

Zoosystematics

and Evolution

98 (1) 2022

Zoosystematics and Evolution

A Bulletin of Zoology since 1898

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Zoosystematics and Evolution

2022. Volume 98. 1 Issue

ISSN: 1435-1935 (print), 1860-0743 (online)

Abbreviated keys title: Zoosyst. Evol.

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Cover design

Pensoft

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Biological Abstracts® (Thompson ISI)

BIOSIS Previews® (Thompson ISI)

Cambridge Scientific Abstracts (CSA/CIG)

Web of Science® (Thompson ISI)

Zoological Record™ (Thompson ISI)

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Grismadox gen. nov., a new Neotropical genus of ant-resembling spiders (Araneae, Corinnidae, Castianeirinae), including the description of two new species from Bolivia and Paraguay

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Academic editor: Danilo Harms ♦ Received 17 October 2021 ♦ Accepted 3 December 2021 ♦ Published 7 January 2022

Abstract

A new genus and two new species of ant-resembling castianeirine spiders are described from the Neotropics. *Grismadox* gen. nov. comprises four species: *Grismadox baueri* sp. nov., and *Grismadox mazaxoides* (Perger & Duperré, 2021) comb. nov. from Bolivia, and *Grismadox karugua* sp. nov. (type species) and *Grismadox mboitui* (Pett, 2021) comb. nov. from Paraguay. All species are diagnosed and the new species are described and illustrated. Available ecological data suggests that all four species are primarily epigeal and inhabit Grassland and savannah type habitats.

Key Words

ant-mimic, Chiquitano, Humid Chaco, myrmecomorph

Introduction

The subfamily Castianeirinae (Araneae, Corinnidae) is represented by slender spiders generally regarded as good examples of Batesian or Müllerian mimics of ants (Cushing 2012; Perger and Rubio 2020; Perger 2021). The subfamily is cosmopolitan and the New World has a rich fauna of accurate ant mimics with similar coloration to their ant models and adaptations to the typical body plan (according to concepts of Pekár and Jarab 2011). New World endemic genera include *Mazax* O. Pickard-Cambridge, 1898, *Myrmecium* Latreille, 1824, *Myrmecotypus* O. Pickard-Cambridge, 1894 and *Psellocoptus* Simon, 1896 (World Spider Catalog 2021). The genera *Apochinomma* Pavesi, 1881, *Castianeira* Keyserling, 1879 and *Spheco-*

typus O. Pickard-Cambridge, 1895 are recorded from the New World, but have intercontinental distributions, being registered from the Oriental Region, and the former two also from the Afrotropical realm (Haddad 2013; World Spider Catalog 2021). However, *Apochinomma* may be restricted to the Afrotropical realm, with Neotropical representatives likely misplaced (Rubio and Arbino 2009).

Castianeirines are notorious for having highly conserved genitalic characters (Rubio et al. 2015), with both sexes showing a reduction or absence of various structures typically found in related families of dionychnan spiders (Ramírez 2014). However, in contrast to the genera *Myrmecium* and *Mazax*, which appear to be monophyletic (Reiskind 1969; Candiani and Bonaldo 2017), the variation in male genitalic structure of *Myrmecotypus* suggests a polyphyletic group

(Reiskind 1969; Perger and Rubio 2020). The level of variation exhibited in the embolus shape, length, and trajectory, as well as the presence or absence of a large to small conductor, retrolateral tibial apophyses (RTAs) of various sizes, including some large and distinct RTAs (Reiskind 1969; Pett 2021a), alludes to the hitherto undescribed generic diversity within the group. In contrast, female genitalia have always been described as relatively uniform in *Myrmecotypus*, with copulatory ducts joining the secondary spermathecae (ST II) and projecting posterolaterally (Reiskind 1969; Rubio and Arbino 2009; Perger and Rubio 2020).

In the current study, two new castianeirine species are described from Bolivia and Paraguay. These species share a combination of several somatic and genitalic characters with *Myrmecotypus mboitui* Pett, 2021 and *M. mazaxoides* Perger & Dupérré, 2021 that is not found in other Neotropical castianeirines. The similarities between these four species and differences to *Myrmecotypus* species warrant the establishment of a new genus, herein described.

Materials and methods

Material is preserved in 70% ethanol. The epigyne was dissected using a custom-made hooked pin and digested by submersion in a glass vial filled with lactic acid, which was placed in boiling water for around thirty minutes. The cleared epigyne was temporarily prepared on a slide and examined with a compound microscope. Examinations were carried out with an AmScope ZM-4T stereomicroscope or an Olympus BX61. Images were taken using either a Leica M125C automontage system or an Olympus BX61 with a DP74 camera. All images were z-stacked, with between 10–30 images merged into a single photomontage, using Helicon Focus 6.7 (www.heliconsoft.com). Habitus illustrations were made by BLP using a ‘Wacom One’ graphics tablet with images overlaid at 60% opacity on the program ‘Autodesk Sketchbook’ (see: Cala-Riquelme 2021). Images were adjusted in Adobe Photoshop version 21.0.1 for contrast and white balance. Plates were also composed in Adobe Photoshop.

The following indices are used following Reiskind (1969): A) carapace index = carapace width/ carapace length \times 100; B) cephalic index = cephalic width/ carapace width \times 100; C) sternum index = sternum width/ sternum length \times 100; D) abdomen index = abdomen width/ abdomen length \times 100. All measurements are in millimetres. The type species is treated first with the remaining species treated alphabetically.

Leg spination follows Rubio and Danisman (2014), with **d** = dorsal; **pl** = prolateral; **plv** = prolateral ventral; **rl** = retrolateral; **rlv** = retrolateral ventral; **v** = ventral. Other morphological terminology is standard for arachnology, with castianeirine specifics from Reiskind (1969). Abbreviations used in the text: **AER** = anterior eye row; **AI** = abdomen index; **AL** = abdomen length; **ALE** = anterior lateral eyes; **AME** = anterior median eyes; **AW** = abdomen width; **BL** = body length; **CD** = copulatory duct; **CI** = carapace index;

CL = carapace length; **CO** = copulatory opening; **CW** = carapace width; **dRTA** = dorsal retrolateral tibial apophysis; **FD** = fertilization duct; **PER** = posterior eye row; **PLE** = posterior lateral eyes; **PME** = posterior median eyes; **SI** = sternum index; **SL** = sternum length; **ST** = spermathecae (ST I is the most posterior (from which the fertilization ducts originate), ST II is the most anterior (into where the copulatory ducts enter)); **SW** = sternum width; **vRTA** = ventral retrolateral tibial apophysis. **Legs I–IV** = leg numbers.

Arachnological collections are abbreviated as follows (curators in parenthesis):

CBF	Colección Boliviana de Fauna, La Paz, Bolivia (R. Perger);
CIPLT-Ar	Colección Científica Para La Tierra- Aracnología (G. Hicks);
SMNK	Staatliches Museum für Naturkunde, Karlsruhe, Germany (H. Höfer).
ZMH	Zoological Museum Hamburg, University of Hamburg, Germany (D. Harms).

Nomenclatural acts. This published work and the nomenclatural acts it contains have been registered in Zoobank: <http://zoobank.org/References/893A-7CA0-CFB1-4687-960B-24D54B863C6C>. <http://zoobank.org/References/9EE84FF4-0803-487E-8997-97C7097007E2>. <http://zoobank.org/References/1EB-5BA81-86AF-48C3-B0FF-040C722F4D6F>. The LSID for this publication is: urn:lsid:zoobank.org:pub:CA75C8DB-013E-42D3-920F-654890CAFCAC.

Ecoregion distribution

The ecoregion affinities of the species were investigated by visualizing the coordinates and a shapefile of the regionalization of Neotropical ecosystems by Olson et al. (2001), by using the geographic information system QGIS (version 2.14.3, <http://www.qgis.org/en/site/>) (Fig. 1). Coordinates of the collection locality for *G. baueri* sp. nov. were approximated according to information from the owner of Espiritu ranch. Geographic coordinates are shown in decimal degrees with reference datum WGS84, and elevation in meters above sea level (m a.s.l.).

Results

Family Corinnidae Karsch, 1880

Subfamily Castianeirinae Reiskind, 1969

Genus *Grismadox* gen. nov.

<http://zoobank.org/893A7CA0-CFB1-4687-960B-24D54B863C6C>

Type species. *Grismadox karugua* sp. nov.

Etymology. The genus name is a patronym in honor of Cristian Grismado, arachnologist of Museo Argentino de

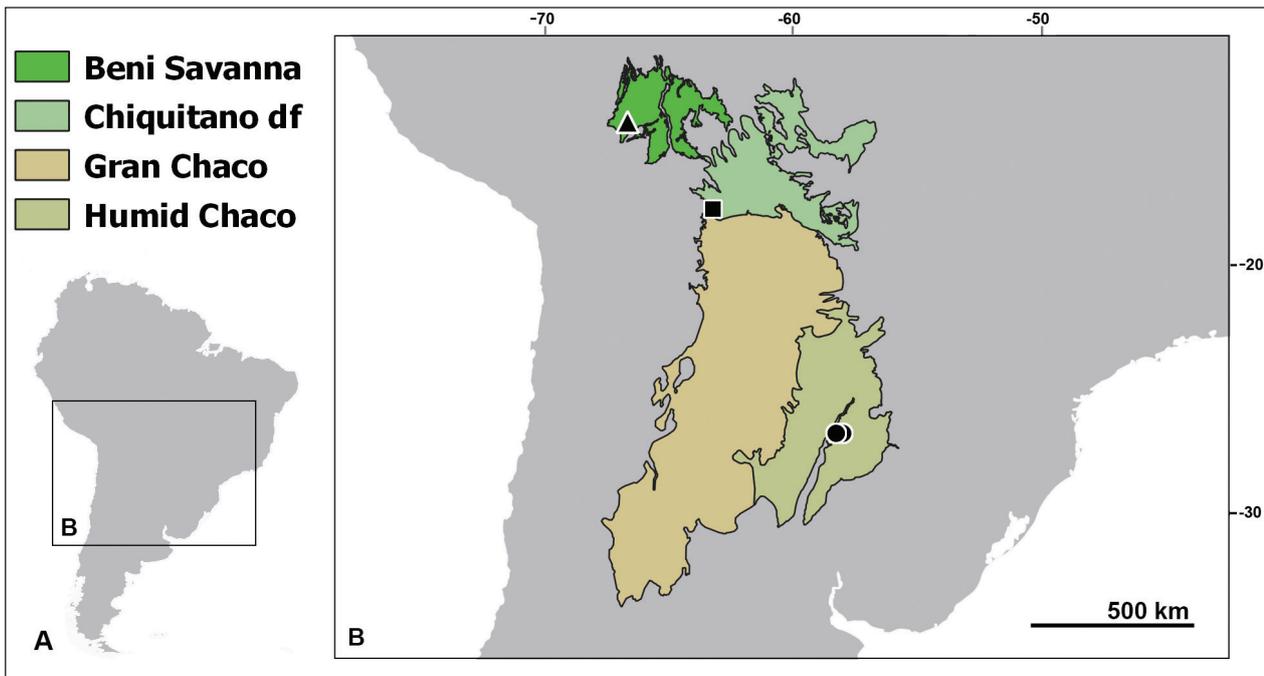


Figure 1. Ecoregion affinities of *Grismadox* gen. nov. spp. Circle = *G. karugua* sp. nov., *G. mboitui* (Pett, 2021); triangle = *G. baueri* sp. nov.; square = *G. mazaxoides* (Perger & Dupérré, 2021); df = dry forest; type locality of *G. mazaxoides* is considered to be situated in Gran Chaco by Olson et al. (2001) and Chiquitano dry forest by Navarro and Ferreira (2007).

Ciencias Naturales “Bernardino Rivadavia”. The letter ‘x’ is taken from the suffix of the genus *Mazax* with which *Grismadox* gen. nov. shares some characteristics. Gender is masculine.

Diagnosis. *Grismadox* gen. nov. can be separated from all other castianeirine genera by the combination of: (i) COs anterior to ST, (ii) male palp with two distinct RTAs, (iii) an embolus with several coils, (iv) a relatively continuous (not constricted) carapace, and (v) an elongated abdomen.

Comparisons. Considering carapace and abdomen shape, arrangement and number of tibia I spines, and the petioled pedicel, *Grismadox* gen. nov. is morphologically intermediate between *Myrmecotypus* and *Mazax* (see Perger and Dupérré 2021 for comments on *G. mazaxoides* comb. nov.). Particularly the carapace shape, elongated and constricted abdomen, and the sclerotized second pair of pedicellate setae indicate species of *Grismadox* gen. nov. are closely related to those of *Mazax*. However, species of *Mazax* have AME smaller than ALE, a distinctly elongated and rugose petiole and COs posterior to ST I.

Grismadox gen. nov. can be reliably differentiated from *Myrmecotypus* and *Mazax*, and other Neotropical castianeirines, by markedly different copulatory structures: embolus with an irregularly multi-coiled screw shape at its apex, coupled with two conspicuous retrolateral apophyses on the palpal tibia, and copulatory openings anterior to the secondary spermathecae. *Myrmecotypus jasmineae* Leister & Miller, 2014a is the only species of the genus with an embolus with several coils (female not known) (cf. Reiskind 1969; Leister and Miller 2014a;

Pett 2021), but it lacks distinct RTAs and an elongate abdomen. *Grismadox* gen. nov. have elongate abdomens and are ground-dwelling, in contrast with the subglobose abdomens and arboreal habits as in *Myrmecotypus*. Additionally, *Myrmecotypus* does not have the second pair of pedicellate setae sclerotized into spines.

Grismadox gen. nov. and some species of *Castianeira* share the multi-coiled embolus tip and elongated abdomens. However, *Castianeira* have straight to procurved AER, COs posterior to ST II, and are without dRTA (although some do have a vRTA or a ventral retrolateral protrusion).

Grismadox gen. nov. shares the general habitus, the arrangement of the eyes with the wide and recurved posterior row, strong dorsal scutum on a constricted abdomen with *Apochinomma*, but can be easily distinguished from this genus by not having a pronounced abdominal petiole a lower number of anterior tibial spines (in *Grismadox* 2–2, 3–2 or 3–3, in *Apochinomma* 4–4 or 5–5).

Some species of *Myrmecium* and *Sphecotypus niger* (Perty, 1833) have male palps with an embolus with several coils and distinct RTAs. Additionally, a few species of *Myrmecium* have anterior positioned COs. However, these genera have a very distinctly constricted carapace (Leister and Miller 2014b; Candiani and Bonaldo 2017).

Other genera with a recurved PER and elongate abdomens include the misplaced Afrotropical species of *Corinnomma* Karsch, 1880 (Haddad 2006; Raven 2015), but *Grismadox* gen. nov. has a strongly recurved PER vs. slight, and dRTA and vRTA on the male palpal tibia (vs. absent in those *Corinnomma* spp.). The monotypic Solomon Islands endemic *Melanesotypus* Raven, 2015 shares with

Grismadox gen. nov. a recurved PER and anterior lateral extremities of carapace subtruncate in females, but has a subglobose abdomen as in *Myrmecotypus*. Additionally *Melanesotypus* has a paracymbial spine on the palp of males, no RTAs, in addition to a wide embolic ridge that arises prolaterally leading into a broad sweeping embolus (Raven 2015), all absent in *Grismadox* gen. nov.

Description. Small, slender spiders with adults between 3.17 mm and 6.20 mm total length. Carapace generally ellipsoid, truncated at posterior margin, with distinct sub-rectangular cephalic region (more rectangular in females). Carapace sloping very gently towards highest point, at posterior half of the fovea. PER wider than AER, recurved; eyes subequal, with AMEs only slightly larger than ALEs, maximally about 1.3× diameter of ALEs; AER recurved. Sternum shield-shaped, between 1.5× to 2× longer than wide, widest between coxa I and II, anterior ridge of sternum truncated to weakly recurved. Abdomen ovoid, longer than wide, drop-shaped in males, pear-shaped (broader posteriorly) in females. Second pair of pedicellate setae thickened into spines. Abdomen constricted at around 1/3 its length. Ventral sclerite present in males, rectangular and covering around ½ total length of venter, just posterior to epigastric sclerite, absent in females. Palpal tibia wider than long, with two RTAs, vRTA disto- laterally oriented at between 10'30 to 11'o clock position and longer, dRTA more distally oriented and shorter. Cymbium with basal retrolateral groove that mirrors angle of vRTA. Sperm duct with convoluted median basal loop and more distal wider retrolateral loop. Embolus screw-like, with between two-and-a-half and five clear irregularly to tightly spaced coils before tapering to embolic apex, apex varies from translucent and relatively blunter (as in *G. karugua* sp. nov.) to well-sclerotized and sharp (all other species). Epigynal plate well-sclerotized. Epigyne relatively simple, ST conspicuous and dark, CO situated anterior to ST, ranging from far anterior (*G. karugua* sp. nov. and *G. mazaxoides*) to anterolateral (*G. mboitui*). Both ST spherical. ST II larger than ST I.

Composition. *Grismadox baueri* sp. nov., *Grismadox karugua* sp. nov. (type species), *Grismadox mazaxoides* (Perger & Duperré, 2021) comb. nov. and *Grismadox mboitui* (Pett, 2021) comb. nov.

Geographical and ecological distribution. The species of this genus are currently known from the Humid Chaco in southwestern Paraguay and two savanna locations in the area of Chiquitano dry forest and Moxos Plains Flooded Savannas in Bolivia. Judging from observations

of live individuals (Perger and Duperré 2021) and the occurrence in pitfall samples of *G. mboitui* comb. nov. (Pett 2021a), and *G. karugua* comb. nov. (this paper), species of this genus appear to be epigeal.

Type material examined for comparison. *Mazax ramirezi* Rubio & Danişman, 2014: 1185, figs 1A–F, 2A–D, 3A–F, 4A–G, 5A–F (Paratypes ♂ and ♀ from ARGENTINA: Buenos Aires, Campana, January 1998, Fuentes & Di Iorio leg (MACN-Ar 30733/30734).

Myrmecotypus haddadi Perger & Rubio, 2021. Holotype ♂ from BOLIVIA: Santa Cruz department, Santa Cruz de la Colina, Urubo (-17.760833, -63.24), 432 m a.s.l., 21 Dec 2019, R. Perger leg. (ZMH-A0015356). Paratypes: 1 ♂, same data as holotype (ZMH-A0015357). 1 ♂, 1 ♀, Santa Maria la Antigua (-17.3719, -63.6563), 13 Apr 2018, R. Perger leg. (IBSI-Ara 1463).

Myrmecotypus rubrofemoratus Perger & Rubio, 2021. Holotype ♂ (IBSI-Ara 1507) and ♀ allotype (IBSI-Ara 1467): BOLIVIA: Santa Cruz department, (-17.469167, -63.6925), 20–22 Jan 2016, R. Perger leg. Paratypes: same data as holotype, 1 ♀ (IBSI-Ara), 3 ♀ (CBF).

