte, Sep. 2009. **ZVC-P 9721**, 7 males 20.1–32.4 mm SL (5 fixed in alcohol 95° 20.1–32.4 mm SL, CAP 282, GP 3318–3323), 5 females 20.4–28.0 mm SL (5 fixed in alcohol 95°, CAP 282, GP 3324–3329), Cañada de las Pajas, 32°07'10"S, 54°06'41"W, M. Loureiro, W.S. Serra, A. Duarte and J. Bessonart, 30 Sep. 2010. **ZVC-P 11622**, 19 males 21.4–42.4 mm SL (2 c&s 21.4–24.0 mm SL; 1 fixed in alcohol 95%, 42.4 mm SL, CAP 1188, GP 3340; 10 fixed in alcohol 95%, 21.7–30.0 mm SL, CAP 1192, GP 3341–3350), 13 females 20.9–40.0 mm SL (2 c&s 20.9- 22.8 mm SL; 2 fixed in alcohol 95%, 21.7–24.5 mm SL, CAP 1192, GP 3351–3352), Paso San Diego, 31°57'57"S, 53°54'52"W, W.S. Serra, A. Duarte and M. Loureiro, 19 Sep. 2012. **ZVC-P 11668**, 7 males 29.2–40.0 mm SL (2 c&s 29.2–37.5 mm SL), 3 females 28.0–29.8 mm SL (2 c&s 28.1–29.8 mm SL), Cañada de las Pajas, 32°07'10"S, 54°06'41"W, W.S. Serra, A. Duarte and M. Loureiro, 30 Jul. 2013. **ZVC-P 13577**, 10 males 25.6–34.5 mm SL, 6 females 22.9–32.2 mm SL, Cañada de las Pajas, 32°07'10"S 54°06'41"W, W.S. Serra, J. Bessonart and M. Loureiro, 2 Sep. 2015. **ZVC-P 13578**, male, 37.0 mm SL, Cañada de las Pajas, 32°07'10"S, 54°06'41"W, W.S. Serra, J. Bessonart and M. Loureiro, 2 Sep. 2015. **Rivera. MHNM 3676** (ex. CLT 1196), 13 males 16.7–33.9 mm SL, 18 females 18.4–30.0 mm SL, Paso Ataques, 31°05'42"S, 55°41'12"W, P. Laurino, T. Litz, E. Perujo, H. Salvia and J. Salvia, 25 Aug. 2004. **ZVC-P 8743**, 12 males 17.0–40.7 mm SL, 16 females 21.1–35.2 mm SL, Río Tacuarembó, Paso Manuel Díaz, 31°32'42"S, 55°40'17"W, M. Loureiro, A. Duarte and W.S. Serra, Oct. 2009. **Tacuarembó. MHNM 2545**, 1 male holotype**,** 35,7 mm SL, pond near Arroyo Tres Cruces, Route 5 km 399.5, 31°39'01"S, 55°54'01"W, L.H. Amato, 3 Nov. 1985. **MHNM 2546**, 21 males paratypes, 27.9–36.2 mm SL (2 c&s disjointed), collected with the holotype. **MHNM 2548**, 28 females paratypes, 24.8–39.5 mm SL (2 c&s 32.2–33.7 mm SL), collected with the holotype. **MHNM 3675** (ex. CTL 1213b), 8 males 20.3–29.1 mm SL, 4 females 23.7–25.3 mm SL, Route 26 y Río Tacuarembó, Pueblo Ansina, 31°52'28"S, 55°28'19"W, P. Laurino, T. Litz, E. Perujo, H. Salvia and J. Salvia, 25 Aug. 2004. **ZVC-P 4322**, 3 males 33.0–37.8 mm SL (1 c&s 37.8 mm SL), 10 females 23.7–36.1 mm SL, pond near Arroyo Tres Cruces, Route 5 km 399.5, 31°39'01"S, 55°54'01"W, M. Loureiro, G. Yemini and C. Hernández, Oct. 1999. **ZVC-P 4323**, 4 males 30.5–43.1 mm SL, 4 females 29.4–35.5 mm SL, Route 26 and Río Tacuarembó, Pueblo Ansina, 31°52'28"S 55°28'19"W, M. Loureiro, F. Teixeira de Mello, A. D'Anatro and L. Bocardi, 26 Sep. 2000. **ZVC-P 8729**, 5 males 36.0–41.5 mm SL (1 fixed in alcohol 95°, 36.0 mm SL, CAP 358, GP 3282), 14 females 26.1–34.2 mm SL (2 fixed in alcohol 95°, 30.1–31.9 mm SL, CAP 358, GP 3283–3284), pond near Arroyo Tres Cruces, Route 5 km 399.5, 31°39'01"S, 55°54'01"W, M. Loureiro, A. Duarte and W.S. Serra, 10 Oct. 2009. **ZVC-P 8753**, 19 males 18.2–45.6 mm SL (3 c&s 22.7–30.3 mm SL; 2 fixed in alcohol 95° 21.6–29.1 mm SL, CAP 342, GP 3285–3286), 29 females 22.4–41.3 mm SL (3 c&s 21.3–31.8 mm SL; 1 fixed in alcohol 95° 27.0 mm SL, CAP 342, GP 3287), Paso Rogerio, Río Tacuarembó, 31°43'32"S, 55°38'46"W, M. Loureiro, A. Duarte and W.S. Serra, 11 Oct. 2009. **ZVC-P 13579**, 20 males 19.4–39.9 mm SL (17 fixed in alcohol 95°, 19.4–28.9 mm SL, CAP 1274, GP 3953–3956), 8 females 18.6–28.0 mm SL (5 fixed in alcohol 95°, 18.6–24.4 mm SL, CAP 1274, GP 3957–3958), Paso de la Laguna, 32°13'27"S, 55°22'32"W, W.S. Serra, J. Bessonart and M. Loureiro, 3 Sep. 2015.

***Austrolebias univentripinnis*: BRAZIL. Rio Grande do Sul. UFRGS 18062**, 25 males 20.7–29.0 mm SL (5 c&s 20.7–26.9 mm SL), 31 females 18.6–23.3 mm SL (5 c&s 19.2–22–3 mm SL), pond near the road Jaguarão-Nossa Senhora da Glória, Jaguarão, 32°22'45"S, 53°26'35"W, L. Malabarba and J. Ferrer, 7 Sep. 2013. **UFRGS 18064**, 7 males 21.9–29.0 mm SL, 2 females 19.4–21.1 mm SL, pond near the road Herval-Pedras Altas, Herval, 31°56'29"S, 53°29'00"W, L. Malabarba and J. Ferrer, 8 Sep. 2013. **UFRGS 18066**, 30 males 15.6–31.6 mm SL (5 c&s 19.4–30.6 mm SL), 47 females 14.2–30.6 mm SL (5 c&s 18.5–29.8 mm SL), pond near the road Jaguarão-Pedras Brancas, Jaguarão, 32°27'49"S 53°26'43"W, L. Malabarba and J. Ferrer, 7 Sep. 2013.

***Austrolebias vandenbergi*: PARAGUAY. Boquerón. MHNM 2557,** 1 male 46.8 mm SL, 6 females 25.0–52.3 mm SL, 100 km al SW de Filadelfia, 12.6 km de la Es-

tancia Heisseque del Dr. Durán, Servicio Forestal Nacional, 13 Jun. 1981. **ANSP 175282,** 5 males 36.7–48.1 mm SL, 11 females 28.4–39.7 mm SL (2 c&s 32.9–34.0 mm SL), pond along road from Filadelfia-Teniente Montania, 22°10'59"S, 60°04'05"W, D.W. Fromm and J. Barnett. **ANSP 175283,** 3 males 33.3–41.3 mm SL, long pool S of road, 21°49'60"S, 60°51'31" W, D.W. Fromm and J. Barnett. **ANSP 175286,** 9 males 35.1–47.4 mm SL (2 c&s 43.3–45.6 mm SL), 1 female 37.5 mm SL, roadside pool on W side of road N from Teniente Montania, 22°03'17"S, 59°57'13"W, D.W. Fromm and J. Barnett. **ANSP 175289**, 10 males 41.4–55.2 mm SL (2 c&s 46.4–46.6 mm SL), 10 females 30.0–38.4 mm SL (2 c&s 30.0–35.5 mm SL), roadside ditch along Filadelfia - Teniente Montania road, 22°10'59"S, 60°04'05"W, D.W. Fromm and J. Barnett. **ARGENTINA. Salta. MHNM 2575**, 1 male 69.1 mm SL, Hickmann, Sr. Pierroti, 20 Jan. 1974.