Myrmecotypus tahyinandu Perger & Rubio, 2020. Holotype ♂ from BOLIVIA: Santa Cruz department: Andrés Ibáñez province, La Guardia (-17.8830, -63.3177), 9 Sep 2015, R. Perger leg. (IBSI-Ara1469). Paratypes: Santa Cruz department: Andrés Ibáñez province: 1 ♂, 3 ♀, same location as holotype, between Sep 2015 and Jan 2017 (IBSI-Ara1465). 5 ♂, 6 ♀, same location as holotype, between Sep 2015 and Jan 2017 (CBF). 4 ♂, 5 ♀, Cotoca (-17.7736, -63.065), 11 Jul 2018, R. Perger leg. (CBF). 1 ♂, 1 ♀, Santa Cruz de la Colina (-17.758889, -63.241667), 3 Mar 2019, R. Perger leg. (CBF); 1 ♂, 2 ♀, Arroyo Urubo (-17.7575, -63.251667), 15 Feb 2020, R. Perger leg. (CBF); Lomas de Arena (-17.925, -63.160833), 14 Feb 2020, R. Perger leg. (CBF). Santa Cruz department: Ichilo province: 1 ♂, Cafetal (-17.468333, -63.700278), 20–22 Jan 2016, R. Perger leg. (CBF).

Myrmecotypus iguazu Rubio & Arbino, 2009. Holotype ♂ (MACN-Ar 19708) and allotype ♀ (MACN-Ar 19709) from ARGENTINA: Misiones Province, Iguazú National Park (25°41'S, 54°26'W), 8 January 2009, G. Rubio and M. Arbino leg. Paratypes: same locality, 15 January 2005, G. Rubio leg., 1 ♂ (CARTROUNNE 7818); same locality, 8 January 2009, G. Rubio and M. Arbino leg. 1 ♂ (CDA 000.806), 3 ♀ (CDA 000.807, CDA 000.808, CDA 000.810), 1 ♀ (MLP 17926); same locality, 20 January 2005, G. Rubio leg. 1 ♂ (CDA 000.811); Misiones Province, Urugua-í Wildlife Reserve (25°59'S, 54°05'W), 7 March 2009, G. Rubio leg., 1 ♀ (CDA 000.809).

Key to the species of *Grismadox* gen. nov.

- | | | |
|---|--|---------------------------|
| 1 | Males..... | 2 |
| – | Females (that of <i>G. baueri</i> sp. nov. unknown)..... | 5 |
| 2 | Coxae II–III pale or light yellow, others dark | 3 |
| – | Coxae II–IV pale or light yellow, other dark | <i>G. baueri</i> sp. nov. |

3	Anterior tibia spination 3-2.....	4
–	Anterior tibia spination 3-3.....	<i>G. karugua</i> sp. nov.
4	Embolus with three coils.....	<i>G. mazaxoides</i> (Perger & Dupérré, 2021) comb. nov.
–	Embolus with four and a half coils.....	<i>G. mboitui</i> (Pett, 2021) comb. nov.
5	Anterior tibia spination 3-2.....	6
–	Anterior tibia spination 3-3.....	<i>G. karugua</i> sp. nov.
6	COs anterolateral of ST.....	<i>G. mboitui</i> (Pett, 2021) comb. nov.
–	COs far anterior of ST.....	<i>G. mazaxoides</i> (Perger & Dupérré, 2021) comb. nov.

Grismadox karugua sp. nov.

<http://zoobank.org/9EE84FF4-0803-487E-8997-97C7097007E2>

Figs 2–11

Type material. Holotype: ♀ • PARAGUAY: Ñeembucú, Estancia Santa Ana, 26°50'16.9"S, 58°01'42.7"W, 07.ii.2020–13.ii.2020, Pitfall traps “Grassland”, Brogan L. Pett & Rufus Wyer leg. (CIPLT–Ar 302). **Paratypes:** PARAGUAY • 1♀; Ñeembucú, Estancia Santa Ana, 30.i.2020–06.ii.2020, co-ordinates same as HT, Pitfall traps “Grassland”, Brogan L. Pett & Rufus Wyer leg. (CIPLT–Ar 300_A). • 1♂ Ñeembucú, Pilar Military Base, 26°50'28.3"S, 58°18'43.6"W, 28.i.2020–16.ii.220, Pitfall traps “Grassland”, Brogan L. Pett & Rufus Wyer leg. (CIPLT–Ar 305).

Diagnosis. Separated from congeners by: embolus that coils four times (vs. three, four-and-a-half, or five) (Figs 10, 11); embolic apex translucent and relatively blunt (vs. sharp and sclerotized); obvious constriction between ST I and ST II (vs. absent or moderate) (Figs 6, 7); carapace color dark brown to black (with greyish tinge, orangish or yellowish in congeners) (Figs 2, 3); coxae II and III light in both sexes (II–IV light in females of *G. mazaxoides* comb. nov. and *G. mboitui* comb. nov., female of *G. baueri* sp. nov. not known) (Figs 2–5, 8, 9); tibia I spines 3–3 (2–2 or 3–2 in congeners).

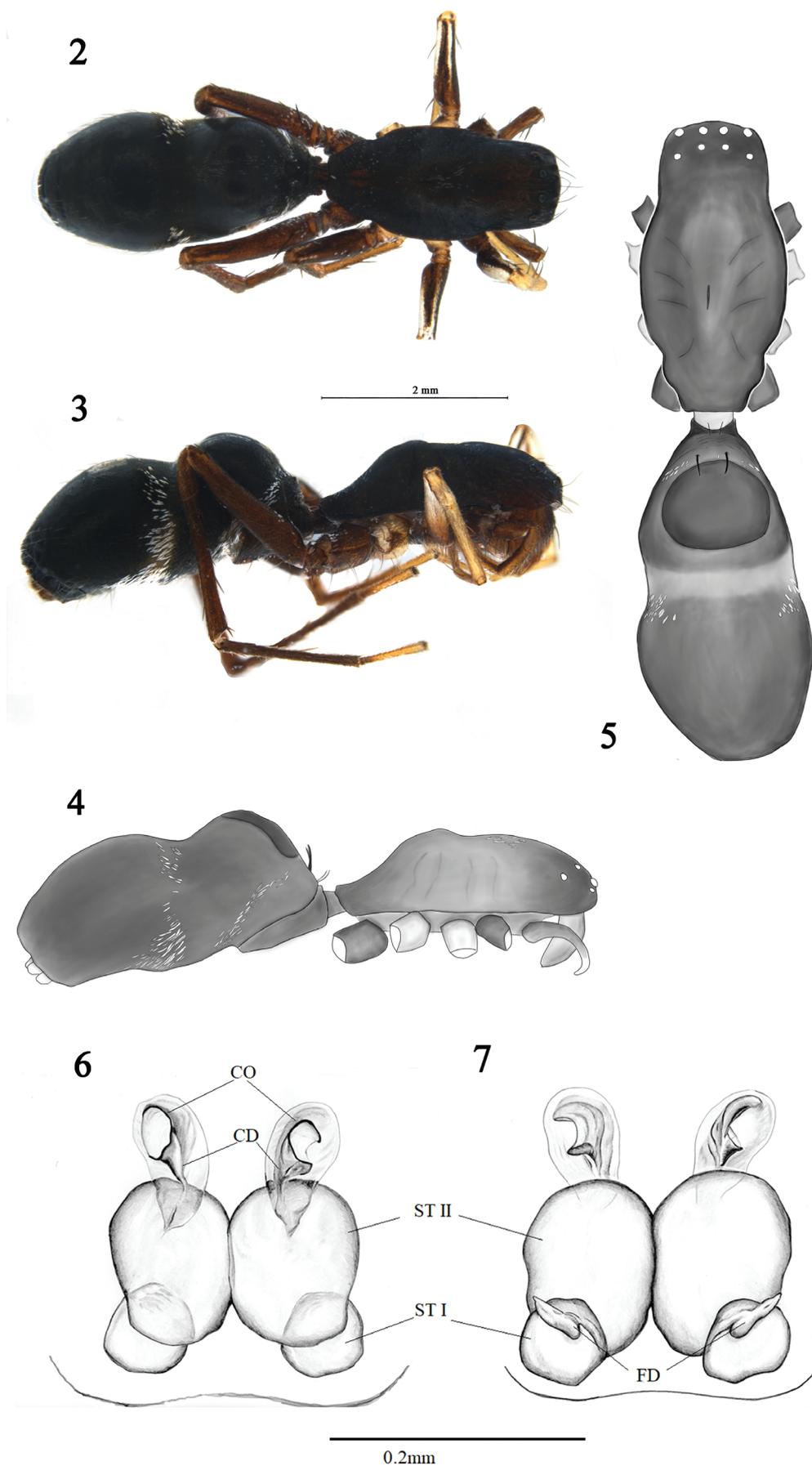
Etymology. The species epithet is a noun in apposition, and refers to the Guarani word for Wetland “karugua”. This refers to the Ñeembucú wetland complex, where the species was discovered.

Description. Female holotype. Figs 2–7.

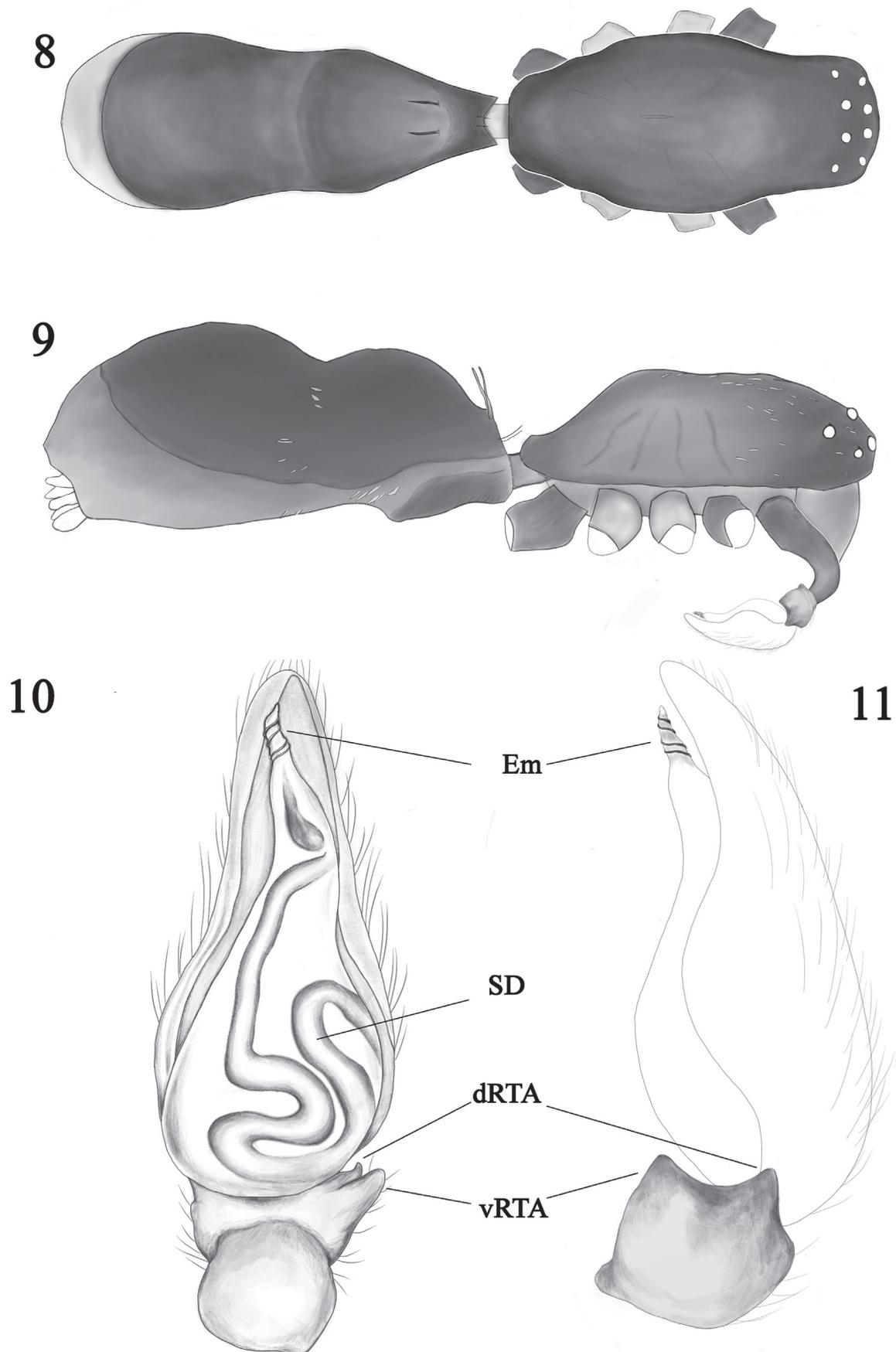
Measurements: TL 5.83, CL 2.64, CW 1.30, CH 0.92, CI 49, AL 3.19 (incl. pedicel), AW 1.58, AI 50, SL 1.04, SW 0.59, SI 57. Legs. I: 1.18, 0.43, 1.04, 0.86, 0.65. II: 0.97, 0.32, 0.73, 0.76, 0.59. III: 1.05, 0.38, 0.89, 0.97, 0.57. IV: 1.50, 0.54, 1.43, 1.38, 0.76. Eyes. AME 0.10, ALE 0.09, PME 0.07, PLE 0.08.

Colouration: Carapace dark brown with heavy black mottling, light orange mottling posteriorly and anteriorly but not medially. Sparse short white setae present in a chevron converging and reaching highest concentration just anterior to the fovea. Base of chelicerae darkest part of cephalothorax. Legs dark orange to brown, granulate. Coxa I and IV dark brown, II and III pale. Sternum orangish-brown with mottled black patches. Abdomen

uniform black, with deep red- brown on epigastric sclerite. Rings of white feathery setae in constriction groove and just posterior to epigastric sclerite. **Carapace:** Generally ovoid, longer than wide by about 2×. Highest point at posterior foveal bump, sloping proximally toward base in lateral view. In dorsal view, carapace moderately truncated anteriorly, with weak curve around cephalic region. **Sternum:** Distinctly shield-shaped, granulated, widest between coxa I and II. Anterior ridge truncated. **Eyes:** Anterior eye row moderately recurved, with ALEs and AMEs less than half an AME diameter apart. Posterior eye row nearly straight in frontal view, clearly recurved in dorsal view, PMEs smallest. **Legs:** Femora with strong, long dorsal spine. Femoral spine IV longer than femoral spine III, III longer than II, and femoral spine on leg I with damaged spines on both legs. However, in paratype female spine of F II larger than spine F I. **Chelicerae:** Small lateral condyle. Two teeth on retromargin, distal tooth larger. Promargin with two teeth, distal tooth much larger than teeth on retromargin and other promarginal tooth, basal tooth about 3× distal tooth width away from distal tooth and much smaller. Strong promarginal rake setae obscure fang and cheliceral furrow teeth. **Abdomen:** Twice as long as it is wide, dorsal scutum convex and covering between 1/4 and 2/3 of abdomen, shiny and granulated. Second pair of pedicellate setae sclerotized into pair of straight spines at anterior part of scutum, running subparallel to bulbous part of scutum. Clear constriction of abdomen around 1/3 of length, distinct when viewed laterally, ring of white longitudinally flattened short feathery setae in region of constriction, almost absent dorsomedially, densest ventrolaterally. Another ring of short feathery white feathery setae present just posterior to spines at the dorsal scutum. Ventral sclerite absent, pale outline of venter clearly not concolorous with rest of abdomen. Concentrated patches of short, fluffy white setae in anterolateral corners of epigastric furrow. Inframamillary sclerite very small, dark brown, epigastric sclerite anteriorly forming petiole, dark brown to black, with moderately lighter orange and red mottling. **Epigyne** (Figs 6, 7). Epigynal plate relatively small. Epigyne with widely spaced copulatory openings anterior to ST. CD horn-like, travelling medially first before reaching spermathecae, CD then tapering laterally from midpoint. ST II touching medially, spherical and diverging posterolaterally into ST I. ST I much smaller and oval, projected laterally, FD arising at ST I anteromedially.



Figures 2–7. *Grismadox karugua* sp. nov. female holotype (CIPLT-Ar 302) habitus. **2.** Micrograph dorsal; **3.** Micrograph lateral; **4.** Drawing lateral; **5.** Drawing dorsal. Figs **6, 7.** cleared female epigyne. **6.** Ventral; **7.** Dorsal.



Figures 8–11. *G. karugua* sp. nov. male paratype habitus and pedipalp (CIPLT-Ar 305). **8.** Dorsal; **9.** Lateral; **10.** Pedipalp ventral; **11.** Pedipalp retrolateral. dRTA = dorsal retrolateral tibial apophysis, Em = embolus, SD = sperm duct, vRTA = ventral retrolateral tibial apophysis.

Leg spination. I: F = pl1 d1, Ti = v = 3–3 (plv3 rlv3), mt = 2–2 (plv 2 rlv 2). II: F = d2, Ti = 3–3 (plv3 rlv3), mt = 2–2 (plv2 rlv2). III: F = d2, pl1, P = d1, Ti = d1 pl2 rl1, mt = 2–2–2–2 (pl2 rl2 plv2 rlv2) 1 distal whorl, IV: F = d2 pl1, P = d1, Ti = d2 pl1 3–3 (plv3 rlv3), mt = d2 2–2 (plv 2 rlv2) 1 distal whorl.

Male paratype. Figs 8, 9. **Measurements.** TL 4.54, CL 2.11, CW 1.08, CH 1.09, CI 51, AL 2.43 (incl. pedicel), AW 1.00, AI 41, SL 1.62, SW 0.97, SI 60. Chelicerae length 0.51, chelicerae width 0.30. Legs. I: 0.97, 0.28, 0.84, 0.80, 0.65. II: Missing. III: 0.95, 0.30, 0.76, 0.86, 0.51. IV: 1.27, 0.31, 1.27, 1.35, 0.68.

Habitus shape, leg formula, spination and general color pattern as in female, male carapace brighter, primarily orangish-brown. Dorsal scutum large, around 3/4 of AL, strong spines on anterior part of dorsal scutum projected posteriorly at 30–45 degree angle, abdominal constriction slight but conspicuous in lateral view, posterior to dorsal scutum. Few short white feathery setae dorsally and laterally at site of constriction and just posterior to spines of dorsal scutum. Ventral sclerite present, clearly defined, covering around 1/2 of venter just posterior to epigastric sclerite, inframamillary sclerite very small, dark brown.

Palp: Spination: femur = v 2, patella = v 2 pl 1, tibia = v 1 pl 1. Tibial spines substantially larger than others, with pl spine about 1.5 times the length of pl ventral spine. Palpal bulb with basally convoluted median loop in sperm duct, second, wider, more distal retrolateral loop. Palpal tibia with two short pointed retrolateral apophyses, ventral one projected disto-laterally at 11'00 position, dRTA shorter and more distally oriented, with tip recurving weakly back towards palp. Embolus screw-like, with three irregularly spaced coils, apical two crossing over, before tapering to pale apex.

Geographical and ecological distribution. This species is only known from the type locality in Estancia Santa Ana, Ñeembucú wetland complex, Paraguay. According to the ecoregion delineation by Olson et al. (2001), the locality is situated in Humid Chaco. The habitat in which *G. karugua* sp. nov. was collected was savanna-like grassland. Judging from the occurrence in pitfall samples, the species is epigeal.

Grismadox baueri sp. nov.

<http://zoobank.org/1EB5BA81-86AF-48C3-B0FF-040C722F4D6F>

Figs 12–15

Type material. *Holotype* ♂; • BOLIVIA: Beni department, Ballivian, Espiritu, 14°12'57.6"S, 66°39'57.6"W), vegetation, 22.5.1986, W. Hanagarth, J. Sarmiento leg. (SMNK-ARA 13505).

Diagnosis. Separated from congeners by: coxae IV in male light (vs. dark) (Figs 12, 13); tibia I spines 2–2 (vs. 3–2 or 3–3); lateral anterior extremities of carapace smooth (vs. squared); embolus with five coils (vs. three – four and a half), apex sharp (vs. pointed or blunt); dRTA that arises at wider position in ventral view (vs. closer to the palp in others) (Figs 14, 15); much smaller size than adult male of

other species (3.17 mm vs. 4.50–5.80); weak constriction of abdomen (vs. clear constriction); sternum index 78, i.e. a broader sternum (much higher than other species).

Etymology. The species epithet is a genitive patronym in honor of German arachnologist Tobias Bauer, of SMNK, for initially facilitating the loan of the holotype of this species and for many instances of arachnological assistance to the first author.

Description. Male holotype. Measurements: TL 3.17, CL 1.52, CW 0.84, CI 55, CH 0.94, AL 1.65 (incl. pedicel), AW 0.72, AI 47, SL 0.64, SW 0.50. SI 78, Chelicera length 0.38, width 0.22. Legs. I: 0.94, 0.20, 0.80, 0.78, 0.70. II: 0.90, 0.16, 0.78, 0.74, 0.58. III: 0.80, 0.24, 0.66, 0.76, 0.54. IV: 1.12, 0.30, 1.10, 1.18, 0.68. Eyes. AME – 0.07, ALE – 0.05, PME – 0.05, PLE – 0.06.

Colouration: In ethanol (c. 1986). Carapace orange with black mottling in lines leading from fovea to carapace margins. Small patch anterior to fovea discolored, potentially harboring setae prior to preservation. Black eye rings, darkest around AMEs. Chelicerae concolorous with carapace. Legs light brown to pale from patella I and II, legs III and IV consistently pale medially with brown margins. Coxae I darker, other three white. Sternum orange with distinct darker stripes converging medially. Abdomen darker orange than carapace, darker at anterior 1/3rd due to moderate sclerotization.

Carapace: Oval, longer than wide by about two times. Very weak depression between fovea and cephalic region, otherwise of uniform height, before sloping proximally towards base in lateral view.

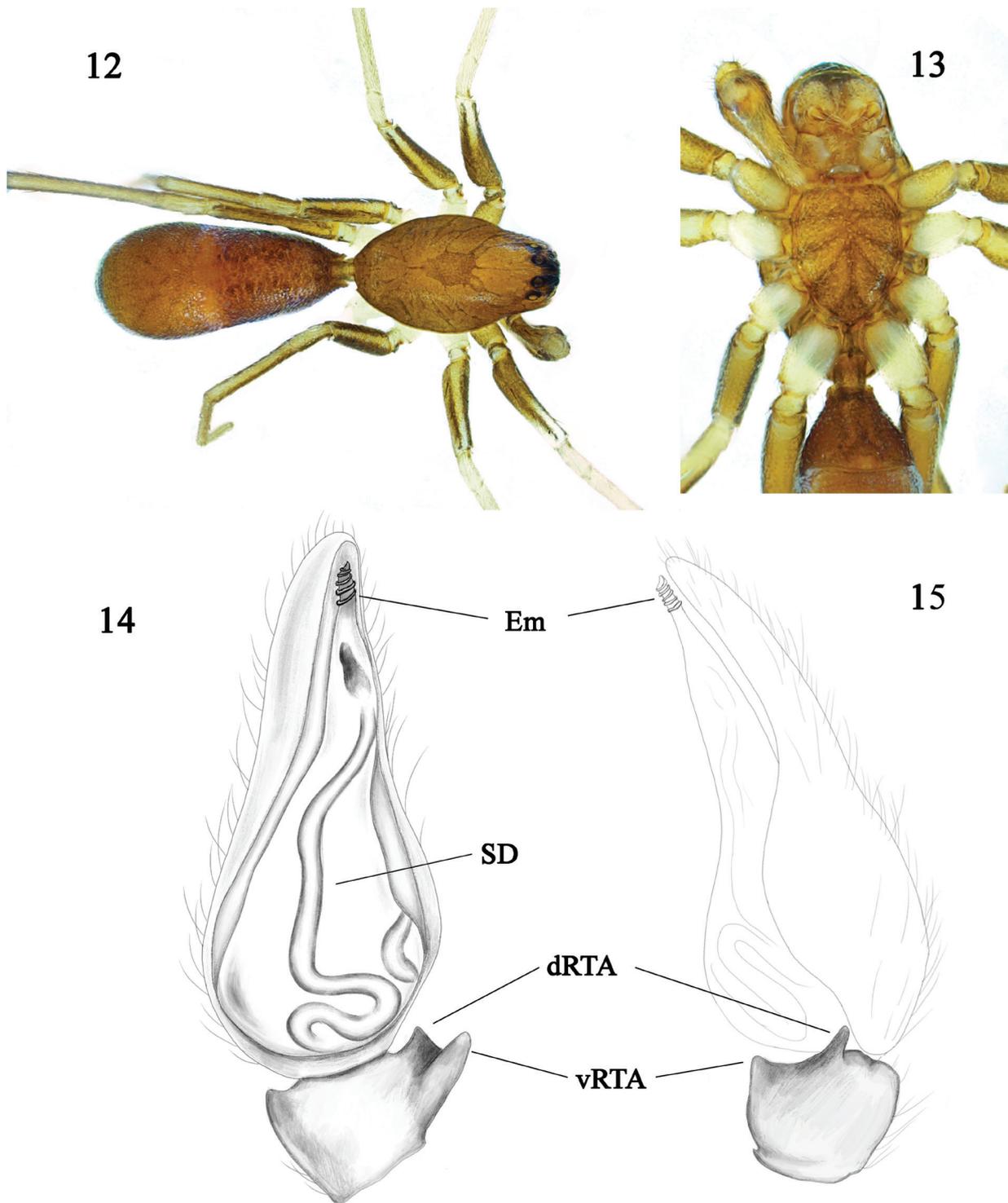
Sternum: Distinctly shield-shaped, relatively broad, 0.8× the length, anterior ridge truncated, widest between coxa I and II. Distinct dark stripe markings beginning adjacent to coxae, converging medially.

Eyes: AER slightly recurved, AMEs largest and black, nearly touching ALEs. PER wider than AER by about 1.4×, PER moderately recurved.

Legs: Femora with strong dorsal spine, all equal length. Femora I and II streaked brown and pale, rest of segments pale, legs III and IV streaked throughout. Short, fine setae throughout.

Chelicerae: Promargin with two teeth, distal almost twice as large. Retromargin with two small teeth, both smaller than smaller promargin tooth. No chilum, lateral condyle not visible.

Abdomen: Drop-shaped, much longer than wide, 2.5×. Dorsal scutum almost entire. Anterior 1/3rd moderately sclerotized, more than scarcely sclerotized posterior 2/3rd's. Weak constriction at 1/3rd, constriction paler, constriction clear when viewed in lateral but is almost indistinct in dorsal view. Second pair of pedicellate setae sclerotized into moderate spines at anterior margin projected at 2' o'clock position when viewed laterally. Two strong setae (much weaker than spines) present, just anterior to spines and projected at 2'30 position when viewed laterally. Large ventral sclerite occupying 2/3 length of venter, barrel-shaped and with recurved posterior margin. Small



Figures 12–15. *Grismadox baueri* sp. nov. male holotype (SMNK-ARA 13505). **12.** Habitus dorsal; **13.** Ventral aspect of carapace; **14.** Pedipalp in ventral; **15.** Pedipalp in retrolateral. dRTA = dorsal retrolateral tibial apophysis, Em = embolus, SD = sperm duct, vRTA = ventral retrolateral tibial apophysis.

patches of white setae at anterior lateral margin of ventral sclerite, bordering posterior lateral edge of epigastric sclerite. Ventral and epigastric sclerites contiguous.

Palp: Tibia with two RTAs, dRTA more pointed than blunter vRTA. In retrolateral view, dRTA apex points ventrally. Embolus relatively thick and elongate, with five coils; three coils from apical third, basal two coils are much wider. Final coil slimmer.

Leg spination: I: F = pl1 d1, T = (2–2) plv2 rlv2, Mt = (2–2) plv2 rlv2. II: F = d1, T = (1–1) plv1 rlv1, Mt = (2–2) plv2 rlv2. III: F = d2, T = pl1 r11 plv1, Mt = (2–2) plv2 rlv2. IV: F = d2, T = (2–2) plv2 rlv2, Mt = pl2 r11 plv2 rlv1.

Geographical and ecological distribution. This species is only known from the type locality in Espiritu, José Ballivián province, Beni Department, Bolivia. According to

the ecoregion delineation by Olson et al. (2001), the locality is situated in Beni savanna (widely recognized as Moxos Plains Flooded Savannas, see Ibisch and Merida 2003). This savanna is comprised of a mosaic of grasslands, swamplands and forest islands (Navarro and Ferreira 2007). Based on the approximated GPS data of the collection locality (according to the information by the owner of Espiritu ranch), it was not possible to determine the accurate ecosystem or habitat associations of *G. baueri* sp. nov. Further studies are needed to determine the latter.

***Grismadox mazaxoides* (Perger & Dupérré, 2021)
comb. nov.**

Myrmecotypus mazaxoides (Perger & Dupérré, 2021). 275, figs. 2A, B, 3A, B, 4A–E, 5A, B, 6A, B, 7A, B. Male holotype (ZMH), male and female paratypes (ZMH and CBF) from Santa Cruz department, Bolivia, examined.

Type deposit. *Holotype* ♂ and ♀ allotype: ZMH-A0014700-14701. *Paratypes*: 2 ♂, 5 ♀: ZMH-A0014707; 6 ♂, 10 ♀: CBF.

Diagnosis. Separated from congeners by: an embolus that coils three times (vs. between four and five times); dRTA that is blunter and translucent (vs. more pointed); constriction between ST I and ST II absent (vs. present, female of *G. baueri* sp. nov. unknown); carapace color with grayish tinge (dark brown to black, orangish or yellowish in congeners).

Description. See: Perger and Dupérré (2021).

Geographical and ecological distribution. Epigeal in Cerrado-like grassland or savanna habitats. According to Navarro and Ferreira (2011), the type locality of this species is situated in Chiquitano forest, while Olson et al. (2001) consider this area Gran Chaco. Judging from the observation of this species in open areas, the species is likely to occur in the Gran Chaco area.

***Grismadox mboitui* comb. nov. (Pett, 2021)**

Myrmecotypus mboitui (Pett, 2021) 79, figs 3–13. Male holotype and female paratypes (CCPLT) from Ñeembucú department, Paraguay, examined.

Type deposit. *Holotype* ♂ CIPLT-Ar 301. *Paratypes* 2♀ CIPLT-Ar 303, 1♀ CIPLT-Ar 300.

Diagnosis. Separated from congeners by: pedipalp with four and a half coils (vs. three, four or five); RTAs in ventral view much larger than that of other species; constriction between ST I and ST II moderate (vs. absent or distinct); COs slightly anterolateral to ST (vs. far anterior); carapace orangish (vs. dark brown to black, greyish or yellowish).

Description. See: Pett (2021).

Geographical and ecological distribution. Epigeal in savanna-like wetland in the Humid Chaco area.

Etymological notes. *G. mboitui* was named after the Guaraní mythological figure Mbói tu'ĩ, and all details of the specific epithet are accurate. However, it was incorrectly stated in parentheses of the etymology section that Mbói = parrot and tu'ĩ = snake (in Guaraní), when in fact this was an incorrect transcription and the opposite is true. Mbói = snake and tu'ĩ = parrot.

Discussion

All *Grismadox* species for which we have detailed records were collected in savannah-like habitat (e.g., seasonally inundated grasslands in Humid Chaco, as in Paraguayan species). The discovery of numerous new species in this habitat in Paraguay and Bolivia illustrates a strong negative sampling bias against Neotropical savannah-like habitats. Indeed, even limited sampling such as in the short pitfall trapping project in the Ñeembucú wetland complex (see: Pett 2019; Pett and Wyer 2020; Pett 2021a, b; Pett et al. 2021) or manual sampling in a Bolivian savanna mosaic (Perger and Dupérré 2021; Perger and Rubio 2021), yielded numerous taxonomic novelties, including three of the species presented here, among other undescribed taxa. It is also of note that two sympatric *Grismadox* exist in the same site in the NWC and at the present time their ecological separation is based on relatively little data but appears to correspond to a forest/grassland edge specialist (*G. mboitui* comb. nov.) and a grassland specialist species (*G. karugua* sp. nov.).

The conservation value of such regions is barely understood in terms of their invertebrate taxa (Rubio et al. 2018; Dickens et al. 2020). We therefore stress the urgent need for further arthropod collections in Neotropical savannahs and wetland regions.

Acknowledgements

Extensive thanks are due to Rufus Wyer, co-collector of the type specimens of the type species. Additional thanks are due to Varvara Vladimirova (Université Laval) and Jack McBride for assistance in the field during the pitfall trapping project in Paraguay. Thanks also to Fundación Para La Tierra for supporting the project through provision of resources during the pitfall trapping project in Paraguay, to Don Odilon Barrios for allowing the pitfall trap project at Estancia Santa Ana, and to the staff at the Coronel Alberto Torres Nuñez for access to the Regimiento de Caballería No 2 “Colonel Felipe Toledo”. The Ministerio del Ambiente y Desarrollo Sostenible granted research permits to the CCPLT for Paraguayan specimens. Thanks to Jorge Ayala Damian Santa Cruz for assisting in Guaraní translation. Thanks to Tobias Bauer and Hubert Höfer (both SMNK) for facilitating loans of Neotropical castianeirine material to the first author, including the holotype of one of the new species. Additionally,

Zoë Simmons is thanked for allowing BLP to use the auto-montage system at Oxford University Museum of Natural History (OUMNH) during a research visit. Charlotte Lawlor (Falmouth University) made the fantastic pedipalp drawings of both new *Grismadox* species for which we are extremely grateful. Amanda Morgan Riley (www.amandarileyart.com) is thanked for epigyne illustration of *G. karugua*. We extend sincere thanks to reviewers Dr. Charles R. Haddad (University of the Free State) and Dr. Martín Ramírez (Museo Argentino Ciencias de Naturales) for substantially improving the manuscript. Subject editor Dr. Danilo Harms (Universität Hamburg) is thanked for handling the manuscript.

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A new catfish of the genus *Trichomycterus* from the Rio Paraíba do Sul Basin, south-eastern Brazil, a supposedly migrating species (Siluriformes, Trichomycteridae)

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Academic editor: Nicolas Hubert ♦ Received 30 July 2021 ♦ Accepted 10 December 2021 ♦ Published 14 January 2022

Abstract

A new species of the catfish genus *Trichomycterus* is described from the Rio Paraíba do Sul, south-eastern Brazil. This species exhibits some morphological character states that are unique amongst congeners, including a robust opercle and a long interopercle with numerous odontodes (50–60 opercular and 90–100 interopercular), a black bar on the basal portion of the caudal fin and a dark brown flank with a well delimited dorsal yellow stripe. It also exhibits some morphological traits that are uncommon amongst congeners, such as the presence of nine pectoral-fin rays. The presence of a shallow hyomandibular outgrowth and a ventrally expanded pre-opercular ventral flap suggests that this species is closely related to *T. melanopygius*, *T. pradensis* and *T. tete*. The new species also differs from *T. melanopygius*, *T. pradensis* and *T. tete* by having an emarginate caudal fin and a single median supra-orbital pore S6. Anecdotal evidence suggests that *T. largoperculatus* and *T. pradensis* have migratory habits, a condition not previously reported for eastern South American trichomycterines.

Key Words

Biodiversity, Brazilian Atlantic Forest, comparative osteology, fish migration, Neotropical Region, systematics

Introduction

The Trichomycterinae, one of the eight subfamilies of the Neotropical catfish family Trichomycteridae, comprises a diversified group with most species inhabiting swift freshwater environments between southern Central America and southern South America (Katz et al. 2018). Taxonomy of the Trichomycterinae has, for long time, been considered particularly problematic due to the traditional non-monophyletic delimitation of the most diverse trichomycterine genus *Trichomycterus* Valenciennes, 1832 (Costa 1992; Costa and Bockmann 1993; de Pinna 1998). Recent studies, using molecular evidence, have consistently indicated that different lineages involving over 150 species, formally placed in *Trichomycterus*, are closer to distinct trichomycterine genera, corroborating the former

view about the paraphyletic nature of *Trichomycterus* (Ochoa et al. 2017; Katz et al. 2018; Costa et al. 2021a, 2021b). This problem was tentatively solved, by restricting *Trichomycterus* (hereafter *Trichomycterus s.s.* [sensu stricto]), to a clade including the type species of the genus, *T. nigricans* Valenciennes, 1832, sister to a clade containing *Cambeva* Katz, Barbosa, Mattos & Costa, 2018 and *Scleronema* Eigenmann, 1917 (Katz et al. 2018). *Trichomycterus s.s.* comprises 60 valid species distributed between the Rio de Contas, in north-eastern Brazil and rivers draining the Baía de Paranaguá in southern Brazil (Costa 2021). However, the greatest species diversity is concentrated in the area of the Atlantic Forest of south-eastern Brazil comprising the Rio Paraíba do Sul Basin and adjacent smaller coastal river basins, with a total of 25 valid species (e.g. Costa et al. 2020a, b; Vilardo et al. 2020).

Species of *Trichomycterus* from the Rio Paraíba do Sul and adjacent coastal basins have been studied and described since the nineteenth century (Valenciennes 1832; Boulenger 1896; Eigenmann and Eigenmann 1889) and sporadic studies in the first half of the twentieth century recorded some new species (Miranda-Ribeiro 1906; Eigenmann 1917, 1918; Miranda-Ribeiro 1943, 1949). However, the great species diversity of this region was revealed only after 1992, following intensive efforts to sample small, swift riverine habitats (Costa 1992; Barbosa and Costa 2003, 2008, 2010a, b, 2012a, b; Lima and Costa 2004; Lima et al. 2008; Costa et al. 2020b; Vilardo et al. 2020).

This study focuses on a new species collected over 10 years ago in the main channel of the Rio Paraíba do Sul, noteworthy by exhibiting a distinctive colour pattern and some unique osteological features amongst eastern South American trichomycterines. Equally remarkable is the report of upstream migration during the collection, which is new for eastern South American trichomycterines. Due to the peculiar combination of morphological character states exhibited by the new species, making its phylogenetic positioning uncertain amongst trichomycterines, a formal description was not made before the conclusion of deeper phylogenetic studies on trichomycterines from eastern South America (Katz et al. 2018; Costa 2021). Herein, we provide a formal description for the new species and discuss morphological variation considered relevant for its phylogenetic positioning, as well as migration in trichomycterines.

Material and methods

Morphometric and meristic data were taken following Costa (1992), with modifications proposed by Costa et al. (2020a); measurements are presented as percentage of standard length (SL), except for those related to head morphology, which are expressed as percentage of head length. Fin-ray counts include all elements; following Bockmann and Sazima (2004), in descriptions, lower case roman numerals indicate unsegmented unbranched rays, upper case numerals indicate segmented unbranched rays and Arabic numerals indicate segmented branched rays. Vertebra counts do not include Weberian apparatus vertebrae and the compound caudal centrum was counted as a single element. Specimens were cleared and stained for bone and cartilage (C&S in lists of specimens) following Taylor and Van Dyke (1985); osteological characters included in the description are those belonging to structures that have informative variability amongst congeners (Costa et al. 2020a, b), including the mesethmoidal region, suspensorium, opercular apparatus and branchial arches. Terminology for bones is according to Costa (2021). Osteological illustrations were made using a stereomicroscope Zeiss Stemi SV 6 with camera lucida. Cephalic latero-sensory system terminology follows Arratia and Huaquin (1995), with modifications proposed by

Bockmann et al. (2004). Specimens are deposited in the ichthyological collection of the Institute of Biology of the Federal University of Rio de Janeiro, Rio de Janeiro City and in the Centre of Agrarian and Environmental Sciences, Federal University of Maranhão, Chapadinha (CIC-CAA). Comparative material is listed in Costa (2021). Geographical names follow Portuguese terms used in the region, thus avoiding common errors or generalisations when translating them to English, besides making it easier to find them in the field.