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## References

Alonso F, Calviño PA, Terán GE, García I (2016) Geographical distribution of *Austrolebias monstrosus* (Huber, 1995), *A. elongatus* (Steindachner, 1881) and *A. vandenbergi* (Huber, 1995) (Teleostei: Cyprinodontiformes), with comments on the biogeography and ecology of Rivulidae in Pampasic and Chaco floodplains. Check List 12(4): 1945: 7. https://doi.org/10.15560/12.4.1945

Alonso F, Terán GE, Calviño P, García I, Cardoso Y, García G (2018) An endangered new species of seasonal killifish of the genus *Austrolebias*(Cyprinodontiformes: Aplocheiloidei) from the Bermejo river basin in the Western Chacoan Region. PLoS ONE 13(5): e0196261. https://doi.org/10.1371/journal.pone.0196261

Berois N, Arezo MA, Papa NG, Chalar C (2016) Life Cycle, Reproduction, and Development in Annual Fishes Cellular and Molecular Aspects. In: Berois N, García G, de Sá RO (Eds) Annual Fishes. Life History Strategy, Diversity, and Evolution. CRC Press, Taylor & Francis Group, Boca Ratón, 33–46.

Boughman JW (2002) How sensory drive can promote speciation. Trends in Ecology and Evolution 17: 571–577. https://doi.org/10.1016/S0169-5347(02)02595-8

Bookstein FL (1991) Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, New York, 435 pp. https://doi.org/10.1002/sim.4780120711

Bracco R, García-Rodríguez F, Inda H, Del Puerto L, Castiñeira C, Panario D (2011) Niveles relativos del mar durante el Pleistoceno final-Holoceno en la costa de Uruguay. In: García-Rodríguez F (Ed.) El Holoceno en la zona costera de Uruguay. Dpto. Publicaciones Udelar, Montevideo, 65–92.

Brazeiro A (2015) Eco-Regiones de Uruguay: Biodiversidad, Presiones y Conservación. Aportes a la Estrategia Nacional de Biodiversidad. Facultad de Ciencias, CIEDUR, VS-Uruguay, SZU. Montevideo, 122 pp.

Calviño P, Nadalin DO, Serio MJ, López HL (2016) Colección Ictiológica del Museo de La Plata: La familia Rivulidae. ProBiota, Serie Técnica y Didáctica 36: 1–21. http://hdl.handle.net/10915/61448

Costa WEJM (2006) The South American annual killifish genus *Austrolebias* (Teleostei: Cyprinodontiformes: Rivulidae): phylogenetic relationships, descriptive morphology and taxonomic revision. Zootaxa 1213: 1–162. http://www.mapress.com/zootaxa/2006/zt01213p162.pdf

Costa WEJM (2010) Historical biogeography of cynolebiasine annual killifishes inferred from dispersal-vicariance analysis. Journal of Biogeography 37: 1995–2004. https://doi.org/10.1111/j.1365-2699.2010.02339.x

Costa WEJM (2014) *Austrolebias araucarianus,* a new seasonal killifish from the Iguaçu river drainage, southern Basilian Araucarian Plateau Forest (Cyprinodontiformes: Rivulidae). Ichthyologycal Exploration of Freshwaters 25(2): 97–101.

Costa WJEM, Cheffe MM, Amorim PF (2017) Two new seasonal killifishes of the *Austrolebias adloffi* group from the Lagoa dos Patos basin, southern Brazil (Cyprinodontiformes: Aplocheilidae). Vertebrate Zoology 67(2): 139–149.

D'Anatro A, Loureiro M (2005) Geographic variation in the annual killifish *Austrolebias luteoflamulatus* Vaz Ferreira, Sierra and Scaglia (Cyprinodontiformes, Rivulidae). Journal of Fish Biology 67: 849–865. https://doi.org/10.1111/j.0022-1112.2005.00791.x

Dingerkus G, Uhler LD (1977) Differential staining of bone and cartilage in cleared and stained fish using alcian blue to stain cartilage and enzymes for clearing flesh. Stain Technologies 52(4): 229–232. https://doi.org/10.3109/10520297709116780

Ferrer J, Malabarba LR, Costa WJEM (2008) *Austrolebias paucisquama* (Cyprinodontiformes: Rivulidae), a new species of annual killifish from southern Brazil. Neotropical Ichthyology 6: 175–180. https://doi.org/10.1590/S1679-62252008000200004

García D, Loureiro M, Machín E, Reichard M (2017) Phenology of three coexisting annual fish species: seasonal patterns in hatching dates. Hydrobiologia 809: 323–337. https://doi.org/10.1007/s10750-017-3484-9

García G, Alvarez-Valin F, Gomez N (2002) Mitochondrial genes: signals and noise in the phylogenetic reconstruction of the annual killifish genus *Cynolebias* (Cyprinodontiformes, Rivulidae). Biological Journal of the Linnean Society 76: 49–59. https://doi.org/10.1111/j.1095-8312.2002.tb01713.x

García G, Gutiérrez V, Ríos N, Turner B, Santiñaque F, López-Carro B, Folle G (2014) Burst speciation processes and genomic expansion in the neotropical annual killifish genus *Austrolebias* (Cy-

prinodontiformes, Rivulidae). Genetica 142(1): 87–98. https://doi.org/10.1007/s10709-014-9756-7

García G, Gutiérrez V, Vergara J, Calviño P, Duarte A, Loureiro M (2012) Patterns of population differentiation in annual killifishes from the Paraná-Uruguay-La Plata basin: the role of vicariance and dispersal. Journal of Biogeography 39(9): 1707–1719. https://doi.org/10.1111/j.1365–2699.2012.02722.x

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. IUCN, Gland, Switzerland and Cambridge, 32 pp.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11: 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Lambeck K, Esat TM, Potter EK (2002) Links between climate and sea levels for the past three million years. Nature 419: 199–206. https://doi.org/10.1038/nature01089

Loureiro M, García G (2008) *Austrolebias reicherti* Loureiro and García, a valid species of annual fish (Cyprinodontiformes: Rivulidae) from Uruguay. Zootaxa 1940: 1–15.

Loureiro M, Duarte A, Zarucki M (2011) A new species of *Austrolebias* Costa (Cyprinodontiformes: Rivulidae) from northeastern Uruguay, with comments on distribution patterns. Neotropical Ichthyology 9(2): 335–342. https://doi.org/10.1590/S1679-62252011000200010

Loureiro M, Borthagaray A, Hernández D, Duarte A, Pinelli V, Arim M (2016) *Austrolebias* in space. Scaling from ponds to biogeographical regions. In: Berois N, García G, de Sá RO (Eds) Annual Fishes – Life History Strategy, Diversity, and Evolution. CRC Press, Taylor & Francis Group, Boca Ratón, 111–132.

Loureiro M, de Sá RO, Serra W, Alonso F, Nielsen D, Calviño P, Volcan MV, Lanés LE, Duarte A, García G (2018) Review of the family Rivulidae (Cyprinodontiformes, Aplocheiloidei) and a molecular and morphological phylogeny of the annual fish genus *Austrolebias* Costa 1998. Neotropical Ichthyology 16(3): e180007. https://doi.org/10.1590/1982-0224-20180007

Martínez S, Rojas A (2013) Relative sea level during the Holocene in Uruguay. Palaeogeography, Palaeoclimatology, Palaeoecology 374: 123–131. https://doi.org/10.1016/j.palaeo.2013.01.010

MVOTMA (2018) MVOTMA. http://www.mvotma.gub.uy/portal/areas-protegidas/item/10006542-area-protegida-con-recursos-manejados-montes-del-queguay-paysandu.html [Accessed may 2018]

Nielsen DTB, Pillet D (2015) *Austrolebias accorsii*, a new annual fish (Cyprinodontiformes: Rivulidae: Cynolebiatinae) from the upper río Grande basin, Amazon basin, Bolivia. Aqua 21(4): 172–179.

Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. Trends in Ecology and Evolution 16: 364–371. https://doi.org/10.1016/S0169-5347(01)02160-7

Rohlf FJ (2003) Morphometrics at SUNY Stony Brook. http://life.bio.sunysb.edu/morph

Volcan MV, Gonçalves ÂC, Lanés LEK (2017) A new annual fish of the genus *Austrolebias* (Cyprinodontiformes: Rivulidae) from Rio Camaquã basin, Laguna dos Patos system, Brazilian Pampa. Zootaxa 4338(1): 141–152. https://doi.org/10.11646/zootaxa.4338.1.7

Volcan MV, Lanés LEK, Gonçalves ÂC (2014) *Austrolebias bagual*, a new species of annual fish (Cyprinodontiformes: Rivulidae) from southern Basil. Aqua 20(4): 161–172.