Results

Trichomycterus largoperculatus sp. nov.

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Figs 1–4, Table 1

Holotype. UFRJ 6987, 77.8 mm SL; Brazil: Estado do Rio de Janeiro: Município de Além Paraíba: Rio Paraíba do Sul just below Ilha dos Pombos Dam, 21°50'36"S, 42°34'46"W, about 105 m a.s.l.; L. P. Bastos, 10 March 2009.

Paratypes. UFRJ 6988, 7, 35.2–91.5 mm SL; UFRJ 6989, 3 (C&S), 55.7–46.2 mm SL; CICC AA 02695, 2, 50.7–51.3 mm SL; all collected with holotype.

Diagnosis. *Trichomycterus largoperculatus* is distinguished from all species of *Trichomycterus* s.s. by having more opercular odontodes (48–62 vs. 11–31), more interopercular odontodes (92–100 vs. 23–72) and a unique colour pattern consisting of a dark brown flank with a longitudinal pale yellow stripe on its dorsal portion (vs. never a similar colour pattern) and the presence of a black bar on the basal portion of the caudal fin (vs. absence).

Description. Morphometric data are in Table 1. Body moderately slender, subcylindrical and slightly depressed anteriorly, compressed posteriorly. Greatest body depth

Table 1. Morphometric data of *Trichomycterus largoperculatus* sp. nov.

	Holotype	Paratypes (n = 8)
Standard length (mm)	77.8	46.6–91.5
Percentage of standard length		
Body depth	16.4	14.3–17.5
Caudal peduncle depth	11.2	9.6–11.4
Body width	9.2	9.6–14.7
Caudal peduncle width	3.9	3.1–4.4
Pre-dorsal length	60.4	58.0–62.9
Pre-pelvic length	53.5	53.3–58.1
Dorsal-fin base length	13.6	11.9–13.4
Anal-fin base length	8.7	8.9–10.4
Caudal-fin length	16.7	15.0–16.8
Pectoral-fin length	15.5	13.8–17.0
Pelvic-fin length	11.5	10.7–12.9
Head length	21.9	21.9–25.1
Percentage of head length		
Head depth	45.2	42.4–48.5
Head width	81.8	73.9–83.6
Snout length	48.4	42.1–45.9
Interorbital length	24.3	21.6–28.3
Pre-orbital length	15.8	13.2–16.4
Eye diameter	11.7	12.2–16.1



Figure 1. *Trichomycterus largoperculatus* sp. nov., UFRJ 6987, holotype, 77.8 mm SL: **A.** Left lateral view; **B.** Dorsal view; **C.** Ventral view.

at vertical just anterior to pelvic fin base. Dorsal profile of head and trunk slightly convex, approximately straight on caudal peduncle; ventral profile straight to slightly convex between lower jaw and end of anal-fin base, straight on caudal peduncle. Anus and urogenital papilla at vertical through middle portion of dorsal-fin base. Head trapezoidal in dorsal view. Anterior profile of snout slightly convex in dorsal view. Eye relatively large, dorsally positioned in head. Minute skin papillae on ventral surface of head. Posterior nostril located nearer anterior nostril than orbital rim. Tip of maxillary and rictal barbels reaching anterior part of interopercular patch of odontodes; tip of nasal barbel reaching posterior part of orbit. Mouth subterminal. Jaw teeth pointed; premaxillary teeth 55–58, slightly curved, arranged in 5 irregular rows; dentary teeth 53–56, slightly curved backwards, irregularly arranged, more concentrated near symphysis. Branchial membrane attached to isthmus only at its anterior point. Branchiostegal rays 7.

Dorsal and anal fins subtriangular; total dorsal-fin rays 13 (iv + II + 7), total anal-fin rays 11 (iv + II + 5); anal-

fin origin posterior to dorsal-fin base. Dorsal-fin origin at vertical through centrum of 16th or 17th vertebra; anal-fin origin at vertical between centrum of 22nd or 23rd vertebra. Pectoral fin subtriangular in dorsal view, posterior margin slightly convex, first pectoral-fin ray terminating in filament, its length about 20% of pectoral-fin length without filament; total pectoral-fin rays 9 (I + 8). Pelvic fin truncate, its posterior extremity reaching urogenital papilla; pelvic-fin bases medially separated by interspace about half-length pelvic-fin base; total pelvic-fin rays 5 (I + 4). Caudal fin emarginated, upper and lower corners rounded to slightly pointed; total principal caudal-fin rays 13 (I + 11 + I), total dorsal procurrent rays 18–22 (xvii–xxi + I), total ventral procurrent rays 14–16 (xiii–xv + I). Vertebrae 35 or 36. Ribs 11 or 12. Two dorsal hypural plates, corresponding to hypurals 4 + 5 and 3, respectively; single ventral hypural plate corresponding to hypurals 1 and 2 and parhypural.

Laterosensory system (Fig. 2). Supraorbital sensory canal continuous, posteriorly connected to posterior section of infra-orbital canal. Supra-orbital sensory canal

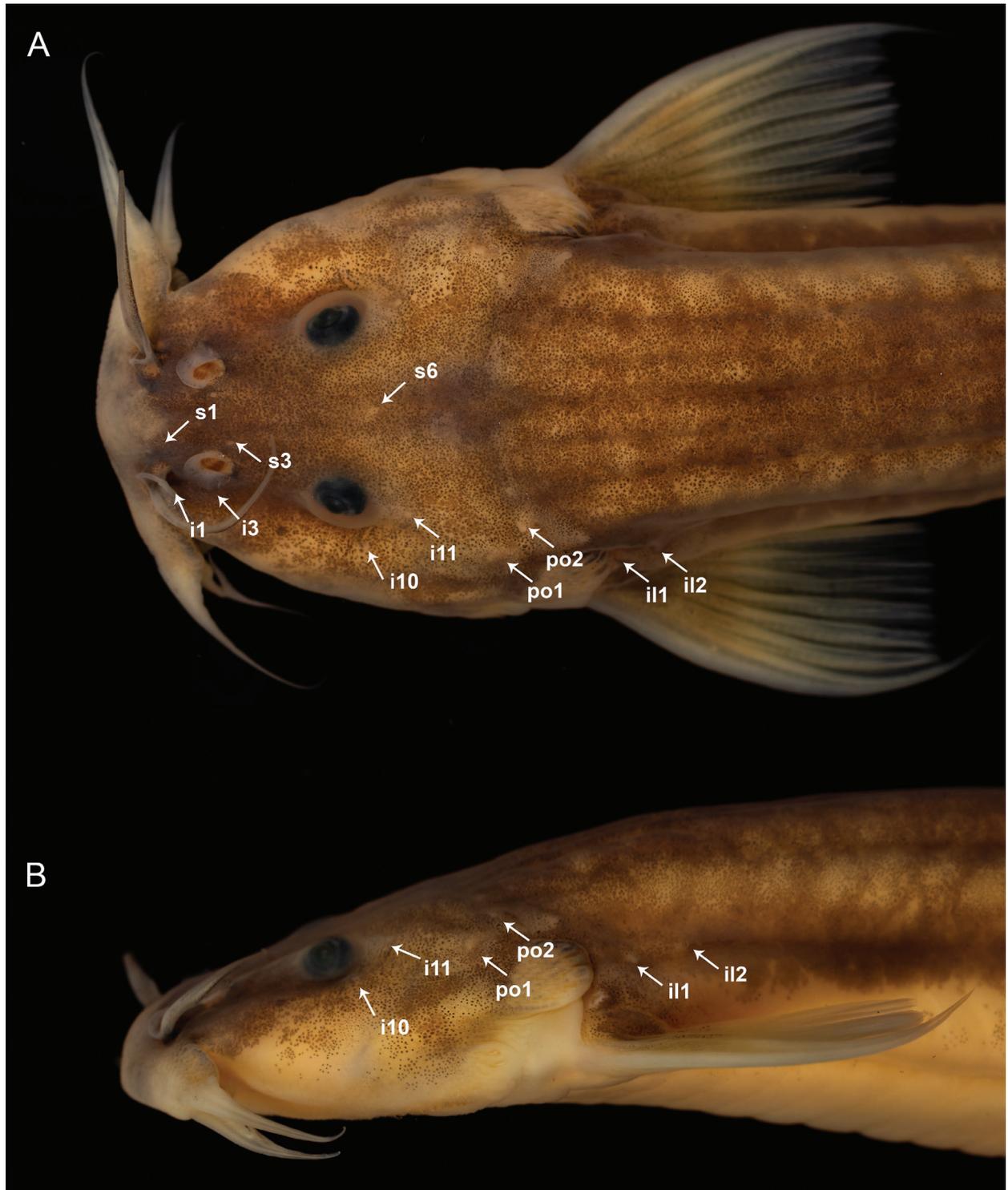


Figure 2. Head of *Trichomycterus largoperculatus* sp. nov., UFRJ 6987, holotype, 77.8 mm SL: **A.** Dorsal view; **B.** Left lateral view.

with 2 paired pores, s1, adjacent to medial margin of anterior nostril and s3, adjacent and just posterior to medial margin of posterior nostril; supra-orbital pore s6 single, on centre of head, at transverse line through posterior half of orbit. Infra-orbital sensory canal arranged in 2 segments, each with two pores; anterior segment with pore i1, at transverse line through anterior nostril and pore i3, at transverse line just anterior to posterior nostril; posterior segment with pore i10, adjacent to ventral margin

of orbit and pore i11, posterior to orbit. Postorbital canal with 2 pores: po1, at vertical line above posterior portion of interopercular patch of odontodes and po2, at vertical line above posterior portion of opercular patch of odontodes. Lateral line of body short, with 2 pores, posterior-most pore at vertical just posterior to pectoral-fin base.

Mesethmoidal region and adjacent structures (Fig. 4A). Anterior margin of mesethmoid nearly straight, mesethmoid cornu robust, subcylindrical, tip



Figure 3. *Trichomycterus largoperculatus* sp. nov., left lateral view: **A.** UFRJ 6988, paratype, 70.0 mm SL; **B.** UFRJ 6988, paratype, 49.0 mm SL.

rounded. Antorbital and sesamoid supra-orbital narrow, rod-like, sesamoid supra-orbital longer, its length about 2.5 times antorbital length. Premaxilla sub-rectangular in dorsal view. Maxilla boomerang-shaped, slender, about equal premaxilla in length, slightly curved, with minute posterior process. Autopalatine sub-rectangular in dorsal view when excluding posterolateral process, narrow, its shortest width about half autopalatine length, lateral and medial margins slightly concave; latero-posterior process of autopalatine subtriangular, long, its length about two thirds of autopalatine length.

Jaw suspensorium and opercular apparatus (Fig. 4B). Metapterygoid trapezoidal, slightly longer than deep. Quadrate robust, dorsoposterior outgrowth continuous to hyomandibular outgrowth. Hyomandibula long, anterior outgrow shallow, slightly concave; postero-dorsal process of hyomandibula pointed. Opercle robust; opercular odontodes 48–62; odontodes pointed, arranged in irregular transverse rows; odontode patch depth about half opercle length; dorsal process of opercle short and blunt, about 2.5 times interopercular odontode patch length. Interopercle long, about three fourths hyomandibula length, with 92–100 odontodes; odontodes pointed, arranged in irregular longitudinal rows; dorsal interopercular process with deep anterior concavity. Pre-opercle compact, with expanded ventral flap.

Branchial arches (Fig. 4C). Basibranchial 2 and 3 sub-cylindrical, approximately equal in length, basibranchial 2 wider anteriorly; basibranchial 4 cartilage sub-pentagonal, longer than wide. Hypobranchial 1 subcylindrical, slightly widening at its distal tip; hypobranchial 2 and 3 subtriangular, anterior portion well-ossified. Ceratobranchial 1 broad in its proximal portion, gradually narrowing

to its distal tip; ceratobranchials 2 and 3 widened in their middle portion, ceratobranchial 3 with deep concavity on posterior margin of basal portion; ceratobranchial 4 sub-rectangular, slightly narrowing proximally; accessory cartilage of ceratobranchial 4 minute; ceratobranchial 5 sub-rectangular, slightly curved, narrower than ceratobranchial 4; medial-proximal portion of ceratobranchial 5 bearing 24–26 small, slightly curved, conical teeth. Epibranchial 1 slender, with well-developed anterior uncinuate process and minute posterior process; epibranchial 2 slender, with rudimentary anterior uncinuate process; epibranchial 3 slender, with well developed, curved posterior uncinuate process; epibranchial 4 broad, sub-rectangular. Pharyngobranchial 3 short, subcylindrical; pharyngobranchial 4 long, bearing broad dentigerous plate with 22–28 fang-shaped teeth.

Colouration in alcohol. Flank dark brown with longitudinal pale yellow stripe on dorsal portion, ventral portion yellowish white; in juveniles and most adult specimens (Fig. 3B), highly contrasting dark and lighter flank zones; in some specimens, including holotype (Fig. 1), dark zone paler and not expanding on venter and on pale yellow stripe, in some others, dark zone intensively pigmented, pale yellow stripe faint (Fig. 3A). Dorsal surface of head and trunk brown, ventral surface yellowish-white. Side of head brown, with unpigmented area on cheek at vertical line just anterior to orbit; dark chromatophores more concentrated between and around nostrils. Maxillary and rictal barbels pale yellow, nasal barbel pale yellow, posterior margin dark brown. Opercular and interopercular patches of odontodes pale yellow, posterior margin of opercle dark grey to black. Unpaired fins yellowish-white; dark brown chromatophores concentrated on basal portion of dorsal

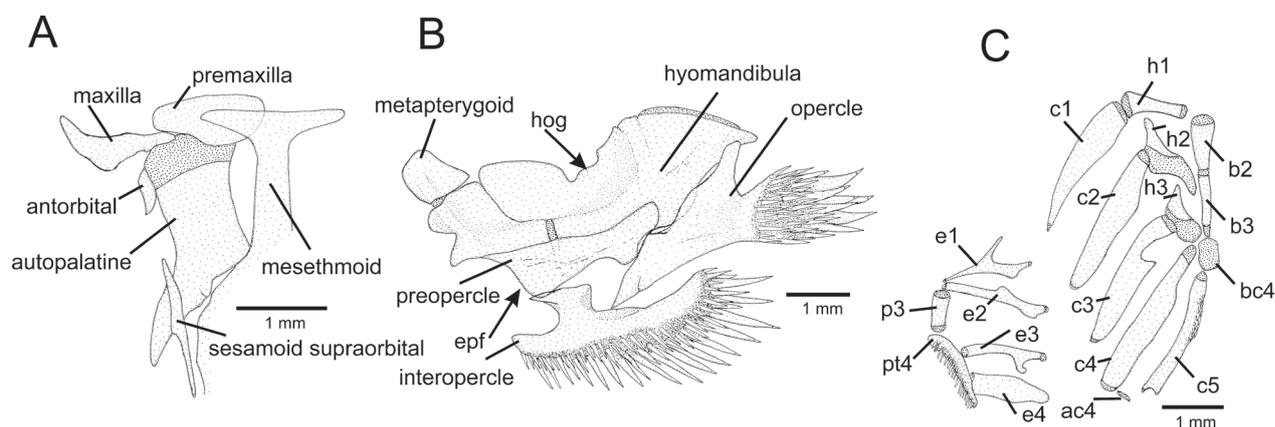


Figure 4. Osteological structures of *Trichomycterus largoperculatus*: **A.** Mesethmoidal region and adjacent structures, middle and left portion, dorsal view; **B.** Left jaw suspensorium and opercular apparatus, lateral view; **C.** Middle and left portion of brachial arches, ventral view of dorsal elements on left, dorsal view of ventral elements on right. Abbreviations: ac4, accessory cartilage basibranchial 4; b2–3, basibranchials 2–3; bc4, cartilaginous basibranchial 4; c1–5, ceratobranchials 1–5; e1–4, epibranchials 1–4; epf, expanded pre-opercular ventral flap; p3, pharyngobranchial 3; h1–3, hypobranchials 1–3; hog, hyomandibular outgrowth; pt4, pharyngobranchial 4 tooth-plate. Larger stippling represents cartilages.

fin, forming diffuse brown area; horizontally elongated brown spot on caudal fin base and black bar on basal portion, almost inconspicuous in some specimens. Paired fins pale yellow, basal portion of pectoral fin dark brown.

Colouration in life. Not recorded.

Distribution. *Trichomycterus largoperculatus* is known only from the type locality, in the middle Rio Paraíba do Sul, south-eastern Brazil (Fig. 5). Specimens of the type series were collected while they were migrating upstream, just below Ilha dos Pombos hydroelectric dam.

Etymology. From the Latin, the name *largoperculatus* (with large opercle) refers to the broad opercular odontode patch resulted from the high number of odontodes (48–62) (Fig. 4B), a unique condition amongst congeners.

Discussion

Comparative morphology

Two conspicuous apomorphic conditions of the external morphology of *T. largoperculatus* include the presence of an emarginate caudal fin and nine pectoral-fin rays. Although these conditions may be present in different trichomycterine lineages, the occurrence of these morphological character states is uncommon amongst eastern South American trichomycterines. In the over 40 species included in the clade comprising *Cambeva* and *Scleroneuma*, which is sister to *Trichomycterus s.s.*, the caudal fin is always truncate or subtruncate and there are eight rays or less in the pectoral fin, never nine (Costa et al. 2020a). According to Costa et al. (2020a), an apomorphic concave posterior margin of the caudal fin yielding an emarginate shape, is synapomorphic for species of the *T. nigricans* group (subgenus *Trichomycterus*), but an emarginate caudal fin is also present in *T. astromycterus* Reis, de Pinna & Pessali, 2020, a species with uncertain phylogenetic

position (Reis et al. 2020; Costa 2021). The *T. nigricans* group is a clade highly supported by molecular data (Costa et al. 2020b), comprising *T. caipora* Lima, Lazzarotto & Costa, 2008, *T. immaculatus* (Eigenmann & Eigenmann, 1889), *T. nigricans* and *T. santaeritae* (Eigenmann, 1918). Interestingly, all of these four species also possess nine pectoral-fin rays, considered to be another synapomorphy for the *T. nigricans* group (Costa et al. 2020a), although independently occurring in *T. giganteus* Lima & Costa, 2004 of the subgenus *Megacambeva* Costa, 2021 and *T. pradensis* Sarmento-Soares, Martins-Pinheiro, Aranda & Chamon, 2005 of the subgenus *Psammocambeva* Costa, 2021 (Costa et al. 2020a, b; Costa 2021). Therefore, the combination of an emarginate caudal fin and nine pectoral-fin rays would suggest that *T. largoperculatus* is a member of the *T. nigricans* group, although these apomorphic conditions are not exclusive of the group, as well as two other congeners not closely related to the *T. nigricans* group. On the other hand, the *T. nigricans* group was also diagnosed by the apomorphic presence of a pronounced posterior process in the maxilla (Costa et al. 2020a: fig. 2A, D and G; Costa 2021), which is not present in *T. largoperculatus* (Fig. 4A). Therefore, considering these conflicting character states, it would not be possible to unambiguously assign *T. largoperculatus* to the *T. nigricans* group of the subgenus *Trichomycterus*.

Trichomycterus largoperculatus has a long maxilla that is conspicuously longer than the premaxilla (Fig. 4A). This character state was considered the only apomorphic condition diagnosing the subgenus *Psammocambeva*, but independently occurring in *T. santaeritae* of the *T. nigricans* group (Costa et al. 2020a; Costa 2021); therefore, not useful to place *T. largoperculatus* in this subgenus. On the other hand, *T. largoperculatus* shares two apomorphic character states, first described by Costa (2021: fig. 3C), with three species of *Psammocambeva* from eastern Brazil (Fig. 5), *T. melanopygius* Reis, dos Santos, Britto, Volpi & de Pinna,

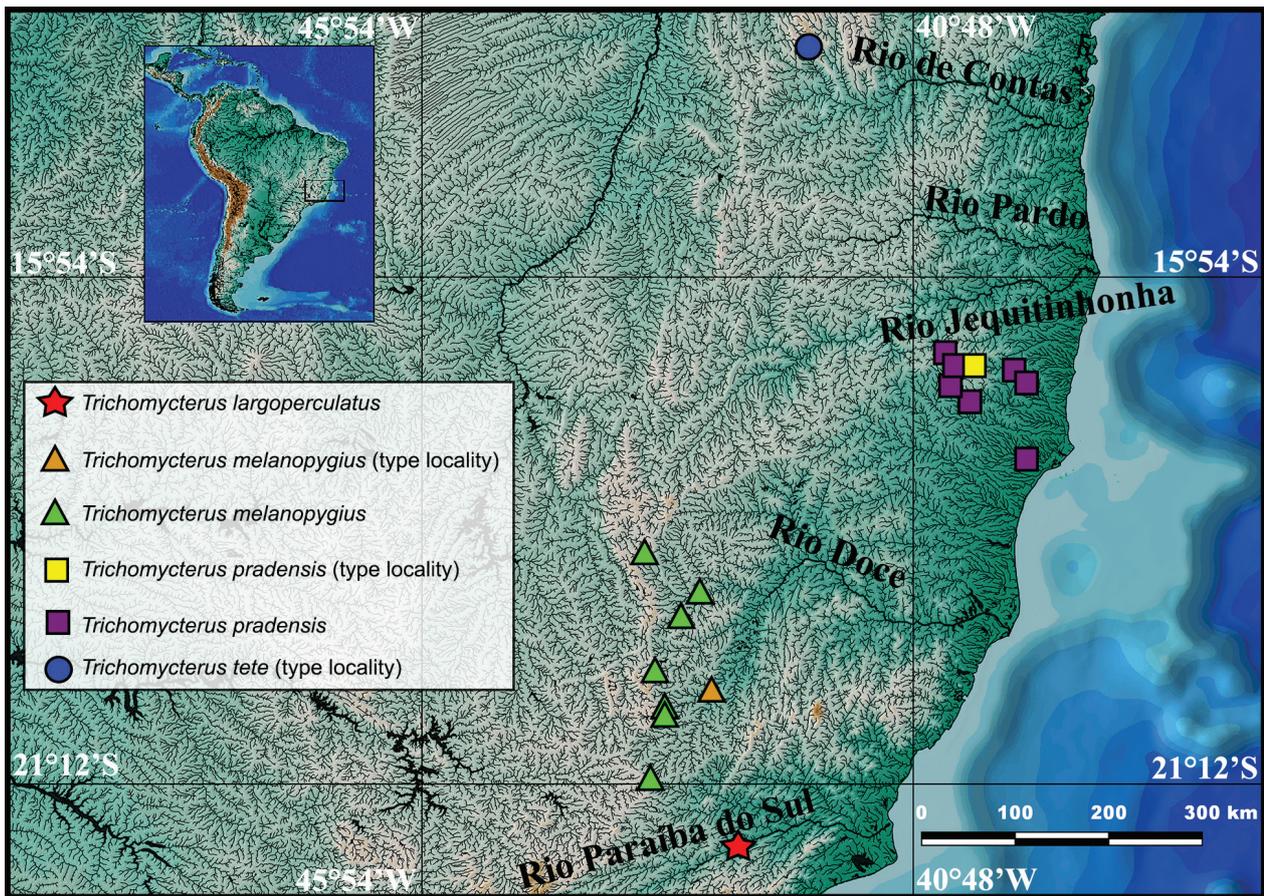


Figure 5. Map of geographical distribution of *Trichomycterus largoperculatus* sp. nov. and closely related species.

2020, *T. pradensis* Sarmento-Soares, Martins-Pinheiro, Aranda & Chamon, 2005 and *T. tete* Barbosa & Costa, 2011, that do not occur in other species of *Trichomycterus* s.s.: the presence of a shallow anterior outgrowth of the hyomandibula and an expanded ventral pre-opercular flap (Fig. 4B). Besides the unique morphological character states listed in the diagnosis (see also discussion below), *T. largoperculatus* also differs from *T. melanopygius*, *T. pradensis* and *T. tete* by having an emarginate caudal fin (vs. subtruncate) and a single median supra-orbital pore S6 (vs. paired). Interestingly, some anecdotal evidence suggests that *T. largoperculatus* and *T. pradensis* have migratory habits (see discussion below), a condition not previously reported to occur in other species of *Trichomycterus* s.s. and the closely related genera *Cambeva* and *Scleronema*, suggesting its being a derived biological condition shared by these species.

Some morphological character states, here recorded as diagnostic for *T. largoperculatus*, are interpreted as autapomorphies. Firstly, in this species, the opercular and interopercular patches of odontodes are broad, with numerous odontodes. There are about 50–60 opercular and 90–100 interopercular odontodes, thus greatly surpassing the maximum of about 30 opercular and 70 interopercular odontodes in all other species of *Trichomycterus* s.s., as well as in all species of its sister group, the clade containing *Cambeva* and *Scleronema*. Secondly, no other trichomycterid has a flank colour pattern consisting of a dark brown flank crossed by

a dorsal yellow stripe like that present in *T. largoperculatus* (Figs 1 and 3). Species of the subgenus *Paracambeva* Costa, 2021 may have a colour pattern superficially similar to that in *T. largoperculatus*. They have a yellow flank with a dark brown to black stripe on the flank mid-line in juveniles that is substituted by a diffuse dark brown zone on the flank mid-line and another on dorsum, resting a yellow unpigmented longitudinal zone on the dorsal part of the flank (Costa and Katz 2021). This pattern differs from the colour pattern of *T. largoperculatus*, in which both juveniles and adults have a dark brown ground colouration with a well-delimited dorsal yellow stripe on the body side (Fig. 3B), indicating that these colour patterns are not homologous. Finally, *T. largoperculatus* has a black bar on the basal portion of the caudal fin, which is not present in any species of the genus. In *T. caudofasciatus* Alencar & Costa, 2004, for example, there are four vertical zones of chromatophores on the caudal fin, producing a colour pattern of faint grey bars (Alencar and Costa 2004: fig. 2), greatly differing from the black bar on the basal portion of the fin occurring in *T. largoperculatus* that is conspicuous in most specimens (Fig. 3).

Migration in trichomycterine catfishes

The type series of *T. largoperculatus* was collected while fish were migrating upstream along the Rio Paraíba do

Sul main channel (V. Abilhoa, pers. com.). Although migration for feeding, reproduction and spawning is a common feature amongst large species of Neotropical pimelodid catfishes (e.g. Barthem et al. 2017), literature reports on migration of trichomycterids are restricted to a detailed record of massive juvenile upstream migration of a species of *Trichomycterus* sensu lato, *T. barbouri* (Eigenmann, 1911), in the Río Beni, Bolivian Amazon, by Miranda-Chumacero et al. (2015). In eastern South America, both juvenile and adults of all class sizes of *Trichomycterus* s.s. are commonly found at the same place, thus excluding occurrence of long range migrations for most species. For example, in the upper Rio Preto drainage, Rio Paraíba do Sul Basin, where one of us (WJEMC) has conducted regular field studies for about four decades, both adults in all reproductive stages of *T. albinotatus* Costa, 1992, *T. auroguttatus* Costa, 1992 and *T. mirissumba* have been collected at the same place and time as small juveniles about 20 mm of total length.

The only exception amongst trichomycterines from eastern South America river basins was observed during field studies on February 2014, when hundreds of specimens of *T. pradensis*, about 20 mm of total length, were seen forming a continuous upstream flow in the lower Rio Jucuruçu (16°23'34"S, 39°17'09"W), eastern Brazil, just about 25 km from the sea (WJEMC, pers. obs.), thus contrasting with larger specimens, between about 40 and 110 mm SL that were only found in the upper section of the basin (Sarmiento-Soares et al. 2005). This upstream flow was recorded during a collecting stop of about one and a half hours and it was continuous and intense during all the time, characterising a migratory movement. However, whereas it is not possible to understand the whole migration cycle based only on this record, the existence of numerous juvenile specimens migrating upstream in a region where large adults are absent, highly suggests a migratory flow. The occurrence of juveniles actively and continuously swimming upstream in a lower altitude region where adults are not present and were not recorded in previous studies (Sarmiento-Soares et al. 2005) is similar to that described for *T. barbouri* in the Bolivian Amazon and consistent with the hypothesis by Miranda-Chumacero et al. (2015) that reproduction occurs in upper areas and eggs are released into the flood, thus reaching lower areas. Further field research is needed to determine if migration is a widespread phenomenon amongst trichomycterines of eastern and south-eastern Brazil or if it is limited to some lineages including at least *T. largoperculatus* and *T. pradensis*.

Acknowledgements

Special thanks are due to Vinicius Abilhoa for donating specimens of the new species for study. Thanks are also due to Donald Taphorn, Heok Hee Ng and Paulo Andreas Buckup for suggestions and criticisms. This work was supported by Conselho Nacional de Desenvolvimento

Científico e Tecnológico (CNPq; grant 304755/2020-6 to WJEMC) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; grant E-26/202.005/2020 to AMK and E-26/201.213/2021 to WJEMC). This study was also supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Finance Code 001) through Programa de Pós-Graduação em: Biodiversidade e Biologia Evolutiva /UFRJ; Genética/UFRJ; and Zoologia, Museu Nacional/UFRJ.

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A new slippery frog (Amphibia, Conrauidae, *Conraua* Nieden, 1908) from the Fouta Djallon Highlands, west-central Guinea

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Academic editor: Johannes Penner ♦ Received 18 October 2021 ♦ Accepted 2 December 2021 ♦ Published 19 January 2022

Abstract

We describe a new species of the genus *Conraua* from the Fouta Djallon Highlands in Guinea. The species is recognised as distinct from nominotypical *C. alleni*, based on morphological evidence and is supported by a recent species delimitation analysis, based on DNA sequence data. The new species is distinguished from its congeners by the unique combination of the following characters: medium body size, robust limbs, only one instead of two palmar tubercles, the first finger webbed to below the first subarticular tubercle, presence of a lateral line system, indistinct tympanum, two subarticular tubercles on fingers III and IV, venter in adults white with dark brown spots or dark brown with grey or whitish spots. The new species differs from all congeners by more than 6% in the DNA sequence of mitochondrial ribosomal 16S. We discuss isolation in Pliocene and Pleistocene forest refugia as a potential driver of speciation in the *C. alleni* complex. We also emphasise the importance of conserving the remaining forest fragments in the Fouta Djallon Region for the preservation of both its unique biodiversity and its valuable water sources for local people.

Resumé

Nous décrivons une nouvelle espèce du genre *Conraua* des hauts plateaux du Fouta Djallon en Guinée. L'espèce est reconnue comme distincte du *C. alleni* nominotypique, sur la base de preuves morphologiques et est soutenue par une analyse récente de délimitation des espèces, basée sur des données de séquence d'ADN. La nouvelle espèce se distingue de ses congénères par la combinaison unique des caractères suivants: taille moyenne du corps, membres robustes, un seul tubercule palmaire au lieu de deux, premier doigt palmaire jusqu'en dessous du premier tubercule subarticulaire, présence d'un système de lignes latérales, tympan indistinct, deux tubercules subarticulaires sur les doigts III et IV, ventre blanc avec des taches brun foncé ou brun foncé avec des taches gris ou blanchâtre chez les adultes. La nouvelle espèce diffère de ses congénères avec plus de 6% de sa séquence d'ADN du ribosome mitochondrial 16S. Nous discutons de l'isolement dans les refuges forestiers du Pliocène et du Pléistocène comme facteur potentiel de spéciation dans le complexe *C. alleni*. Nous soulignons également l'importance de conserver les fragments de forêt restants dans la région du Fouta Djallon pour préserver à la fois sa biodiversité unique et ses sources d'eau précieuses pour les populations locales.

Key Words

Anura, conservation, forest refugia, Upper Guinea forest zone, West Africa

Introduction

The Fouta Djallon is a poorly studied mountainous region in west-central Guinea (Rödel et al. 2021). It is characterised by plateaus with irregular profiles, permanent rivers in deep valleys, steep slopes with waterfalls and an elevational gradient of 1000–1600 m a.s.l. (Young and Young 1992). The proximity to the Atlantic Ocean and south-westerly winds generate higher levels of precipitation than in other parts of West Africa (Kamara et al. 2002; Descroix et al. 2020). Together, the topographic and climatic complexity of Fouta Djallon creates a unique ecoregion with patches of tropical and subtropical moist broadleaf forests in valleys, surrounded by tropical and subtropical grasslands, savannahs and shrublands (ecoregions, *sensu* Dinerstein et al. 2017).

For decades, it has been stated that the Fouta Djallon harbours a large and unique floral diversity and that its conservation should be a priority (Hepper 1968; Schnell 1968). However, the habitats of this area have experienced severe degradation due to agricultural expansion and cattle ranching (Couch et al. 2019, 2020). Several plant species, endemic to the Fouta Djallon, have not been seen in over 60 years and are likely to be extinct (Couch et al. 2019). In contrast to botanical inventories, the fauna of the Fouta Djallon has been largely neglected though there has been some recent progress. For instance, two candidate species of barb fishes, genus *Enteromius*, have been recognised in different basins of the Fouta Djallon (Schmidt et al. 2019). To date, 94 amphibian species are known from Guinea (Channing and Rödel 2019), with 25 species recorded from the Fouta Djallon (Hillers et al. 2008). However, Hillers et al. (2008) suggested this diversity might be higher and this is supported by the subsequent discovery and description of a new *Arthroleptis* (Rödel et al. 2011) and two *Odontobatrachus* species (Barej et al. 2015b). Hillers et al. (2008) also briefly mentioned and figured an enigmatic, likely undescribed species of *Conraua*. This taxon was subsequently listed as a distinct taxon in Channing and Rödel (2019), but it has not yet been formally described.

Seven species of *Conraua* are currently recognised as valid (Neira-Salamea et al. 2021). They are distributed in East (*C. beccarii*), Central (*C. goliath*, *C. crassipes*, *C. robusta*) and West Africa (*C. alleni*, *C. derooi*, *C. sagyimase*). A recent phylogenetic analysis of the genus *Conraua* indicated that the frogs, known as *C. alleni*, actually comprise a species complex, including lineages from the Fouta Djallon (Blackburn et al. 2020). Here, we take a step forward in disentangling the taxonomy of this species complex. Through a combination of comparative morphology and the recent molecular phylogenetic analyses of Blackburn et al. (2020), we describe a new species of *Conraua* that is known from Konkouré Fetto, Hörè Binti and Chute de Ditinn in the Fouta Djallon Highlands, central Guinea.

Materials and methods

We examined four *Conraua* specimens from Konkouré Fetto, five specimens from Hörè Binti and five specimens from Chute de Ditinn, all of which are from the Fouta Djallon Region in Guinea and all are deposited in the collection of the Museum für Naturkunde Berlin, Germany (ZMB). The holotype and two paratypes of the new species were included in the study by Blackburn et al. (2020). All individuals were preserved in 75% ethanol.

For comparison, we examined specimens of all other species of *Conraua*, including 19 specimens of *C. alleni*, three *C. beccarii*, four *C. goliath*, four *C. robusta*, nine *C. crassipes*, 34 *C. derooi* and eight *C. sagyimase*. The comparative material included the holotype of *C. alleni* from the Museum of Comparative Zoology (Harvard University, Cambridge, USA; MCZ), four paratypes of *C. derooi* from the Royal Museum for Central Africa (Tervuren, Belgium; MRAC) and the Muséum national d'Histoire naturelle (Paris, France; MNHN), the holotypes of *C. crassipes*, *C. sagyimase* and *C. robusta* from the ZMB and the holotype of *Rana Griaulei* Angel, 1934 (a synonym of *C. beccarii*) from the MNHN. All comparative material is listed in Appendices 1 & 2. MOR measured the *C. alleni* holotype, the four *C. derooi* paratypes and the *R. Griaulei* holotype. All other data were collected by KNS. To account for potential individual differences when measuring museum specimens, we included only the data collected by KNS in statistical comparisons.

We measured the following morphometric variables: snout–vent length (SVL, from tip of snout to posterior end of vent), head length (HL, from tip of snout to posterior end of head protuberance), head width (HW at corners of the mouth), snout length (SL, from anterior edge of orbit to tip of snout), eye diameter (ED, maximum horizontal diameter), interorbital distance (IOD, shortest distance between upper eyelids), upper eyelid width (UEW, maximum width of upper eyelid), eye to nostril distance (EN, from anterior edge of eye to centre of nostril), eye to snout distance (ES, from anterior edge of eye to tip of snout), internarial distance (IND, between centre of nostrils), tympanum diameter (TD, maximum horizontal diameter), eye to tympanum distance (ETD, from posterior edge of eye to anterior edge of tympanum), crus (tibiofibula) length (TL, from the bent knee to heel), foot length (FL, from the proximal end of tarsus to tip of fourth toe), toe IV length (T4), hand length (HAL, from the proximal edge of the palm to the tip of the finger III), finger III length (F3) and forearm length (FLL). All measurements were taken with a digital calliper (± 0.1 mm) and/or a dissecting microscope and are given in millimetres (mm). Measurements of the type series are presented in Table 1, summarised measurements for the populations of Hörè Binti and Chute de Ditinn are given Table 2.

Sex and maturity were assessed by examination of gonads through an incision in the lateral body wall. Additional qualitative morphological characters that we examined include: tympanum detectability (distinct/indistinct), webbing condition (complete/incomplete), head shape in lateral view (round/pointed/truncated),

Table 1. Measurements [mm] of the type series of *Conraua kamancamarai* sp. nov. (holotype in bold); m = male, f = female, s = sub-adult; SVL = snout–vent length, HW = head width, HL = head length, SL = snout length, ED = horizontal eye diameter, EN = eye to nostril distance, ES = eye to snout distance, IND = internarial distance, IOD = interorbital distance, UEW = upper eyelid width, TD = tympanum diameter, ETD = eye to tympanum distance, TL = crus length, FL = foot length including toe IV, T4 = toe IV length, HAL = hand length, F3 = finger III length, FLL = forearm length; ZMB = Museum für Naturkunde, Berlin.