Williams DD (2006) The Biology of Temporary Waters. Oxford University Press, New York. https://doi.org/10.1093/acprof:oso/9780198528128.001.0001

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# Multigene analysis of the catfish genus *Trichomycterus* and description of a new South American trichomycterine genus (Siluriformes, Trichomycteridae)

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## Abstract

*Trichomycterus* comprises about 170 valid species, but its monophyly has been challenged in the last decades. Bayesian Inference and Maximum Likelihood analyses comprehending mitochondrial genes COI and CYTB and nuclear genes GLYT, MYH6 and RAG2 from 71 Trichomycterinae terminal taxa and eight outgroups were performed. The analyses highly supports a clade containing *Trichomycterus nigricans*, the type species of the genus, and several other congeners endemic to eastern and northeastern Brazil, herein considered as the genus *Trichomycterus*, the sister clade the southern Brazil and adjacent areas clade; the latter clade comprises two subclades, one comprising species of the genus *Scleronema* and another comprising species previously placed in *Trichomycterus*, herein described as a new genus. *Cambeva* **gen. n.** is distinguished from all other trichomycterines by the presence of a bony flap on the channel of the maxillo-dentary ligament, the interopercle shorter than the opercle, a deep constriction on the basal portion of the antero-dorsal arm of the quadrate, absence of teeth in the coronoid process of the dentary, the maxilla shorter than the premaxilla, the cranial fontanel extending from the the medial posterior of frontal to the medial region of supraoccipital, and absence of the postorbital process of the sphenotic-prootic-pterosphenoid.

## Introduction

Siluriformes (catfishes) is among of the most diverse vertebrate orders, with about 3700 species in 39 families (Nelson et al. 2016, Frick et al. 2018). Trichomycteridae, the second most diverse catfish family, with about 300 species (Frick et al. 2018 ), is characterized by having a modified opercular system, involving the presence of odontodes in the interopercular and opercular bones (Baskin 1973, de Pinna 1992, 1998). It occurs between southern Central America, in Costa Rica, and southern South America, in Patagonia (Baskin 1973, de Pinna 1998), exhibiting an impressive species diversity and endemism around higher elevations (de Pinna 1992). Presently, Trichomycteridae comprises eight subfamilies: Trichomycterinae Bleeker, 1858, Vandelliinae Bleeker, 1862, Stegophilinae Günther, 1864, Tridentinae Eigenmann, 1918, Glanapteryginae, Myers 1944, Sarcoglanidinae Myers & Weitzman, 1966, Trichogeninae Isbrücker, 1986 and Copionodontinae de Pinna, 1992. Trichomycterinae is the most species rich subfamily, with 220 species in eight genera: *Bullockia* Arratia, Chang, Menu-Marque & Rojas, 1978; *Eremophilus* Humboldt, 1805; *Hatcheria* Eigenmann, 1909; *Ituglanis* Costa & Bockmann, 1993; *Rhizosomichthys* Miles, 1943; *Scleronema* Eigenmann, 1917; *Silvinichthys* Arratia, 1998; and *Trichomycterus* Valenciennes, 1832 (Frick et al. 2018).

Among the Trichomycterinae, *Trichomycterus* is the most species diverse genus, comprising about 170 valid species (Frick et al. 2018) and occurring in a geographical range coincident to that described for the whole family (Arratia 1998; de Pinna 1998). Inhabiting mainly high-al-

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titude rapid stream rivers, a remarkable feature occurring among species of *Trichomycterus* is the ability to climb waterfalls (Eigenmann 1918, Arratia 1983) with the support of the interopercle and opercle odontodes, in an 'elbowing' behaviour (de Pinna 1992, Adriens et al. 2010).

Since Baskin (1973), monophyly of *Trichomycterus* has been challenged by several authors (Arratia et al. 1978, de Pinna 1989, Arratia 1990a, Costa and Bockmann 1993, Datovo and Bockmann 2010, Ochoa et al. 2017), but its phylogenetic status still remains unclear. Morphological taxonomic studies could not find unique diagnostic characters for *Trichomycterus* (de Pinna 1989), whereas some species traditionally placed in *Trichomycterus* have been reallocated in other trichomycterine genera (e.g. Costa and Bockmann 1993, Arratia 1998, Henschel et al. 2017). Furthermore, recent molecular analyses have suggested that species still today placed in *Trichomycterus* do not form a monophyletic group (Ochoa et al. 2017).

In addition to the uncertain status of *Trichomycterus*, two other trichomycterine genera, *Scleronema* and *Ituglanis*, have unclear relationships. They are often considered more related to non-trichomycterine trichomycterids (Myers and Weitzman 1966, de Pinna 1989, Costa and Bockamnn 1994, DoNascimiento 2015), but more recent studies indicated that they are members of the Trichomycterinae (Datovo and Bockmann 2010, Henschel et al. 2017, Ochoa et al. 2017).

The objective of this paper is to provide the more inclusive molecular based phylogenetic analysis of *Trichomycterus*, assigning the phylogenetic position of its type species, *Trichomycterus nigricans* Valenciennes, 1832 and describing a new genus, sister to *Scleronema*, that was hidden under the lack of consistent information about *Trichomycterus* relationships.

## Materials and methods

### Taxon sampling

Specimens were euthanized by sub-merging them in a buffered solution of tricaine methanesulphonate (MS-222) at a concentration of 250 mg/L, for a period of 10 min, following the guidelines of the Journal of the American Veterinary Medical Association (AVMA Guidelines) (Leary et al. 2013) and European Commission DGXI consensus for fish euthanasia (Close et al. 1996, 1997). Molecular data were obtained from specimens fixed and preserved in absolute ethanol. Specimens used for morphological comparisons were fixed in formalin for a period of 14 days and then transferred to 70% ethanol. All the collected material was deposited in the fish collection of Institute of Biology, Federal University of Rio de Janeiro (UFRJ).

### DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissues using the DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer's protocol. The analyses included a set of partial sequences of four nuclear genes: recombination activating 2 (RAG2), myosin heavy chain 6 (MYH6), SH3 and PX domain containing 3 (SH3PX3), glycosyltransferase (GLYT); and partial sequences of two mitochondrial encoded genes: cytochrome c oxidase I (COI) and cytochrome b (CYTB). Amplification of the target DNA fragments was made through the polymerase chain reaction (PCR) method, using the following primers: Cytb Siluri F, Cytb Siluri R, L5698-ASN, H7271-COI (Villa-Verde et al. 2012), Glyt_F577, Glyt_F559, Glyt_R1562, Glyt_R1464, myh6_F459, myh6_F507, myh6_R1322, myh6_R1325, SH3PX3_F461, SH3PX3_R1303, SH3PX3_F532, SH3PX3_R1299 (Li et al. 2007); RAG2 MCF, RAG2 MCR (Cramer et al. 2011), MHRAG2-F1 and MHRAG2-R1 (Hardman & Page, 2003). Double-stranded PCR amplifications were performed in 60 µl reactions with reagents at the following concentrations: 5× GreenGoTaq Reaction Buffer (Promega), 3.2 mm MgCl2, 1 µm of each primer, 75 ng of total genomic DNA, 0.2 mm of each dNTP and 1 U of standard Taq polymerase or Promega GoTaq Hot Start polymerase. The thermocycling profile was as follows: initial denaturation for 2 min at 94–95 °C; 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min–90 s at 48.0–64.0 °C and extension for 1–2 min at 72 °C; and terminal extension for 4 min at 72 °C. Negative controls were used to check on contaminations. The PCR products were then purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 20 µl reaction volumes containing 4 µl BigDye, 2 µl sequencing buffer 5× (Applied Biosystems), 2 µl of the amplified products (10 -40 ng), 2 µl primer and 10 µl deionized water. The thermocycling profile was: (1) 35 cycles of 10 seconds at 96 °C, 5 seconds at 54 °C and 4 minutes at 60 °C.