Voucher	ZMB	Sex	SVL	HW	HL	SL	ED	EN	ES	IND	IOD	UEW	TD	ETD	TL	FL	T4	HAL	F3	FLL
78432		f	71.7	23.7	23.5	7.0	6.5	4.3	8.2	4.0	5.2	3.8	4.3	4.0	33.3	44.8	29.3	16.8	9.8	12.4
78429		m	56.8	20.3	19.1	5.9	5.8	3.5	6.7	4.6	4.5	3.2	-	-	25.2	35.0	21.1	14.2	8.2	10.4
78430		s	48.6	16.6	17.3	5.4	5.0	3.0	6.3	3.9	4.1	3.1	-	-	21.6	29.1	20.9	12.4	6.4	8.4
78431		s	49.4	19.4	16.8	6.1	5.0	3.7	7.5	4.1	5.0	3.0	-	-	22.3	30.6	22.6	12.6	7.5	8.9

Table 2. Summary measures [mm] of *Conraua kamancamarai* sp. nov. from Höre Binti and Chute de Ditinn (referred material; total of four males and females, respectively). SVL = snout–vent length, HW = head width, HL = head length, SL = snout length, ED = horizontal eye diameter, EN = eye to nostril distance, ES = eye to snout distance, IND = internarial distance, IOD = interorbital distance, UEW = upper eyelid width, TD = tympanum diameter, ETD = eye to tympanum distance, TL = crus length, FL = foot length including toe IV, T4 = toe IV length, HAL = hand length, F3 = finger III length, FLL = forearm length.

	Sex	SVL	HW	HL	SL	ED	EN	ES	IND	IOD	UEW	TD	ETD	TL	FL	T4	HAL	F3	FLL
mean	f	81.7	28.3	25.1	8.0	7.5	5.4	10.3	6.5	5.8	4.5	5.1	4.9	35.1	52.9	32.2	19.1	11.0	14.6
sd	f	5.6	2.4	3.3	0.6	1.0	0.5	0.9	0.9	1.0	0.9	0.4	2.5	2.7	7.1	6.4	1.6	0.8	0.6
max	f	86.6	31.5	27.6	8.7	8.8	6.1	11.4	7.4	6.7	5.5	5.3	6.7	38.5	62.2	38.3	21.3	12.1	15.2
min	f	74.3	25.9	20.3	7.2	6.5	5.0	9.2	5.3	4.4	3.3	4.8	3.1	32.1	46.5	24.3	17.6	10.2	13.8
mean	m	78.8	26.9	26.2	8.2	7.5	5.3	9.9	6.1	5.9	5.0	4.3	6.3	34.2	42.9	31.7	21.6	13.3	13.4
sd	m	9.7	3.5	2.3	0.6	1.0	0.4	1.3	1.1	0.7	0.7	0.3	3.3	1.5	7.5	8.7	4.0	3.4	2.0
max	m	88.6	30.3	28.3	9.0	8.4	5.7	11.2	7.6	6.9	6.0	4.5	8.6	35.7	49.4	44.0	26.9	18.1	16.4
min	m	70.1	23.2	23.4	7.5	6.6	4.7	8.6	5.0	5.3	4.5	4.1	4.0	32.8	32.2	24.4	17.5	10.1	12.0

relative length of fingers (i.e. comparative lengths were assessed by pressing fingers together and fingers were numbered from pre-axial to postaxial I to IV), presence of an interorbital stripe, colour and shape (curved/straight/slightly-curved) of the supra-tympanic ridge, presence or absence of subarticular tubercles, undivided or divided palmar tubercles, belly and throat colouration in alcohol and presence of a lateral line system (see Lamotte and Perret 1968; Fritsch et al. 1987). Concerning the nomenclature of these lines (infra-orbital line, supra-orbital line, upper lateral line, lower lateral line, median lateral line, caudal lateral line and jugular line, anterior lower lateral line, posterior lower lateral line and mandibular lateral line), we followed Escher (1925) and Shelton (1970). Colouration of the back in preservation was only specified as

being comparatively light or dark. The variation of qualitative morphological characters amongst all *Conraua* species is summarised in Table 3.

For statistical comparisons of morphological characters with the new taxa, we focused on populations of the *C. alleni* complex that were included in the recent phylogeny of the genus (see Blackburn et al. 2020). Our analyses included thirteen individuals of *C. alleni sensu lato* (Table 4, Appendix 2), three individuals from Soyah, Fouta Djallon (Appendix 2) and 10 from Konkouré Fetto, Höre Binti and Chute de Ditinn, Fouta Djallon. We performed a principal component analysis (PCA) to reduce morphometric variables and explore intraspecific differences of morphometric measurements amongst individuals (Neff and Marcus 1980). A subsequent discriminant function analysis (linear

Table 3. Comparison of qualitative morphological characters of all currently recognised *Conraua* species. All species have completely webbed feet.

Characters	<i>C. kamancamarai</i> sp. nov.	<i>C. alleni</i>	<i>C. sagyimase</i>	<i>C. derooi</i>	<i>C. goliath</i>	<i>C. beccarii</i>	<i>C. crassipes</i>	<i>C. robusta</i>
Webbing hands	finger I webbed	absent						
Palmar tubercle	single	double	double	double	single	single	single	absent
Belly colouration	white with brown spots	white or white with brown mottling	white or white with brown mottling	white or white with brown mottling	mostly yellow	uniform white or grey	uniform white or grey	white or white with brown mottling
Throat colouration	white with brown spots or brown with white or grey spots	white	white with brown mottling	white with brown mottling	white or white with brown mottling	white or white with brown mottling	white or white with brown mottling	white or white with brown mottling
Relative length of fingers	III>IV>II>I	III>IV>II>I	III>IV>II≈I	III>IV>II>I	III>IV>II>I	III>IV>II>I	III>IV>II>I	III>IV>II>I
Snout shape	rounded	rounded	rounded	rounded	pointed	rounded	rounded	rounded
Interorbital stripe	mostly present	mostly present	mostly present	mostly absent	mostly absent	mostly present	mostly present	mostly absent
Supratympanic fold	slightly curved	straight or slightly curved	slightly curved	curved	curved	curved	curved	curved
Lateral line system	present	present	present	present	absent	present	absent	absent
Tympanum visibility	indistinct	indistinct	indistinct	indistinct	distinct	distinct	distinct	distinct

Table 4. Summary measures (in mm) of adult *Conraua alleni*; m = male, f = female, N = sample size; SVL = snout–vent length, HW = head width, HL = head length, SL = snout length, ED = horizontal eye diameter, EN = eye to nostril distance, ES = eye to snout distance, IND = internarial distance, IOD = interorbital distance, UEW = upper eyelid width, TD = tympanum diameter, ETD = eye to tympanum distance, TL = crus length; FL = foot length including toe IV, T4 = toe IV length, HAL = hand length, F3 = finger III length, FLL = forearm length.

	Sex	N	SVL	HW	HL	SL	ED	EN	ES	IND	IOD	UEW	TD	ETD	TL	THL	FL	T4	HAL	F3	FLL
mean	f	5	58.4	22.3	20.7	5.6	6.5	3.7	7.3	5.0	4.9	3.7	4.0	2.9	28.0	27.6	38.4	24.0	14.9	8.6	10.0
sd	f	5	25.1	9.6	8.8	2.3	2.8	1.7	3.2	2.1	2.1	1.6	1.9	1.5	12.1	11.7	16.6	10.1	6.4	3.7	4.3
max	f	5	68.4	26.4	24.1	6.4	7.5	5.0	9.0	6.0	6.3	4.4	4.4	4.0	33.4	31.9	46.9	28.5	17.8	10.1	12.3
min	f	5	50.9	19.5	18.2	5.0	5.2	3.0	6.3	4.2	4.4	3.4	3.4	2.4	23.6	24.3	32.4	21.8	12.8	7.2	8.0
mean	m	8	55.5	21.5	20.1	5.9	5.9	3.7	7.3	4.5	4.9	3.6	3.5	2.8	26.6	27.3	36.4	23.3	13.5	7.9	10.3
sd	m	8	7.7	2.8	2.9	0.9	0.8	0.5	1.2	0.7	0.7	0.2	0.4	0.5	3.6	3.7	6.7	3.8	2.0	1.1	1.6
max	m	8	71.6	27.3	26.1	7.0	7.4	4.5	9.5	5.7	6.2	4.0	4.1	3.5	34.0	34.0	47.3	30.8	16.5	9.8	14.0
min	m	8	48.3	18.9	16.9	4.7	5.0	3.2	6.2	3.6	4.0	3.3	3.0	2.4	23.4	23.7	28.9	18.6	11.4	6.5	8.8

discriminant analysis, LDA) was used to calculate the reliability of morphological quantitative traits in assigning individuals to the species groups, identified by Blackburn et al. (2020). To visualise morphometric variation independent of SVL, PCA was run on residuals of linear regressions of the measured variables. LDA was applied on raw morphometric measurements including SVL, because the aim of the LDA was to determine the use of all morphometric measurements to distinguish the different lineages (Funk et al. 2012). Both analyses were performed on log-transformed data. In some analyses, we investigated both sexes together because sex is difficult to determine, based on external morphology for most *Conraua* species (see Neira-Salamea et al. 2021). Tympanum diameter (TD) and eye to tympanic distance (ETD) were excluded from the analysis, as the tympanum was not visible in most specimens. Statistical analyses were performed in R version 3.5.1 (R Core Team 2018) using the *prcomp* function for PCA (with values scaled and centered) and *lda* function for LDA from the MASS package (v.7.3-51.3; Venables and Ripley 2002) and for PCA visualisation, we used the function *autoplot* of the *ggplot2* package (Wickham 2016).

Results

Based on the multi-locus phylogeny, published by Blackburn et al. (2020), the *Conraua* populations from Fouta Djallon are distinct from *C. alleni*, *C. derooi* and *C. sagyimase*, all of which form a large clade. Uncorrected p-distances of the mitochondrial 16S rRNA fragment (569 bp) revealed high genetic divergence between the populations from Fouta Djallon and all other *Conraua* species. Uncorrected p-distances between the undescribed species from Fouta Djallon and other species is as follows: *C. derooi*, 6%; *C. sagyimase*, 7%; *C. alleni*, 7%; *C. goliath*, 9%; *C. robusta*, 9%; *C. beccarii*, 11%; and *C. crassipes*, 12%. These values are higher than the 3% threshold, usually applied for recognising anuran candidate species (Fouquet et al. 2007; Vieites et al. 2009). Barcoded specimens within the type series (ZMB 78432, 78429, 78430) of the undescribed species had uniform 16S sequences. The genetic distance between these frogs and a population from Soyah (ZMB 90175, 90176), a site well situated within the Fouta

Djallon, was about 3% (mean \pm sd: 0.0295 ± 0.008 , range: 0.028–0.030). Therefore, we excluded the Soyah population from the description of the new species and suggest further bioacoustics analysis to confirm if it belongs to the species described below. The frogs of the type series further showed genetic differences to other populations from the Fouta Djallon. For instance, a specimen from Höre Binti (ZMB 90177) differed from the type series by 0.9% (this specimen differed from the Soyah population by $0.029\% \pm 0.0008$, 0.028–0.030%). Although we believe that specimens from Höre Binti and Chute de Ditinn represent the new species, we list and describe material from these locations only as additional referred material.

We analysed morphological characters (Tables 1, 2 and 4) of all lineages of the *C. alleni* complex included in the genus phylogeny (for details, see ‘Material and Methods’ and Blackburn et al. 2020; for morphological comparisons with other *Conraua* species, see ‘Diagnosis’ below). Five principal components with eigenvalues > 1.0 (PC1–PC5) accounted for 72.8% of the total character variation (Fig. 1; Table 5). PC1 accounted for 29.3% of the variation and loaded most strongly for eye to snout distance (ES) and crus (tibiofibula) length (TL). PC2 accounted for 14.8% of the variation and loaded negatively most strongly for finger III length (FE) and eye to nostril distance (EN). PC3 was mainly impacted by upper eyelid width (UEW), eye diameter (ED) (both negatively). PC4 was dominated by hand length (HAL, negatively) and interorbital distance (IOD). PC5 loaded most strongly for head length (HL, negatively) and internarial distance (IND). A plot of PC1 versus PC2 showed both large variation amongst individuals, as well as a large amount of overlap between the four genetic lineages included (Fig. 1). However, a subsequent linear discriminant analyses (LDA) of the morphological raw data correctly assigned all individuals to their respective genetic lineage. The characters contributing most to the species assignment were SVL, HL and TL. Coefficients of linear discriminant analysis (LDA) are provided in Table 6.

An unexpected diagnostic character was the number of palmar tubercles. Whereas *C. derooi*, *C. sagyimase* and *C. alleni* have a divided, double palmar tubercle (an outer and a middle one), all *Conraua* populations from the Fouta Djallon exhibit only an undivided palmar tubercle

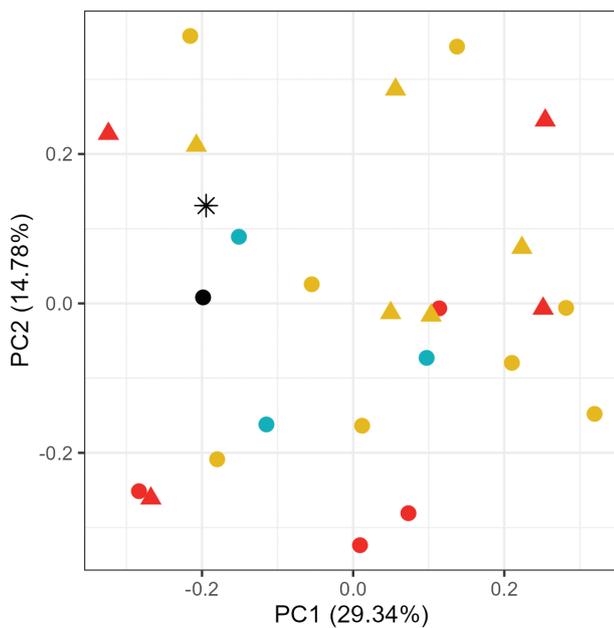


Figure 1. Axes I and II from the Principal Component Analyses (PCA), based on 15 size-corrected morphometric variables of four *Conraua* lineages from West Africa; only adult frogs included. Black = *Conraua kamancamarai* sp. nov. types from Konkouré Fetto (asterisk = female holotype), red = *Conraua kamancamarai* sp. nov. populations from Hörè Binti and Chute de Ditinn, blue = *Conraua* populations from Soyah, Fouta Djallon, yellow = *C. allenii*; triangle = females, circle = males; compare Table 5.

Table 5. Principal component analysis on morphometric measurements for *Conraua kamancamarai* sp. nov., *C. allenii* and *Conraua* populations from Soyah. Character loading, percentage (%) and cumulative percentage of explained variance for principal components with eigenvalues > 1.0, (PC) I–V; variables with the highest loadings are given in bold; compare Fig. 1; for abbreviations of morphological measures, see ‘Materials and Methods’ section or Tables 1, 2 and 4.

Variable	PC1	PC2	PC3	PC4	PC5
HW	0.33	0.08	-0.03	0.16	-0.07
HL	0.26	0.06	-0.19	0.15	-0.62
SL	0.23	-0.31	-0.04	0.13	0.31
ED	0.10	0.30	-0.44	0.10	-0.13
EN	0.13	-0.45	0.19	0.34	0.15
ES	0.37	-0.07	0.04	0.25	0.10
IND	0.26	0.05	-0.35	0.11	0.45
IOD	0.30	-0.04	0.10	0.40	-0.22
UEW	-0.20	-0.29	-0.49	0.06	-0.09
TL	0.35	0.08	-0.04	-0.33	-0.17
FL	0.31	0.20	0.19	-0.39	0.24
T4	0.30	0.32	0.19	0.02	0.09
HAL	0.26	-0.25	-0.33	-0.45	0.01
F3	0.17	-0.49	-0.04	-0.28	-0.13
FLL	0.07	-0.23	0.41	-0.16	-0.31
Eigenvalue	4.40	2.22	1.75	1.35	1.20
%	29.34	14.78	11.64	8.98	8.02
Cumulative %	29.34	44.12	55.75	64.74	72.75

(Fig. 2). In *C. derooi*, the divided palmar tubercles are nearly connected and the thenar tubercle is clearly separated from the middle palmar tubercle. In *C. allenii*, the divided palmar tubercles (outer and middle) are very close

Table 6. Coefficients of linear discriminant analysis (LDA) based on the 16 morphometric variables of three *Conraua* populations from West Africa: *Conraua kamancamarai* sp. nov., *Conraua* populations from Soyah, Fouta Djallon and *C. allenii*; those characters that contribute most are given in bold; for abbreviations of morphological measures, see ‘Materials and Methods’ section or Tables 1, 2 and 4.

	LD1	LD2
SVL	-33.06	-6.11
HW	5.43	6.73
HL	0.67	31.97
SL	-13.74	-9.47
ED	4.93	-9.75
EN	-5.73	15.57
ES	19.41	-0.79
IND	1.26	3.27
IOD	10.45	-18.10
UEW	3.23	-2.18
TL	23.24	-14.45
FL	8.12	-2.75
T4	-12.27	6.19
HAL	-8.53	5.04
F3	-5.55	-2.96
FLL	-1.84	-9.82

to each other, but not connected and the thenar tubercle is separated from the middle palmar tubercle. In *C. sagyimase*, the divided palmar tubercles (outer and middle) are close but separated and the middle palmar and the thenar tubercle are distinctly separated (Fig. 2).

The most conspicuous morphological character of diagnostic value is the ventral pattern. Whereas most West African *Conraua* species have a predominantly white or light grey venter, often with dark mottling, but never with distinct spots or blotches, the new species exhibits a white ventral colour with well-defined dark brown spots and blotches (or dark colour with scattered grey or whitish spots) (Figs 3, 5 and 6). This ventral colour patterning is most distinct in adults, but already developed in some subadults.

Based on the genetic differences and the above diagnostic characters, we describe the *Conraua* populations from Konkouré Fetto, Hörè Binti and Chute de Ditinn as a species new to science. Due to their genetic divergence from other Fouta Djallon populations, we exclude the population from Soyah until larger sample size and call recordings are available (e.g. compare Neira-Salamea et al. 2021). This population might represent another cryptic species that would be described separately.

Description of the new species

Conraua kamancamarai sp. nov.

<http://zoobank.org/625E8A78-5BB3-4EB1-8405-159C93135699>

Figs 2–6

Holotype. ZMB 78432 (field and tissue #: GN11-140; GenBank # for 16S: MT669400) adult female, Guinea, Fouta Djallon Region, Konkouré Fetto, 10°20'28.21"N,



Figure 2. Ventral view of hands of four *Conraua* species showing palmar tubercles differences. Whereas three species possess a divided (outer and middle) palmar and a thenar tubercle, *Conraua kamancamarai* sp. nov. has an undivided palmar and a thenar tubercle. **a.** *Conraua kamancamarai* sp. nov. holotype (ZMB 78432); **b.** *C. derooi* (MNHN-RA 1993 4087); **c.** *C. sagyimase* (UWBM Herp 05841, paratype); **d.** *C. alleni* (ZMB 90390); Scale bars: 5 mm.

12°10'16.82"W, 650 m a.s.l., 20 June 2011, collected by Laura Sandberger-Loua & Joseph Doumbia.

Paratypes. ZMB 78429 (field and tissue #: GN11-130; GenBank # for 16S: MT669399) adult male; ZMB 78430, ZMB 78433 (field and tissue #: GN11-133, GN11-136; GenBank # for 16S: KF693389) subadults, all other data as holotype.