### Phylogenetic analyses

In-group included seven subfamilies of Trichomycteridae lineages. Since this study was directed to searching for relationships and monophyly of *Trichomycterus*, the analysis included a total of 50 terminal taxa presently placed in this genus, as well as 21 terminal taxa belonging to the trichomycterine genera *Bullockia*, *Eremophilus*, *Ituglanis* and *Scleronema*. Out-group selection was directed to sample representatives of other lineages of the Trichomycteridae, comprising six terminal taxa representing each of the remaining subfamilies, as well as representatives of other families of the Loricarioidea, including the nematogenyid *Nematogenys inermis* Guichenot, 1848 and the loricariid *Pareiorhina rudolphi* (Miranda-Ribeiro, 1911), in which the analyses were rooted. A list of specimens and its respective GenBank accession numbers is provided in Suppl. material 1. Sequences were aligned and edited in Mega 7 software (Kumar et al. 2016) using the ClustalW algorithm (Chenna et al. 2003). Gaps were considered as informative characters. The data set was partitioned by gene, and the best-fit evolutionary model selection was performed under the Akaike information criteria in the software jmodeltest 2 (Darriba et al. 2012) for each partition. Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were conducted using the softwares Mrbayes 3.2 (Ronquist et al. 2012) and Garli 2.0 (Zwickl 2006), re-

spectively. The BI analysis was conducted with the following parameters: two independent Markov chain Monte Carlo (MCMC) runs of two chains each for 20 million generations, with a tree sampling frequency of every 100 generations. The convergence of the MCMC chains and the proper burn-in value were assessed by evaluating the stationary phase of the chains using tracer v. 1.5 (Rambaut et al. 2013). The BI final consensus tree and its Bayesian posterior probabilities were generated with the remaining tree samples after removing the first 25% samples as burn-in. To test the support of the nodes in the ML analysis, 1,000 bootstrap (Felsenstein 1985) replicates were made.

### Morphological comparisons

Morphological comparisons were focused on the external morphology and osteological features of cleared and stained specimens prepared according to Taylor and Van Dyke (1985). Terminology for osteological nomenclature follows Arratia (1998). Measurements and counts follow Barbosa and Costa (2003). A list of material analysed appears in Suppl. material 2.

## Results

### Phylogenetic analyses

The concatenated matrix comprised 4380 bp after alignment (522 bp for COI, 857 for CYTB, 890 for GLYT, 542 for MYH6, 884 for RAG2 and 680 for SH3PX3). The best-fit evolutionary models found are shown in Suppl. material 3. The best log-likelihood score for the ML analysis was -31473.209522 and the mean for BI was -lnl -32013.626.

Both analyses exhibit similar topologies (Figs 1, 2). In all analyses, monophyly of the Trichomycterinae was high-

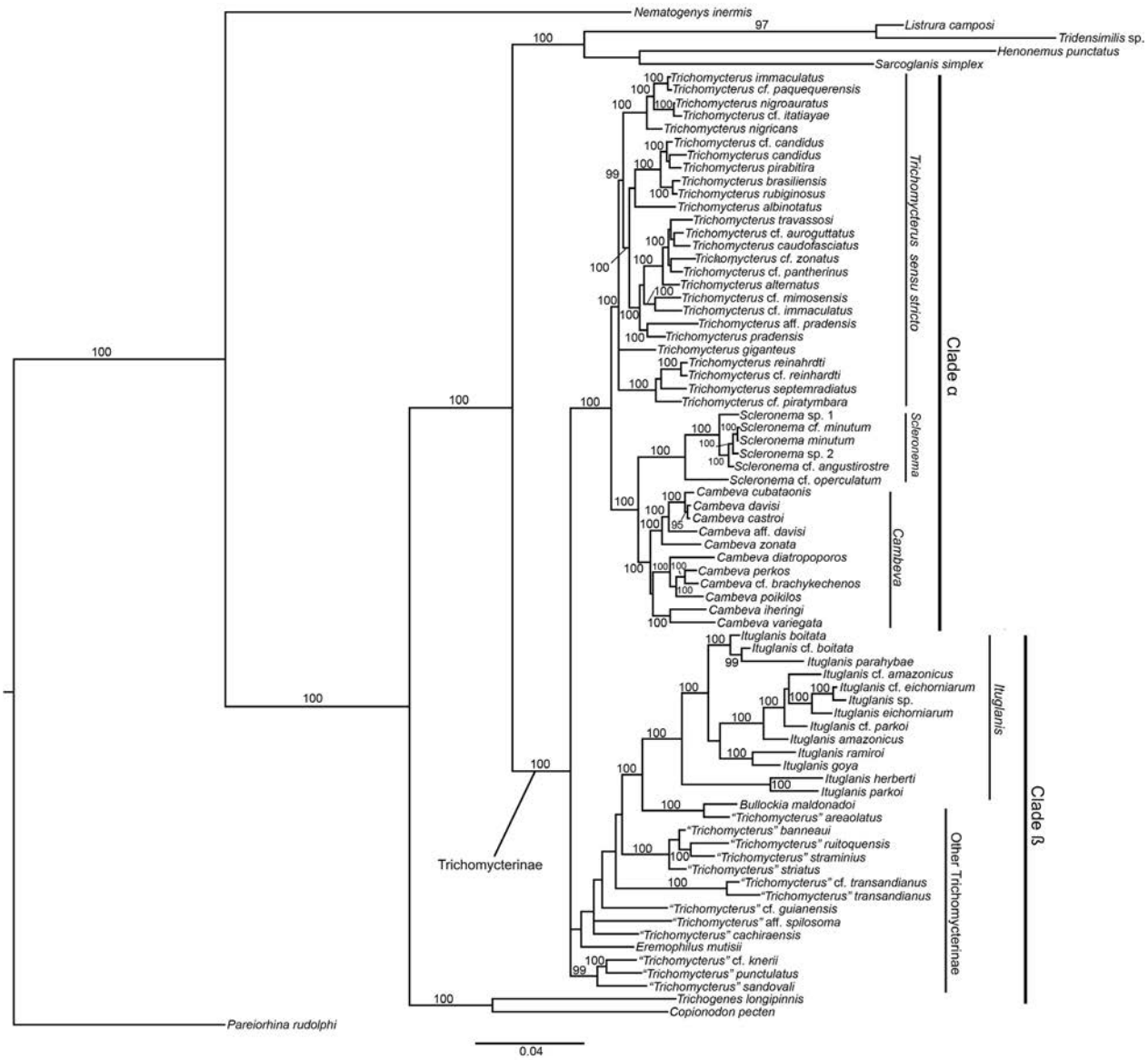


**Figure 1.** Phylogenetic positioning of *Cambeva* among the Trichomycteridae, inferred by Bayesian Inference from the analysis of molecular data, total of 4380 bp comprising segments of nuclear genes for GLYT, MYH6, RAG2 and SH3PX3 and the mitochondrial genes COI and CYTB. Numbers on each node represent posterior probabilities.

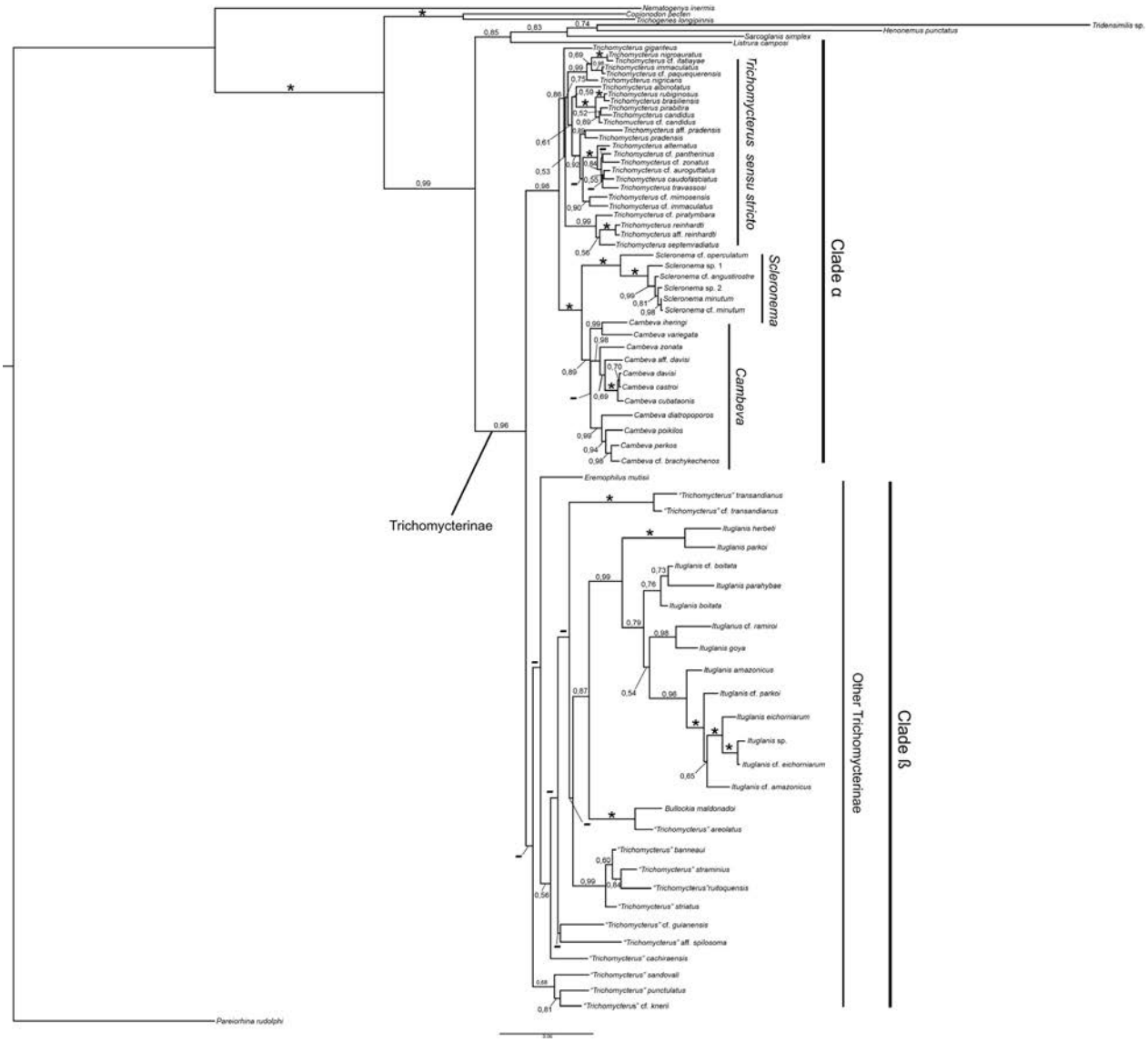


**Figure 2.** Phylogenetic positioning of *Cambeva* among the Trichomycteridae, inferred by Maximum Likehood from the analysis of molecular data, total of 4380 bp comprising segments of nuclear genes for GLYT, MYH6, RAG2 and SH3PX3 and the mitochondrial genes COI and CYTB. Numbers on each node are bootstrap percentages of the Maximum Likelihood analysis; asterisks indicate maximum support value and hyphen values under 50.

ly supported, but nominal species of *Trichomycterus* did not form a single monophyletic group. Two major clades were found in Trichomycterinae. The first one, the trichomycterine clade a, is highly supported in both analyses (Figs 1, 2) and comprises species geographically restricted to an area encompassing eastern, south-eastern and southern Brazil, which have been traditionally placed in two genera, *Scleronema* and *Trichomycterus*. Both analyses highly corroborate three major subclades (Figs 1, 2), one comprising *T. nigricans*, the type species of *Trichomycterus*, and other congeners, therefore herein recognised as the true *Trichomycterus*; another corresponding to the genus *Scleronema*; and another clade, sister to *Scleronema* and comprising several nominal species of *Trichomycterus*, herein recognised as a new genus (see taxonomic accounts below).

The second trichomycterinae group, the clade b, is weakly supported in the BI analysis (Fig. 1), but not supported in the ML analysis (Fig. 2). It includes taxa endemic to a broad geographical area, including the Andean region, Amazon, and Patagonia, formally placed in the genera *Bullockia*, *Eremophilus*, *Ituglanis* and *Trichomycterus*, which is herein graphed "*Trichomycterus*" by being distantly related to that clade including the type species of the genus. Relationships among basal lineages of the clade b were weakly supported (BS < 50; BI < 95).

## Taxonomic accounts

### *Cambeva* gen. n.

http://zoobank.org/C26BEA49-7714-44FB-957B-678D8C6C9DCC

**Type species.** *Pygidium davisi* Haseman, 1911 (Fig. 3)

**Diagnosis.** *Cambeva* is similar to *Scleronema* and distinguished from all other genera of the Trichomycteri-

**Figure 3.** *Cambeva davisi*, topotype, UFRJ 9759, 67.6 mm SL; Brazil: Paraná: Balsa Nova. Photograph by A. M. Katz.

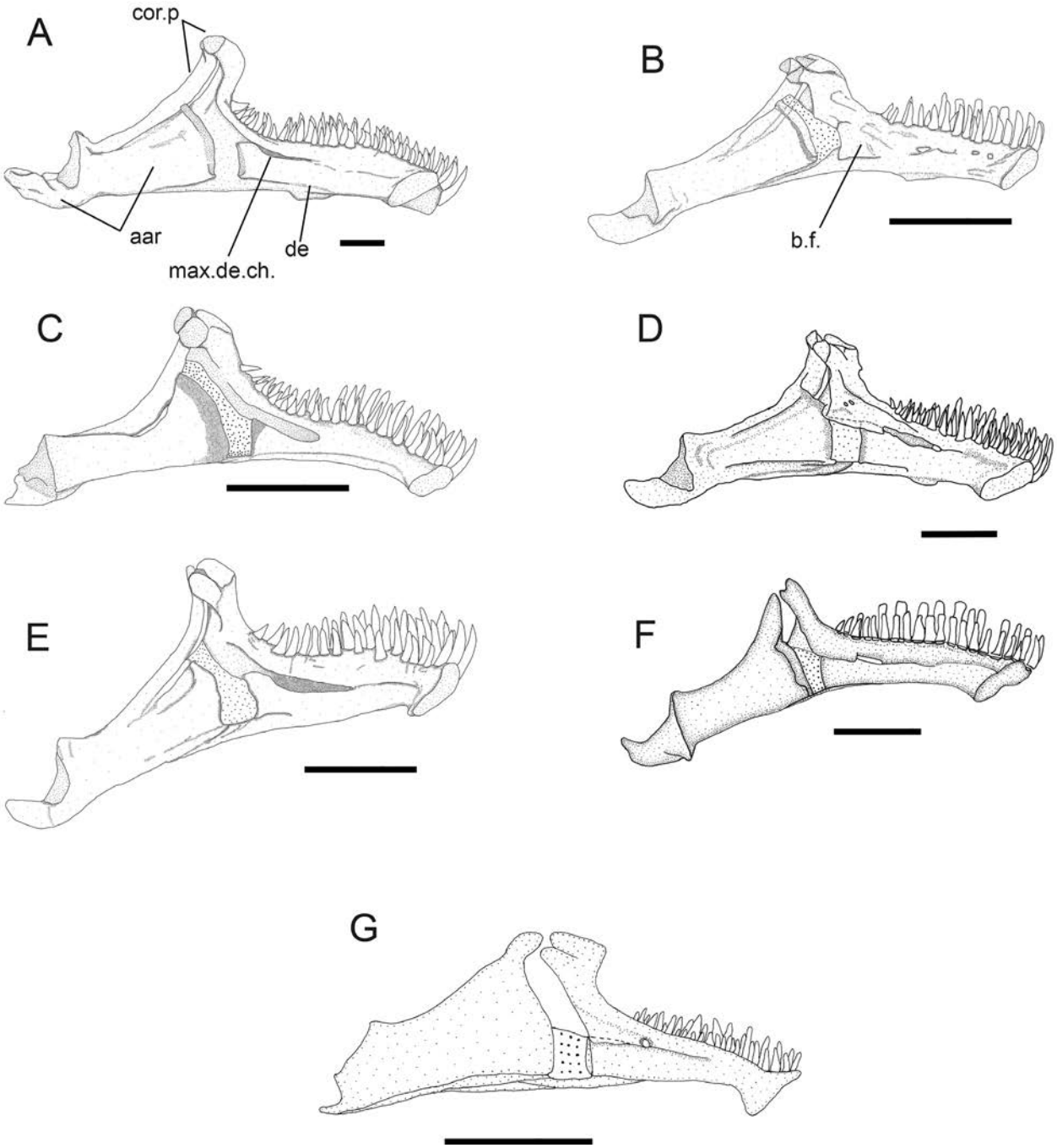


**Figure 4.** Left lower jaw in medial view. **A** *Trichomycterus giganteus*, UFRJ 5732, paratype. **B** *Cambeva davisi*, UFRJ 10713. **C** *Trichomycterus alternatus*, UFRJ 9900. **D** *Cambeva zonata*, UFRJ 11900. **E** *Trichomycterus brasiliensis*, UFRJ 4834. **F** *Cambeva brachykechenos*, UFRJ 10586. **G** *Scleronema operculatum*, UFRJ 11856. Scale bar: 1 mm aar, anguloarticuloretroarticular; corp.p, coronoid process; de, dentary; max.de.ch., maxillo-dentary channel; b.f. bone flap.