Additional referred material. For the description of the new species, we restrict the type series to the population from Konkouré Fetto. We do so because there may be additional undescribed diversity within the group identified as “*C. alleni* 1b” by Blackburn et al. (2020). The vouchers from Hörè Binti, Pita and Chute de Ditinn, Dalaba (all from Fouta Djallon, Guinea) are, therefore, listed as additional referred material (see Table 2).

Hörè Binti, Pita. ZMB 90301, ZMB 90304 (field and tissue #: CB2010-055, CB2010-061), adult females; ZMB 90302 (field and tissue #: CB2010-056), adult male; ZMB 90177 (field and tissue #: CB2010-057; GenBank # for 16S: MT669401), subadult; ZMB 90303 (field and tissue #: CB2010-059), subadult, 10°51'04.8"N, 12°31'14.1"W, 657 m a.s.l.; 22 July 2010, collected by Christian Brede & Joseph Doumbia.

Chute de Ditinn, Dalaba. ZMB 90305, ZMB 90307, ZMB 90309 (field and tissue #: CB2010 082, CB2010 089, CB2010 091), adult males; ZMB 90306, ZMB 90308 (field and tissue #: CB2010 088, CB2010 090), adult females, 10°49'13.1"N, 12°11'30.7"W, 760 m a.s.l.; 24 July 2010, collected by Christian Brede & Joseph Doumbia.

Diagnosis. The new species resembles other members of the genus *Conraua* Nieden, 1908. *Conraua kamancamarai* sp. nov. is an aquatic frog with the following traits: smooth dorsal skin, covered with scattered small, rounded warts on back and longitudinal ridges on dorsal part of hind legs; venter skin smooth; three odontoid projections on lower jaw, one at symphysis and one to each side on dentary; vocal sacs absent; fully webbed feet, i.e. to end of last phalanx of toe. *Conraua kamancamarai* sp. nov. is

closely related to a clade including *C. alleni sensu stricto*, *C. derooi* and *C. sagyimase* (see Blackburn et al. 2020). Genetic distances between the new species and all other



Figure 3. *Conraua kamancamarai* sp. nov., female holotype (ZMB 78432) in lateral, dorsal and ventral view. Scale bar: 20 mm.



Figure 4. *Conraua kamancamarai* sp. nov., female holotype (ZMB 78432) lateral view of head and ventral view of left foot. Scale bar: 10 mm.

Conraua species were higher than 6% in the examined part of the 16S gene.

Conraua kamancamarai sp. nov. can be distinguished from *C. goliath* by a rounded snout (pointed in *C. goliath*), the absence of short dorsal skin ridges, a white venter with dark brown blotches (yellow venter in *C. goliath*), the presence of a lateral line system, an indistinct tympanum, a wide tarsal fold and by having more than one subarticular tubercle on fingers (one in *C. goliath*). *Conraua kamancamarai* sp. nov. differs from *C. crassipes* by a white venter with dark brown blotches (uniform

white or cream in *C. crassipes*), an indistinct tympanum, the presence of a lateral line system, by a conspicuous outer metatarsal tubercle (less conspicuous in *C. crassipes*) and by lacking a dermal fold near the elbow. *Conraua kamancamarai* sp. nov. differs from *C. beccarii* by the absence of a transverse fold behind the eyes and across the interorbital region, by lacking a swollen post-occipital and suprascapular region in adult males, by a white-coloured venter with dark brown blotches (no spots in *C. beccarii*) and by having a head that is as wide as long (wider than long in *C. beccarii*). *Conraua kamancamarai*



Figure 5. *Conraua kamancamarai* sp. nov., female holotype (ZMB 78432) in life.

sp. nov. differs from *C. robusta* by having a head that is as wide as long (wider than long in *C. robusta*), by having a U-shaped notched tongue-tip (tip of tongue rounded in *C. robusta*), by a white venter with dark brown blotches (uni-

formly white or with dark mottling in *C. robusta*) and the presence of a lateral line system. *Conraua kamancamarai* sp. nov. differs from *C. alleni sensu stricto* by having an undivided palmar tubercle, by having a white-coloured



Figure 6. Dorsal and ventral views of paratypes of *Conraua kamancamarai* sp. nov., male (ZMB 78429), subadults (ZMB 78430, ZMB 78431). Scale bars: 20 mm.

venter with dark brown blotches (uniform light or light with dark mottling in *C. alleni*), by a larger inner metatarsal tubercle, a wider tarsal fold and by the presence of webbing between fingers I and II. *Conraua kamancamarai* sp. nov. differs from *C. derooi* by having a more slender body and limbs, a slightly curved supratympanic fold (distinctly curved in *C. derooi*), two subarticular tubercles on finger III (one in *C. derooi*), by lacking a swollen postoccipital and suprascapular region in adult males, by the absence of a divided palmar tubercle, by a white venter with dark brown blotches (uniform whitish or with dark mottling in *C. derooi*) and by the presence of webbing between fingers I and II. *Conraua kamancamarai* sp. nov. differs from *C. sagyimase* by having narrower fingertips, a wider tarsal fold, by the absence of a divided palmar

tubercle, by a white venter with dark brown blotches (uniform pale or with dark mottling in *C. sagyimase*) and by the presence of webbing between fingers I and II.

Description of the holotype (Figs 2–5; measurements in mm). Adult female; slightly dorsoventrally flattened, short and rounded body; snout rounded in dorsal and lateral view, with upper lip slightly projecting forward; SVL 71.7; head width 23.7, approximately equal to head length 23.5; head length 33% of SVL; snout length 7.0, 30% of head length; eye–nostril distance 4.25; eye–snout distance 8.1; internarial distance 4.0, slightly larger than interorbital distance 5.2; nostrils protuberant, directed dorsolaterally, visible in lateral and dorsal view; large eyes, projecting laterally beyond margins of head in dorsal view; eyes

projecting slightly above dorsal margin of head in lateral view; eye diameter 6.5, horizontal diameter of tympanum 4.3; upper eyelid width 3.8, 73% of interorbital distance; eye–tympanum distance 3.9; tympanum indistinct; canthus rostralis distinct and rounded; loreal region concave; slightly curved supratympanic fold extending from posterior edge of eye to shoulder, joining the lateral fold; upper lip slightly protruding; premaxillary and maxillary teeth slender and pointed, three odontoid projections on lower jaw, one on at symphysis and one to each side on dentary; vomerine teeth pointed; about half of anterior tongue attached to floor of mouth, tongue-tip with U-shaped notch.

Forelimbs robust; forearm length 12.4, 74% of hand length 16.8; thenar and palmar tubercle oval and protruding, palmar tubercle larger than thenar tubercle; shape of fingers conical, wider at bases and narrower towards tips; finger tips rounded, non-expanded; one subarticular tubercle on fingers I and II; two subarticular tubercles on fingers III and IV; subarticular tubercles absent on base of fingers; relative length of fingers: III > IV > II ≈ I, length of finger III 9.8; fingers I and II webbed to first subarticular tubercle.

Hind limbs moderately robust; crus length 33.3, 46% of the SVL; foot including longest toe 44.8, 62% of SVL; elongated, prominent oval inner metatarsal tubercle, more than twice as long (4.4) as wide (1.7); outer metatarsal tubercle absent; supernumerary plantar tubercles absent; subarticular basal tubercles absent; one subarticular tubercle on toes I and II; two subarticular tubercles on toes III and V, three subarticular tubercles on toe IV; toe tips rounded, forming small discs, as broad as subarticular tubercles; relative lengths of toes: VI > III > V > II > I; length of toe IV 29.3; webbing complete, i.e. to end of last phalanx of toe; dermal fringing on outer surfaces of toes I and V, forming lateral skin folds; wide tarsal fold.

Skin texture on dorsal parts of head, body, flanks and limbs smooth with scattered, small, rounded warts; upper eyelid skin with many warts; inner surface of upper arm smooth; dorsal surface of crus with 12 rows of longitudinal ridges; ventral skin smooth, throat with longitudinal folds; a post-gular (thoracic) fold extending to level of forelimbs insertion; lateral line system with jugular line, upper lateral line, lower lateral line, median lateral line, caudal lateral line, infra-orbital line, supra-orbital line, mandibular lateral line and anterior lower lateral line (see Shelton 1970).

Colouration in preservative (after 10 years in 75% ethanol; Figs 2–4). Dorsum brown with scattered small cream warts, warts more abundant on posterior surface; light interorbital stripe; upper eyelids brown with abundant lighter spots; lips brown or brown with lighter mottling; supratympanic fold brown; lateral fold light; upper arm fold light; dorsal surface of flanks brown; ventral surface of flanks white or light grey with brown spots; dorsal surface of legs brown with dark scattered spots; dorsal surface of toes I, II, III and IV with brown mottling; dorsal surface of toe V brown; dorsal surface of arms brown

with scattered light brown warts; dorsal surface of fingers I and II light brown with dark mottling, fingers III and IV brown; ventral surface of throat and belly whitish with dark brown blotches; ventral surface of crus and feet light brown with dark mottling; ventral surface of hands brown.

Colouration in life (Fig. 5). Dorsum dark brown with scattered lighter dots and dark spots; lips same colour as dorsum, paler on lateral surfaces; flanks dark; iris gold; ventral surfaces white with dark brown spots; ventral surface of hands dark, tubercles lighter; ventral surfaces of legs with scattered reddish dots; lateral fold light brown.

Variation (Figs 6–7; Tables 2 and 3). Overall, the paratypes are similar to the holotype in external appearance and colouration. Dorsal colouration ranges from uniform dark brown (ZMB 78432) to predominantly brown with dark mottling (ZMB 78430, ZMB 78431) or predominantly brown with dark spots. In the male paratype (ZMB 78429), the jugular lateral line is more conspicuous than in the holotype and the upper lip is light brown. Ventral colour pattern of all specimens similar: whitish with distinct brown blotches, however, these blotches are lighter in the subadult paratypes (ZMB 78430, ZMB 78431).

Referred specimens from Hörè Binti: Dorsal colouration varies from similar to the type series (ZMB 90177, ZMB 90303) to darker (ZMB 90301, ZMB 90302, ZMB 90304) or a dark dorsum with light brown spots (ZMB 90302). Ventral colouration of some individuals deviates from that of the type series by a greyish belly with dark mottling and a dark throat with lighter spots (ZMB 90304, ZMB 90301). Ventral colouration of ZMB 90302 is difficult to define due to its preservation condition. Ventral colouration of juveniles is showing fewer dark spots than in adults (ZMB 90177, ZMB 90303). Lateral line system in ZMB 90304 is more conspicuous than in the type series.

Referred specimens from Chute de Dittinn: Dorsal colouration of all individuals darker than that of type series, some individuals with light brown spots (ZMB 90307, ZMB 90308) that are absent in the type series. Some individuals present a different colouration pattern than type series on the throat with a dark base colour and lighter spots (ZMB 90306, ZMB 90307, ZMB 90308). Belly of ZMB 90305 greyish with dark mottling, different to the colour pattern of the type series, probably due to preservation. The lateral line system in all individuals is more conspicuous than in the type series. A posterior lower lateral line (see Shelton 1970) is present in ZMB 90307 and ZMB 90309. This part of the lateral line system was not detected in the type series.

For variation in life colouration of *Conraua* specimens from various localities in the Fouta Djallon Region, see Figure 7.

Distribution (Fig. 8). So far, the known range of *Conraua kamancamarai* sp. nov. is restricted to the type locality at Konkouré Fetto and to two other sites, Hörè Binti and

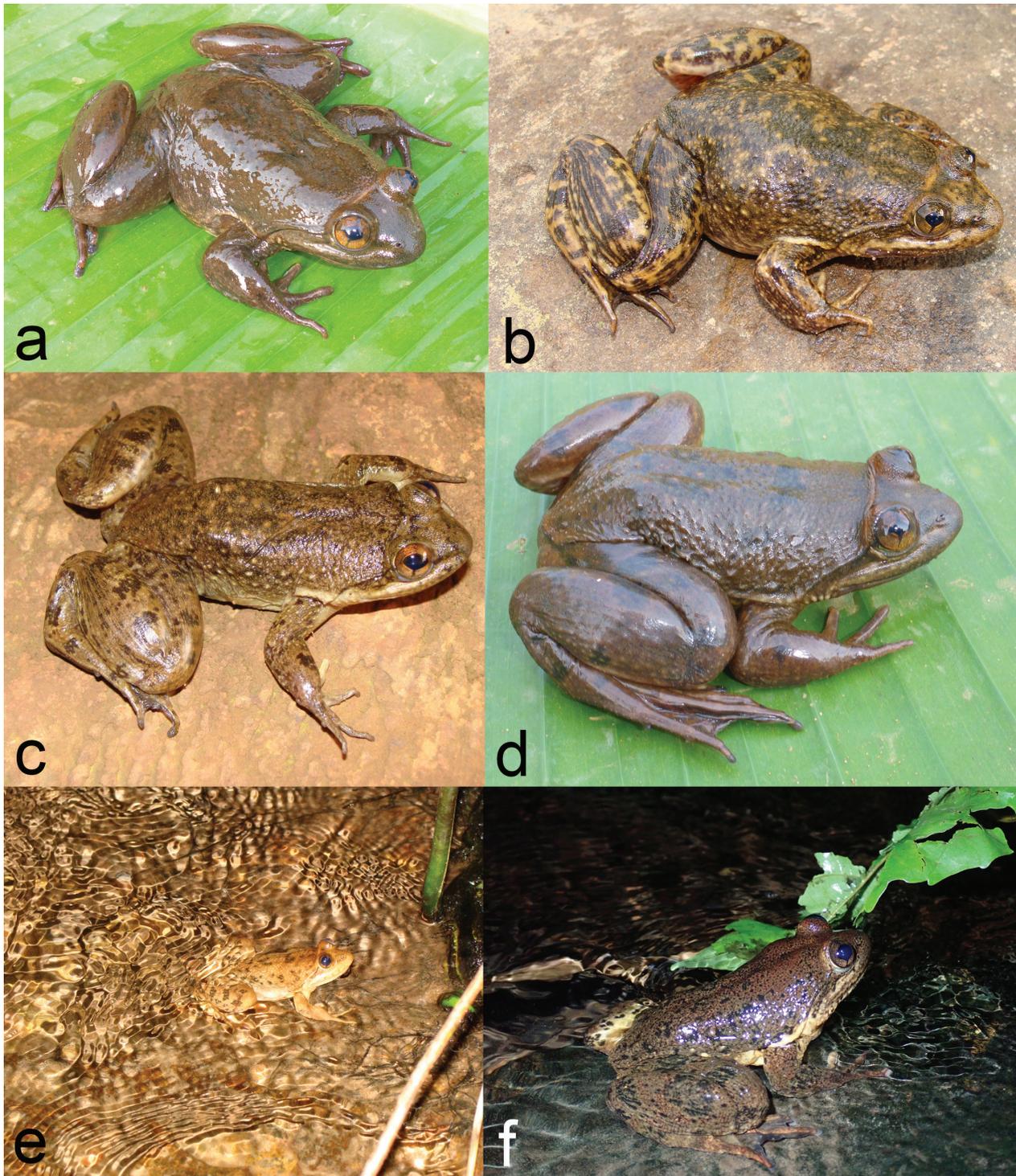


Figure 7. Colouration of life *Conraua kamancamarai* sp. nov. from the Fouta Djallon and surrounding region, Guinea, illustrating variation in colour pattern and skin texture. **a.** From Dubreka, River Bindinbandan ($10^{\circ}22'21.9''\text{N}$, $13^{\circ}9'16.8''\text{W}$, 199 m a.s.l.); **b.** From Dalaba, Chute de Ditinn; **c.** From Hòrè Binti; **d.** From Dubreka, River Bindinbandan ($10^{\circ}22'21.9''\text{N}$, $13^{\circ}9'16.8''\text{W}$, 199 m a.s.l.); **e.** From Dalaba, Chute de Ditinn; **f.** From Téliimélé, locality Kourakoto, river Didounpouriguè ($10^{\circ}55'30.4''\text{N}$, $13^{\circ}47'39.4''\text{W}$, 238 m a.s.l.); frogs in lower row in typical calling position, sitting in shallow water; specimens either not collected or not assignable to a voucher specimen, whereas the frogs from Hòrè Binti and Chute de Ditinn can be assigned to *Conraua kamancamarai* sp. nov. without doubt; the other frogs may represent an undescribed *Conraua*.

Chute de Ditinn. Although the species probably occurs in a few more rivers that have not yet been surveyed, the entire range almost certainly will not extend beyond the Fouta Djallon, where it is very likely patchily distributed.

Biology and habitat (Figs 9 and 10). The new species occupies fast-flowing rocky streams with waterfalls within riverine forest in mountainous areas in the Fouta Djallon, Guinea (Figs 9 and 10). Like other frogs of the genus, they

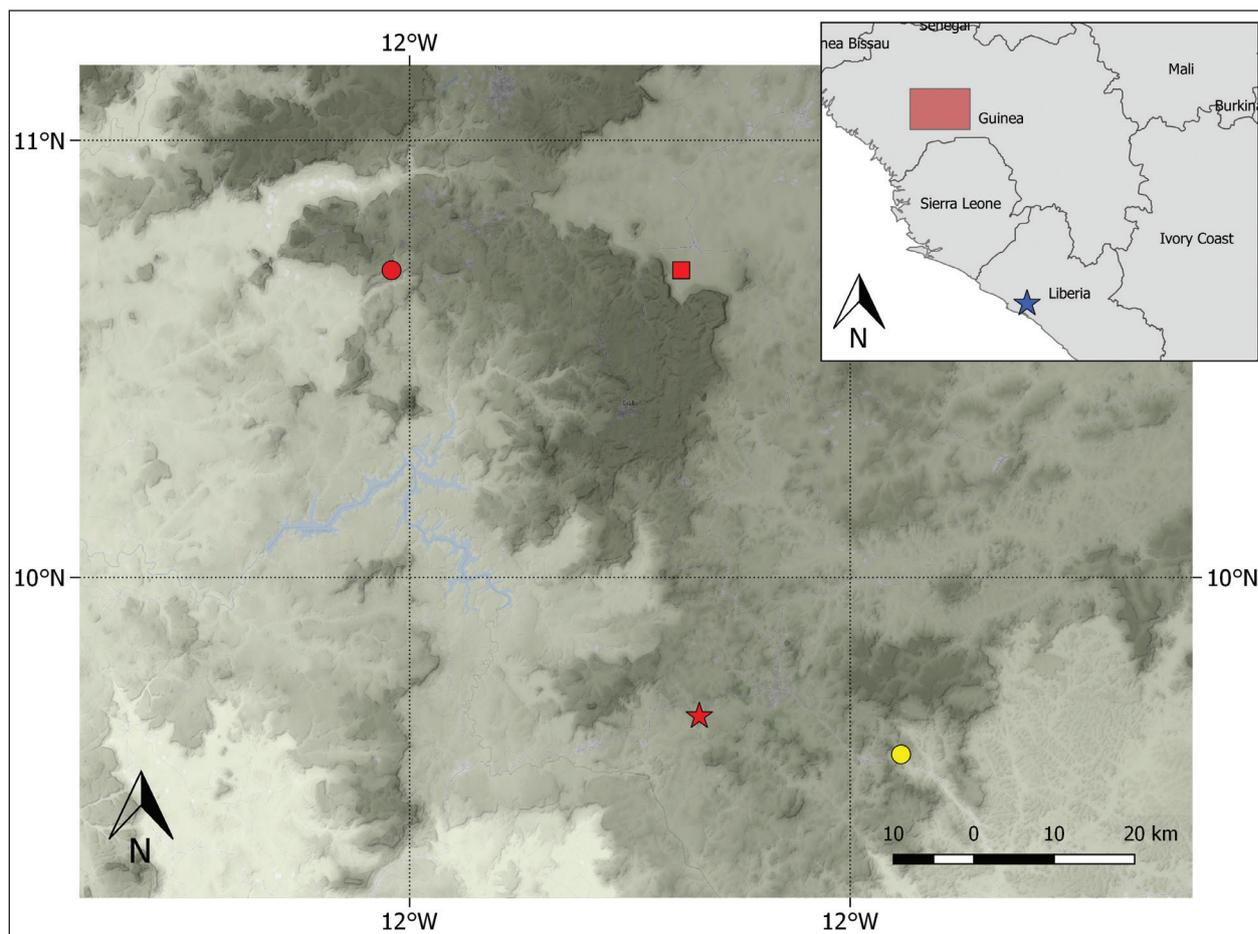


Figure 8. Map of known localities of *Conraua kamancamarai* sp. nov. in the Fouta Djallon Highlands, Guinea. Inset (upper right) shows a map of West Africa indicating in red, the area of occurrence of the new species in the Fouta Djallon and in blue, the assumed type locality of *C. allenii*. Known localities of *Conraua kamancamarai* sp. nov. are shown in red (Konkouré Fetto, type locality: star; Hôre Binti: circle; Chute de Ditinn: square), the population from Soyah, potentially representing another undescribed *Conraua*, is given in yellow. Altitudinal range is indicated with light shading from lowlands (112 m a.s.l.) to dark shading highlands (1089 m a.s.l.). Sources: OpenStreetMap (2020), U.S. Geological Survey (2020).

are predominately nocturnal and aquatic. Despite their occurrence in fast flowing streams, adults show a preference for calmer river sections, where turbulent water is absent. Usually, frogs are encountered at least partly submerged in shallow water, facing the riverbanks. When outside of the water, they remain within jumping distance to water. Disturbed frogs seek shelter on the ground of pools, sometimes trying to burrow deeper into them and cover themselves with gravel or substrate (Fig. 11). This behaviour is similar to what Knoepffler (1985) described for *Conraua crassipes*. They call (whistle) with an open mouth, sitting in shallow water (Fig. 7; compare Amiet 1990). Mating has never been observed by the authors; however, single observations of clutches and jelly remnants of spawn indicate that oviposition sites are small puddles or depressions on the riverbanks near the spray water zone of cascades and waterfalls. *Conraua* tadpoles usually were observed in silted calm ponds where up to 50 tadpoles of about the same size have been encountered. If this and other species of the genus show breeding behaviour comparable to *Conraua goliath* (compare Schäfer et al. 2019), remains to be researched.