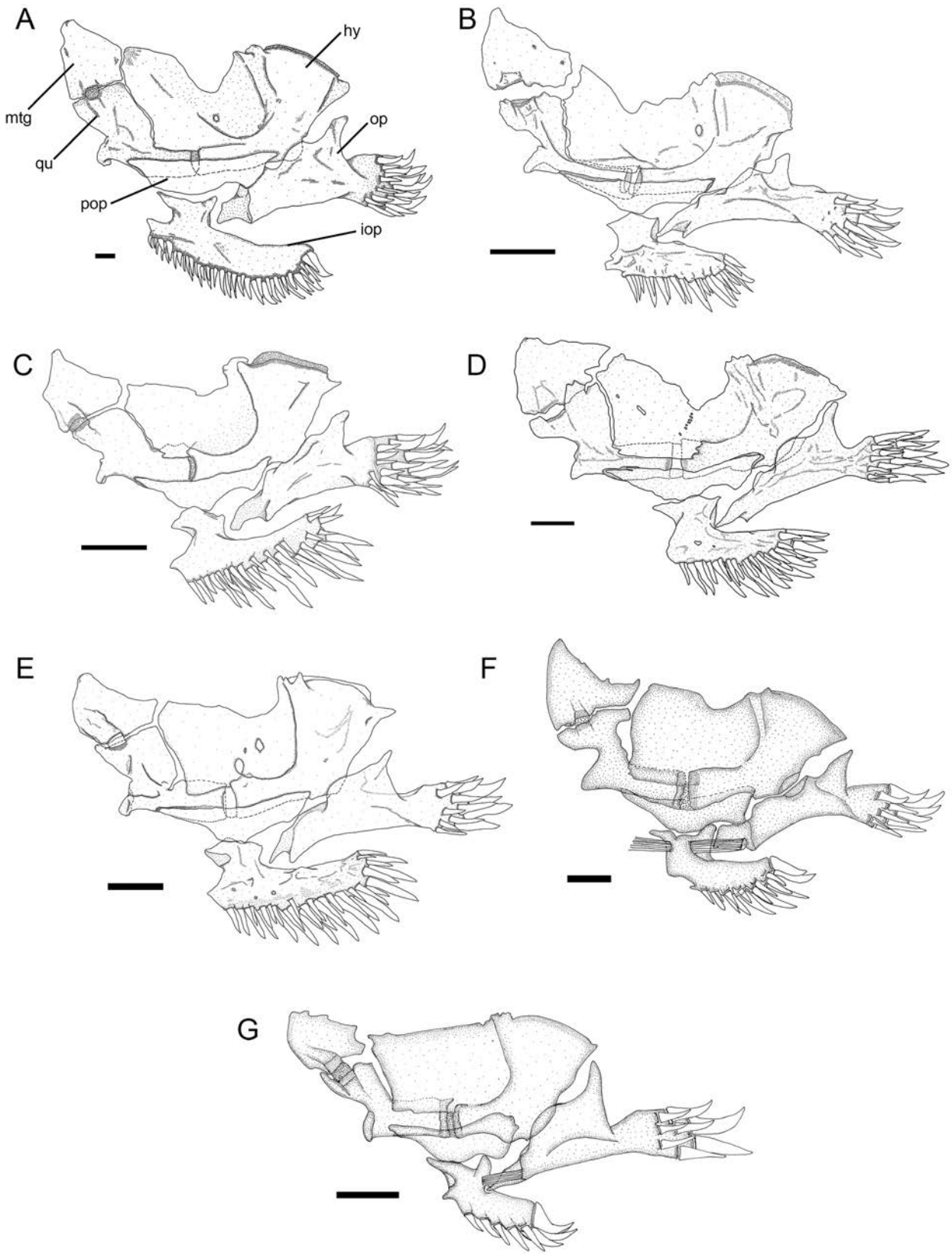


**Figure 5.** Left suspensory in dorsal view. **A** *Trichomycterus giganteus*, UFRJ 5732, paratype. **B** *Cambeva davisi*, UFRJ 10713. **C** *Trichomycterus alternatus*, UFRJ 5673. **D** *Cambeva zonata*, UFRJ 11900. **E** *Trichomycterus brasiliensis*, UFRJ 4834. **F** *Cambeva brachykechenos*, UFRJ 10586. **G** *Scleronema operculatum*, UFRJ 11856. Scale bar: 1 mm hy, hyomandibula; iop, interopercle; mtg, metapterygoid; op, opercle; pop, preopercle.

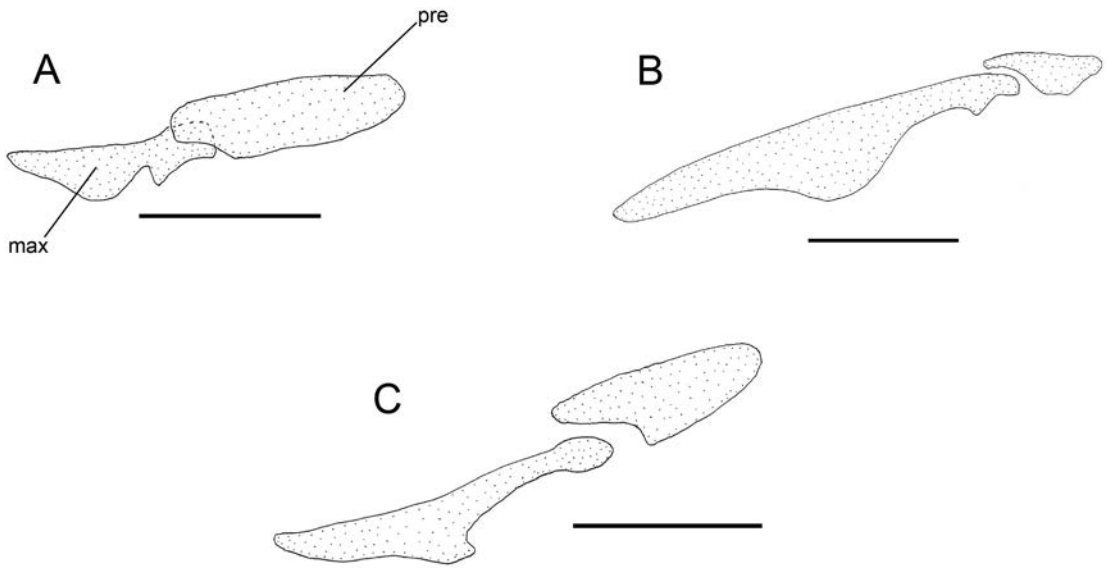


**Figure 6.** Dorsal view of premaxilla and maxilla. **A** *Cambeva davisi*, UFRJ 10713 **B** *Scleronema operculatum*, UFRJ 11856. **C** *Scleronema* sp1., UFRJ 10645. Scale bar: 1 mm max, maxilla; pre, premaxilla.

nae by the presence of a bony flap covering the posterior segment of the maxillo-dentary ligament channel in the dentary (Fig. 4B, D, F, G vs. flap absent, Fig. 4A, C, E; see also Arratia 1998: fig. 9a). *Cambeva* also differs from most Trichomycterinae except *Scleronema* by the presence of a short interopercle, shorter than the opercle (Fig. 5B, D, F, G; vs. longer, Fig. 5A, C, E; see also Arratia 1998: fig. 9b, Schaefer & Fernández 2009: fig. 3, Adriens et al. 2010: fig. 5 c,d), a deep constriction on the basal portion of the antero-dorsal arm of the quadrate in lateral view, its width less than 50% quadrate width at its dorsal limit (Fig. 5B, D, F, G; vs. more than 50%, Fig. 5A, C, E; see also Arratia 1990b: fig. 10a-c, Arratia 1998: fig. 8a, b), and absence of teeth in the coronoid process of the dentary (vs. presence), (Fig. 4B, D, F, G, Arratia 1998: fig. 9a). *Cambeva* differs from *Scleronema* by having the maxilla shorter than the premaxilla (maxilla with 30-60% length of premaxilla (Fig. 6A) vs. 90% or more (Fig. 6B, C)), absence of a transverse skin fold between the anterior nostrils and the maxillary barbel (vs. presence), 4–6 abdominal vertebrae (vs. 1–3), and absence of a fleshy projection posteriorly extending to opercular patch of odontodes (vs. presence). *Cambeva* is also distinguished from *Ituglanis* by possessing the cranial fontanel extending from the medial posterior of frontal to the medial region of supraoccipital (vs. restricted to the posterior region of the parieto-supraoccipital) (Costa & Bockmann 1993: fig. 3A), absence of the postorbital process of the sphenotic-prootic-pterosphenoid (vs. presence of an anteriorly directed postorbital process on the sphenotic-prootic-pterosphenoid) (Costa & Bockmann 1993: fig. 3A, B) and a smooth medial rim of the autopalatine (vs. deeply concave) (Costa & Bockmann 1993: Fig. 6).

**Included species.** *Cambeva davisi* (Haseman, 1911), *Cambeva iheringi* (Eigenmann, 1917), *Cambeva zonata* (Eigenmann, 1918), *Cambeva brachykechenos* (Ferrer & Malabarba, 2013), *Cambeva castroi* (de Pinna, 1992), *Cambeva cubataonis* (Bizerril, 1994), *Cambeva diatropoporos* (Ferrer & Malabarba, 2013), *Cambeva poikilos* (Ferrer & Malabarba, 2013), *Cambeva variegata* (Costa, 1992). Other species, not included in the molecular analysis but exhibiting generic morphological diagnostic character states and congruent geographical distribution are: *Cambeva stawiarski* (Miranda Ribeiro, 1968), *Cambeva balios* (Ferrer & Malabarba, 2013), *Cambeva concolor* (Costa, 1992), *Cambeva crassicaudata* (Wosiacki & de Pinna, 2008), *Cambeva diabola* (Bockmann, Casatti & de Pinna, 2004), and *Cambeva naipi* (Wosiacki & Garavello, 2004). The following species, were not examined or lack the necessary osteological information on their original descriptions, but have the general external appearance and occur in the same basins that *Cambeva* is distributed, so they are tentatively included in the new genus: *Cambeva paolence* (Eigenmann 1917), *Cambeva guaraquessaba* (Wosiacki, 2005), *Cambeva igobi* (Wosiacki & de Pinna, 2008), *Cambeva mboycy* (Wosiacki & Garavello, 2004), *Cambeva pascuali* (Ochoa, Silva, Silva, Oliveira & Datovo, 2017), *Cambeva perkos* (Datovo, Carvalho & Ferrer, 2012), *Cambeva plumbea* (Wosiacki & Garavello, 2004), *Cambeva tropeiro* (Ferrer & Malabarba, 2011), *Cambeva tupinamba* (Wosiacki & Oyakawa, 2005), and *Cambeva ytororo* (Terán, Ferrer, Benitez, Alonso, Aguilera & Mirande, 2017).

**Distribution.** Species of *Cambeva* gen. n. occur in the Paraná, São Francisco, Ribeira de Iguape, and Uruguay river basins, as well as in smaller isolated coastal river basins of south-eastern and southern Brazil.

**Etymology.** *Cambeva*, probably derived from the Tupi-Guarani, is a popular name for trichomycterid fishes in southern and south-eastern Brazil. Gender: feminine.

# Discussion

### Monophyly of Trichomycterinae

Since the first objective phylogenetic analysis of the Trichomycteridae by Baskin (1973), monophyly of the Trichomycterinae has been challenged by several authors. Besides not finding unique diagnostic features for the subfamily, supposed derived traits shared by some trichomycterines and species of other trichomycterid subfamilies made uncertain the position of some trichomycterine members. de Pinna (1989) suggested that the trichomycterine genus *Scleronema* is more closely related to the subfamily Sarcoglanidinae than to other trichomycterines, as already discussed by Myers & Weitzman (1966), due to the common possession of an enlarged maxilla; Arratia (1990) also reported a maxilla enlargement for the trichomycterine genera *Bullockia* and *Hatcheria*, besides species of '*Trichomycterus*'. Our results clearly indicate that taxa having long maxilla (e.g., sarcoglanidines, *Bullockia*, *Scleronema*) do not form a monophyletic group. Considering the psammophilic habits exhibited by species of Sarcoglaninidae and *Scleronema*, with specimens digging into sand or gravel (Zuanon and Sazima 2004, Schaefer et al. 2005, AM Katz and WJEM Costa pers. obs.), the long maxilla exhibited by these taxa may be interpreted as an adaptive convergence generating homoplasies in both groups.

Costa and Bockmann (1993) considered *Ituglanis* and *Scleronema* more closely related to the TSVSG clade, comprising the Tridentinae, Sarcoglanidinae, Vandelliinae, Stegophilinae and Glanapteryginae (Costa and Bockmann 1994), than to other trichomycterids based on a thin tip of the lateral process of the urohyal and a slightly reduced interopercular patch of odontodes. However, as noted by subsequent authors (e.g. Fernández and de Pinna 2005; Datovo and Bockmann 2010; the present study) other trichomycterines also exhibit in some extent those character states. More recently, Datovo and Bockmann (2010), in their myological analysis of trichomycterids, found evidence indicating that *Ituglanis* and *Scleronema* are more closely related to other Trichomycterinae than to the TSVSG clade. A similar result has been proposed in molecular phylogenies (e.g. Henschel et al. 2017, Ochoa et al. 2017). In our multigene analysis, using a broader genetic sample, the phylogenetic position of *Ituglanis* and *Scleronema* within a monophyletic Trichomycterinae is highly corroborated (Figs 1, 2). *Ituglanis*, with species distributed in the main tropical and subtropical Cis-Andean river basins of South America, did not appear as closely related to *Scleronema*, but sister to a clade comprising Andean, Patagonian and Amazon species of '*Trichomycterus*' (Figs 1, 2).

# Monophyly of Cambeva

All species here included in *Cambeva* were previously placed in *Trichomycterus*, instead of being considered as more closely related to *Scleronema* (Eigenmann 1918, Bizerril 1994, Wosiacki and Garavello 2004). Datovo and Bockmann (2010) examined myological characters of several trichomycterids, among which were two species of *Cambeva*, *C. davisi* and *C. stawiarski*, which according those authors share a unique apomorphic condition consisting of the extensor tentaculi originating exclusively from the ethmoidal region of the neurocranium. We have searched this derived condition in several species of *Trichomycterus* and *Cambeva* herein examined (see Suppl. material 2), but it was only found in species endemic to some coastal rivers of southern Brazil, and to the Iguaçu, Ribeira do Iguape and Parapanema river basins (*C. castroi*, *C. cubataonis*, *C. davisi*, *C. naipi* and *C. zonata*), suggesting that this derived condition is synapomorphic for a subclade of *Cambeva*.

*Cambeva* is here supported by high values of bootstrap and posterior probability as monophyletic and sister to *Scleronema* (Figs 1, 2). However, in spite of the clade comprising *Cambeva* and *Scleronema* being diagnose by morphological synamoporphies, we did not find unique character states for *Cambeva*, which is only distinguishable from other trichomycterine genera by a combination of derived and primitive morphological character states (see Diagnosis above).

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# References

Aedriens D, Baskin JN, Coppens H (2010) Evolutionary morphology of trichomycterid catfishes: About hanging on and diggingin. In: Nelson JS, Schultze HP, Wilson MVH (Eds) Origin and phylogenetic interrelationships of teleosts. Verlag Dr. Friedrich Pfeil, München, 337–362.

Arratia G (1990a) The South American Trichomycterinae (Teleostei: Siluriformes), a problematic group. In: Peters G, Hutterer R (Eds) Vertebrates in the tropics. Museum Alexander Koenig, Bonn, 395–403.

Arratia G (1990b) Development and diversity of the suspensorium of trichomycterids and comparison with loricarioids (Teleostei: Siluriformes). Journal of Morphology 205: 193–218. https://doi.org/10.1002/jmor.1052050208

Arratia G (1998) *Silvinichthys*, a new genus of trichomycterid catfishes from the Argentinian Andes, with redescription of *Trichomycterus nigricans*. Ichthyological Exploration of Freshwaters 9: 347–370.

Arratia G, Chang A, Menu-Marque S, Rojas G (1978) About *Bullockia* gen. n., *Trichomycterus mendozensis* n. sp. and revision of the family Trichomycteridae (Pisces, Siluriformes). Studies on Neotropical Fauna and Environment 13: 157–194. https://doi.org/10.1080/01650527809360539

Barbosa MA, Costa WJEM (2003) Validade, relações filogenéticas e redescrição de *Eremophilus candidus* (Ribeiro, 1949) (Siluriformes: Trichomycteridae). Arquivos do Museu Nacional 61: 179–188.

Baskin JN (1973) Structure and relationships of the Trichomycteridae. Supplementary material of de Pinna (2016). Neotropical Ichthyology, 14: S1–S62.

Bizerril CRSF (1994) Descrição de uma nova espécie de *Trichomycterus* (Siluroidei, Trichomycteridae) do Estado de Santa Catarina, com uma sinopse da composição da família Trichomycteridae no leste Brasileiro. Arquivos de Biologia e Tecnologia 37: 617–628.

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignmentwith the Clustal series of programs. Nucleic Acids Research 31: 3497–3500. https://doi.org/10.1093/nar/gkg500

Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D (1996) Recommendations for euthanasia of experimental animals: Part 1. Laboratory Animals 30: 293–316. https://doi.org/10.1258/002367796780739871 Close B, Banister K, Baumans V, Bernoth EM, Bromage N, Bunyan J, Erhardt W, Flecknell P, Gregory N, Hackbarth H, Morton D (1997) Recommendations for euthanasia of experimental animals: Part 2. Laboratory Animals 31: 1–32. https://doi.org/10.1258/002367797780600297

Costa WJEM, Bockmann FA (1993) Un nouveau genre néotropical de la famille des Trichomycteridae (Siluriformes: Loricarioidei). Revue Française d'Aquariologie et Herpétologie 20: 43–46.

Costa WJEM, Bockmann FA (1994) A new genus and species of Sarcoglanidinae (Siluriformes: Trichomycteridae) from south-eastern Brazil, with a re-examination of subfamilial phylogeny. Journal of Natural History 28: 715–730. https://doi.org/10.1080/00222939400770331

Cramer CA, Bonatto SL, Reis RE (2011) Molecular phylogeny of the Neoplecostominae and Hypoptopomatinae (Siluriformes: Loricariidae) using multiple genes. Molecular Phylogenetics and Evolution 59: 43–52. https://doi.org/10.1016/j.ympev.2011.01.002

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Datovo A, Bockmann FA (2010) Dorsolateral head muscles of the catfish families Nematogenyidae and Trichomycteridae (Siluriformes: Loricarioidei): comparative anatomy and phylogenetic analysis. Neotropical Ichthyology 8: 193–246. https://doi.org/10.1590/S1679-62252010000200001

de Pinna MCC (1989) A new sarcoglandine catfish, phylogeny of its subfamily, and an appraisal of the phyletic status of the Trichomycterinae (Teleostei, Trichomycteridae). American Museum Novitates 2950: 1–39.

de Pinna MCC (1992) A new subfamily of Trichomycteridae (Teleostei: Siluriformes), lower loricarioid relationships and a discussion on the impact of additional taxa for phylogenetic analysis. Zoological Journal of the Linnean Society 106: 175–229. https://doi.org/10.1111/j.1096-3642.1992.tb01247.x

de Pinna MCC (1998) Phylogenetic relationships of neotropical Siluriformes (Teleostei: Ostariophysi): Historical overview and synthesis of hypotheses. In: Malabarba LR, Reis, RE, Vari RP, Lucena ZMS, Lucena CAS (Eds) Phylogeny and classification of Neotropical Fishes. Edipucrs, Porto Alegre, 279–330.

DoNascimiento C (2015) Morphological evidence for the monophyly of the subfamily of parasitic catfishes Stegophilinae (Siluriformes, Trichomycteridae) and phylogenetic diagnoses of its genera. Copeia 103: 933–960. https://doi.org/10.1643/CI-14-132

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fernandez L, de Pinna MCC (2005) A phreatic catfish of the genus *Silvinichthys* from southern South America (Teleostei, Siluriformes, Trichomycteridae). Copeia 2005:100–108. https://doi.org/10.1643/CI-03-158R2

Fricke R, Eschmeyer WN, van der Laan R (Eds) (2018) Catalog of fishes: genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [Accessed on 20/05/2018]

Henschel E, Mattos JLO, Katz AM, Costa WJEM (2017) Position of enigmatic miniature trichomycterid catfishes inferred from molecular data (Siluriformes). Zoologica Scripta 00: 1–10. https://doi.org/10.1111/zsc.12260

Hardman M, Page LM (2003) Phylogenetic relationships among Bullhead Catfishes of the genus *Ameiurus* (Siluriformes: Ictaluridae). Copeia 2003: 20–33. https://doi.org/10.1643/0045-8511(2003)003[0020:PRABCO]2.0.CO;2

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evo-lutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Leary S, Underwood W, Anthony R, Cartner S, Corey D, Grandin T (2013) AVMA guidelines for the euthanasia of animals. http://works.bepress.com/cheryl_greenacre/14

Li C, Ortí G, Zhang G, Lu G (2007) A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. BMC Evolutionary Biology 7: 44. https://doi.org/10.1186/1471-2148-7-44

Myers GS, Weitzman SH (1966) Two remarkable new trichomycterid catfishes from the Amazon basin in Brazil and Colombia. Journal of Zoology 149: 277–287. https://doi.org/10.1111/j.1469-7998.1966.tb04049.x

Nelson JS, Grande T, Wilson MVH (2016) Fishes of the world. 5th ed. John Wiley and Sons Inc., Hoboken. https://doi.org/10.1002/9781119174844

Ochoa LE, Roxo FF, DoNascimiento C, Sabaj MH, Datovo A, Alfaro M, Oliveira C (2017) Multilocus analysis of the catfish family Trichomycteridae (Teleostei: Ostariophysi: Siluriformes) supporting a monophyletic Trichomycterinae. Molecular Phylogenetics and Evolution 115: 71–81. https://doi.org/10.1016/j.ympev.2017.07.007

Rambaut A, Suchard MA, Xie D, Drummond AJ (2013) Tracer v1.5. Retrieved from: http://beast.bio.ed.ac.uk/Tracer.

Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Schaefer SA, Provenzano F, de Pinna MCC, Baskin JN (2005). New and noteworthy Venezuelan glanapterygine catfishes (Siluriformes, Trichomycteridae), with a discussion on their biogeography and psammophily. American Museum Novitates 3496: 1–27. https://doi.org/10.1206/0003-0082(2005)496[0001:NANVGC]2.0.CO;2

Schaefer SA, Fernández F (2009) Redescription of the pez graso, *Rhizosomichthys totae* (Trichomycteridae), of Lago de Tota, Colombia, and aspects of cranial osteology revealed by microtomography. Copeia 2009: 510–522. https://doi.org/10.1643/CI-08-186

Taylor WR, Van Dyke OC (1985) Revised procedures for staining and clearing small fishes and others vertebrates for bone and cartilage study. Cybium 9: 107–109. https://doi.org/10.1111/j.1096-3642.1944.tb00219.x

Tchernavin V (1944) A revision of some Trichomycterinae based on material preserved in the British Museum (Natural History). Proceedings of the Zoological Society of London 114: 234–275.

Villa-Verde L, Lazzarotto H, Lima SQM (2012) A new glanapterygine catfish of the genus *Listrura* (Siluriformes: Trichomycteridae) from southeastern Brazil, corroborated by morphological and molecular data. Neotropical Ichthyology 10: 527–538. https://doi.org/10.1590/S1679-62252012000300005

Wosiacki WB, Garavello JC (2004) Five new species of *Trichomycterus* from the rio Iguaçu (rio Paraná Basin), southern Brazil (Siluriformes: Trichomycteridae). Ichthyological Exploration of Freshwaters 15: 1–16.

Xia X (2013) DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. Molecular Biology and Evolution 30: 1720–1728. https://doi.org/10.1093/molbev/mst064

Xia X, Lemey P (2009) Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vandamme AM (Eds) The phylogenetic handbook. Cambridge University Press, Cambridge, 615–630. https://doi.org/10.1017/CBO9780511819049.022

Zuanon J, Sazima I (2004) Natural history of *Stauroglanis gouldingi* (Siluriformes: Trichomycteridae), a miniature sanddwelling candiru from central Amazonia streamlets. Ichthyological Exploration of Freshwaters 15: 201–208.

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Thesis, University of Texas, Austin.

# Supplementary material 1

## GenBank accession codes

Authors: Axel Makay Katz, Maria Anais Barbosa, José Leonardo de Oliveira Mattos, Wilson José Eduardo Moreira da Costa

Data type: MS Word document

Explanation note: Terminal taxa for molecular phylogeny and respective GenBank accession numbers.

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Link: https://doi.org/10.3897/zse.94.29872.suppl1

# Supplementary material 2

## Occurences and morphological data

Authors: Axel Makay Katz, Maria Anais Barbosa, José Leonardo de Oliveira Mattos, Wilson José Eduardo Moreira da Costa

Data type: MS Word document

Explanation note: List of material examined for the analysis of morphological characters.

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Link: https://doi.org/10.3897/zse.94.29872.suppl2

# Supplementary material 3

## Genetics data

Authors: Axel Makay Katz, Maria Anais Barbosa, José Leonardo de Oliveira Mattos, Wilson José Eduardo Moreira da Costa

Data type: MS Word document

Explanation note: Best-fit evolutionary models found for each mitochondrial gene and for each codon position of nuclear genes.

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Link: https://doi.org/10.3897/zse.94.29872.suppl3