The surroundings of the forest fragments where the species occurs are generally degraded by anthropogenic disturbance, particularly peanut and rice crops and cattle grazing. The type locality (Fig. 10) is located between Konkouré and the largest city within the Fouta Djallon, Mamou, within a relatively short distance to the connection road and was surveyed on 20 June 2011. Along the national route one (N1), one of the largest roads connecting the East with the West of Guinea, houses are numerous, but already within a relatively short distance to the road, human presence may be considerably scarcer. Slopes are either covered by an open, short, dry forest with signs of cattle grazing and used for charcoal production (Fig. 10) or comparatively large fields for peanuts or rice crops. Only steep slopes surrounding rivers had sometimes larger trees and denser vegetation with higher humidity levels than the surroundings. The type locality is at a river within denser forest, with large boulders and some cascades, allowing for a diverse river site with fast and slow flowing parts and comparatively clear water (Fig. 10). These forests are not protected and were in the past burned by the population as protest against government decisions in Conakry.



Figure 9. The Fouta Djallon, Guinea and habitats of *Conraua kamancamarai* sp. nov. Hörè Binti landscape (upper photo) and typical river habitats.

The classified forest (partly protected areas allowing forestry) of Hörè Binti is located within a mountainous area containing several freshwater sources. It was surveyed from 22–23 July 2010. Many fast-flowing streams with cascades have its source on the mountain. The habitat degradation due to anthropogenic alterations was dramatic and only very small forest fragments remained. The anthropogenic pressure consisted of cultivations/fields (mainly peanut and rice) and grazing cattle. Only streams were surrounded by some remaining larger trees. The Di-

tinn / Dalaba site was within a small fragment of gallery forest with a stream, next to the waterfall of Ditinn. It was surveyed from 24–25 July 2010. Although there is a small village next to the forest, only minor anthropogenic alterations were detectable.

Threat status. *Conraua kamancamarai* sp. nov. should be considered Data Deficient (DD) because more information is required to make an adequate assessment of the species' extinction risk. However, if the species range is



Figure 10. The type locality of *Conraua kamanamarai* sp. nov. near Konkouré Fetto, Fouta Djallon, Guinea ($10^{\circ}20'28.21''\text{N}$, $12^{\circ}10'16.82''\text{W}$, 650 m a.s.l.). The frogs live in clear, fast flowing streams, with riverine forest. The surroundings are heavily degraded by agriculture, cattle grazing and charcoal production (inset figure).

indeed restricted to the sites of Konkouré Fetto, Hörè Binti and Chute de Ditinn, the species should be categorised as Endangered (EN) following the IUCN (2012) criteria B2bi (continuing decline, observed, inferred or projected in extent of occurrence) and B2biii (continuing decline, observed, inferred or projected, in area, extent and/or quality of habitat).

Etymology. This species is dedicated to Kaman Camara (Fig. 12), our long-term field assistant and friend, who started working with MOR in 2002 on a survey to the

Simandou Range that was organised by Conservation International (Rödel and Bangoura 2004). From 2007 until his recent death, Kaman was a member of our Guinean team, investigating the amphibians of the Nimba Mountains and other Guinean areas. Kaman had outstanding skills in detecting and catching frogs, and, more importantly, an unswerving positive attitude. A day could be completely exhausting and frustrating, but with a simple joke from Kaman all was good again! Kaman was born and lived in a remote village at the western foothills of



Figure 11. *Conraua alleni* hiding on river bottom, the new species shows an identical behaviour when trying to escape.



Figure 12. Kaman Camara in June 2007 on Mount Nimba, Guinea. Inset figure taken on a Rapid Assessment to south-eastern Guinea, organised by Conservation International and Kaman’s first experience with frog work, from left to right: Mark-Oliver Rödel, Mohamed Alhassane Bangoura and Kaman Camara.

the Simandou Range. He never received any formal education. Still, he repeatedly rejected other better paying job offers from mining companies, preferring instead to work with his frog team whenever it was possible. Kaman died in June 2020 after a short severe disease. These frogs shall be a permanent memory to an outstanding person! We suggest ‘Kaman Camara’s Slippery Frog’ as the English common name, ‘la grenouille glissante de Kaman Camara’ in French and in the local language Poular: ‘Tôti bhowroundi de Kaman Camara’.

Discussion

Surveys in West Africa over the past 20 years have revealed previously unknown populations of the genus *Conraua* and prompted the need for re-evaluating the taxonomy of this genus, including exploring the potential for undescribed and morphologically-cryptic species (Rödel and Branch 2002; Rödel and Bangoura 2004; Hillers et al. 2009; Leaché et al. 2020). Based on comparative morphology and molecular phylogenetic data, we describe a new species, *Conraua kamancamarai* from three sites in the Fouta Djallon, Guinea, thus confirming the existence of an Unconfirmed Candidate Species (UCS) (*sensu* Vieites et al. 2009), as suggested by Blackburn et al. (2020).

The uncorrected p-distances of mitochondrial 16S between the new species and all other *Conraua* species was greater than 6% and, thus, well above the usual threshold (~ 3%) for potential anuran candidate species (Fouquet et al. 2007; Vieites et al. 2009). Although we had no bio-acoustic data available and individuals overlap in their body shape with those of *C. alleni*, these populations are diagnosable as a distinct species by various characters, including the absence of a divided palmar tubercle, having a first finger webbed to below the first subarticular tubercle and the unique ventral colour pattern in adults.

Conraua kamancamarai is the most westerly distributed *Conraua* species. Its closest congeners are found to the east, including the *C. alleni* complex from Guinea (east of the Fouta Djallon to lowland forests in western Ghana, west of the Atewa Forest Range), *C. sagyimase* in the Atewa Range Forest in Central Ghana and *C. derooi* along the escarpment of the montane border in western Ghana and eastern Togo. Similar patterns of distribution are also found in other pairs or groups of closely related vertebrates; for instance, toothed frogs (*Odontobatrachus*; Barej et al. 2015b), reed frogs (*Hyperolius*; Rödel et al. 2010; Channing and Rödel 2019), horseshoe bats (*Rhinolophus*; Fahr et al. 2002) and suckermouth catfishes (*Chiloglanis*; Schmidt et al. 2017).

Although the fauna of the Fouta Djallon has been poorly studied compared to other forests in West Africa (Rödel et al. 2021), studies of its flora have identified the region as a centre of endemism and species richness (Porembski et al. 1994; Poorter et al. 2004). These two metrics are commonly used to recognise areas that may have been

forest refugia (Hillers 2008; Tchouto et al. 2009), i.e. forests that persisted during cold and dry climatic periods (Haffer 1969; Couvreur et al. 2021). By investigating the phylogeography of several puddle frog species, *Phrynobatrachus*, Hillers (2008) confirmed the presence of macro- and micro-forest refugia in the highlands of Fouta Djallon during the Pleistocene. This finding, together with the results of Blackburn et al. (2020), which found the divergence of *C. alleni* into various lineages during the Pliocene and Pleistocene, suggests allopatric speciation, associated with isolation in Pleistocene forest refugia, led to the evolution of one or more endemic *Conraua* species in the Fouta Djallon. Pliocene–Pleistocene speciation events are well documented for other African forest taxa (e.g. rodents: Nicolas et al. 2010; Bohoussou et al. 2015; legumes: Duminil et al. 2013; bats: Hassanin et al. 2015).

Today the ecosystems of the Fouta Djallon are heavily degraded (Wilson 1992; Couch et al. 2019), threatening the area’s unique biodiversity (Schmidt 2014; Barej et al. 2015a) including this newly-described *Conraua* species. A preliminary assessment of the conservation status of vascular plants identified Guinea as amongst the top countries in tropical Africa with the highest proportion of threatened species (Stévant et al. 2019). In Fouta Djallon, there are still patches of forest (Wilson 1992) that seem sufficient to facilitate the survival of endemic frog species, such as the new *Conraua* or the endemic *Odontobatrachus* species (Barej et al. 2015b; this study). The conservation of preserved small areas can be key to the survival of regional biodiversity and these isolated forests that served as refugia in the past may also be more resistant to future climatic changes (Hillers 2008). *Conraua kamancamarai* occurs in classified forests (Konkouré Fetto and Binti classified forests), i.e. areas designated for sustainable management of forest resources (Brugiere and Kormos 2008). However, these forests have historically been given low priority, have been illegally degraded (Brugiere and Kormos 2008), being ineffective in mitigating the effects of anthropogenic intervention on vegetation change (Liu et al. 2017). A more rigorous protection of these forests and potentially upgrading of the conservation status of these forests would be important steps towards the preservation of the region’s biodiversity, as well as for the long-term maintenance of this important water source for local people. The Fouta Djallon is known as the “Water Tower” of West Africa, being the principal water supply for several countries in the region. For instance, Mauritania and Niger rely on this source for 96% and 70% of their water, respectively (Descroix et al. 2020).

With the description of *Conraua kamancamarai*, we continue to refine the taxonomy of the West African slippery frogs. However, there still remains work to be done. Further data and analyses are required to re-evaluate the larger *C. alleni* complex and to identify whether other populations in the Fouta Djallon, such as at Soyah (subclade 1a; see Blackburn et al. 2020), might represent additional undescribed species.

Acknowledgements

We thank Joseph Martinez (MCZ), Annemarie Ohler (MNHN), Danny Meirte (MRAC), Peter S. Miller (UWBM), as well as Claudia Koch, Dennis Rödder and Wolfgang Böhme (ZFMK) for providing access to voucher specimens under their care. Frank Tillack assisted with access to specimens and data at ZMB and took the photos of the vouchers of the new species. Amanda Quezada (Tropical Herping) helped with photo editing. Guillaume Demare assisted with the map. We particularly thank our assistants, Kaman Camara, Francois Gbê mou, Blaise Pivi and Ouou Kolié for their invaluable support in the field. We gratefully acknowledge the Guinean ‘Ministère en charge de l’Agriculture Élevage Environnement Eaux et Forêts, the ‘Ministère de l’Environnement et du Développement Durable’ and the ‘Ministère de l’Enseignement Supérieur et de la Recherche Scientifique’ for research, collection and export permits (Certificat d’Origine N° 00136, 00139, 00602, 0000189, 0002707, 0002712, 0003227, 0003255; 0003312, 0003314; n° 061, 072, 177/MERS/DNRST). Belén Ceollar and Virgile Lescure helped with the translation of the abstract into French. We thank Alan Channing, Václav Gvoždík and Werner Conradie for constructive feedback in their reviews of this manuscript.

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Appendix 1

Additional *Conraua* specimens examined for qualitative comparisons. MCZ = Museum of Comparative Zoology at Harvard University, Cambridge; MNHN = Muséum national d'Histoire naturelle, Paris; MRAC = Musée Royal de l'Afrique Centrale, Tervuren, Belgium; UWBM = Burke Museum of Natural History and Culture, Seattle; ZFMK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, ZMB = Museum für Naturkunde, Berlin.

***Conraua alleni* (sensu lato = all *Conraua* from the western Upper Guinea forest zone, morphologically assignable to *C. alleni*).** MCZ A-11991, subadult, holotype, Liberia, Firestone Plantation No. 3, Du River, Liberia; ZMB 90351, adult male, Guinea, Mont Béro Classified Forest, 08°08'30.9"N, 08°34'09.6"W; ZMB 90443, adult female, Sierra Leone, Tingi Hills, 08°53.4"N, 10°47.4"W; ZMB 90426, adult female, Sierra Leone, Loma Mountains Forest Reserve, 9°12.752"N, 11°08.623"W; ZMB 90178, adult female, Sierra Leone, Nimini Forest Reserve, 8°30.035"N, 11°08.800"W; ZMB 90388, ZMB 90389, adult males; ZMB 90179, adult female, Liberia, Putu Range, 05°39'44.6"N, 08°09'39.2"W, 306 m a.s.l.; ZMB 90304, adult male, Guinea, Fouta Djallon, 10°49'13.1"N, 12°11'30.7"W, 513 m a.s.l.; ZMB 90357, adult male and ZMB 90358, adult female, Ivory Coast, Haute Dodo, 04°59'14"N, 07°19'39"W; ZMB 90310, ZMB 90313, adult females, ZMB 90311, ZMB 90312, adult males, Guinea, Forêt Classée de Zياما, 8°21'02.7"N, 9°25'01.8"W, 467 m a.s.l.; ZMB 90325, adult female, ZMB 90326, adult male, Guinea, Nimba Range, 07°38'56.3"N, 8°25'25.56"W, 680 m a.s.l.; ZMB 90388, ZMB 90389, adult males, Liberia, Slabbert's Ville, 05°39'44.6"N, 08°09'39.2"W, 316 m a.s.l.; ZMB 90342, adult male, Liberia, Gola National Forest, 07°27'17"N, 10°41'52"W, 299 m a.s.l.

***Conraua beccarii*.** ZFMK 15749–15750, Ethiopia, Il-lubator; MNHN 1933. 21, adult male, holotype of *Rana Griaulei* Angel, 1934, Ethiopia, Gondar, 2,200 m a.s.l.

***Conraua crassipes*.** ZMB 8360, holotype, "Abo", north of Douala, Cameroon; ZFMK 73216, Gabon,

Barrage de Kinguélé, Tchimbélé; ZFMK 69351, 69353, 69354, Cameroon, Mt. Nlonako, Nguengue, Campsite; ZMB 90400–90403, Gabon, Moukalaba-Doudou National Park.

***Conraua derooi*.** MRAC 112077–112078, paratypes, Togo, Missahohe; MRAC 112079–112080, paratypes, Togo, Missahohe; ZMB 71293, adult male and ZMB 71294, adult female, Ghana, Biakpa, 06°50.652"N, 00°25.280"E; ZMB 71298–71300, ZMB 71302 adult males and ZMB 71301, adult female, Togo, Missahohe, 6°57.094"N, 0°33.878"E; UWBM:Herp 09599–09603, adult females and UWBM:Herp 09604, adult male, Ghana, Volta Region, Adaklu-Anyigbe; MNHN 1978.2027, 1978.2029, adult males and MNHN 1978.2030, 1978.2031, adult females and MNHN 1978.2028, adult with unknown sex, Togo, Dangi Atiba; MNHN 1993.2627, 1993.2629, adult males and MNHN 1993.2630, 1993.2631, adult females and MNHN 1993.2628 adult with unknown sex, Togo, Klotto; MNHN 1993.4084–1993.4087, Togo, Missahohe; MNHN 1995.5726, 1995.5727, Togo, Kluto; MNHN 1987.2026, Togo, Dangi Atigba.

***Conraua goliath*.** ZFMK 77927, 77928, 77930, 77932, Cameroon, Mt. Nlonako, Ekomtolo, 500 m a.s.l.

***Conraua robusta*.** ZMB 20085, holotype, Cameroon; ZMB 78427, Cameroon; ZMB 90174, Cameroon, Manengouba Village, Mt. Manengouba; ZFMK 67288, Cameroon, Bakossi Mts., Kodmin.

***Conraua sagyimase*.** UWBM:Herp 5839, holotype, adult male, Ghana, Eastern Region, Atewa Range Forest Reserve, 06°13'57.79"N, 0°33'07.08"W, 633 m a.s.l.; ZMB 91136, paratype, adult female, Ghana, Asiakwa South, 06°15'44.3"N, 0°33'18.8"W, 783 m a.s.l.; ZMB 91137–91138, paratypes, adult females, Asiakwa North, 06°16'16.1"N, 0°33'52.7"W, 814 m a.s.l.; UWBM:Herp 5840–5843, paratypes, adult male and female and two subadults, Ghana, Eastern Region, Atewa Range Forest Reserve, 06°13'57.79"N, 0°33'07.08"W, 633 m a.s.l.

Appendix 2

***Conraua* specimens examined for quantitative comparisons.** ZMB = Museum für Naturkunde, Berlin.

***Conraua* sp. from Soya, Fouta Djallon.** ZMB 90341, ZMB 90175, ZMB 90176, adult males, Guinea, Soya, 10°17'50.9"N, 11°56'32.4"W, 515 m a.s.l.

Conraua alleni. ZMB 90310, ZMB 90313, adult females, ZMB 90311, ZMB 90312, adult males, Guinea, Forêt Classée de Ziama, 8°21'02.7"N, 9°25'01.8"W, 467 m a.s.l.; ZMB 90325, adult female, ZMB 90326, adult male, Guin-

ea, Nimba Range, 07°38'56.3"N, 8°25'25.56"W, 680 m a.s.l.; ZMB 90388, ZMB 90389, adult males, ZMB 90390, adult female, Liberia, Slabbert's Ville, 05°39'44.6"N, 8°09'39.2"W, 316 m a.s.l.; ZMB 90427, ZMB 90428, adult males, Guinea, Nimba Range, 07°42'82"N, 8°21'70"W, 848 m a.s.l.; ZMB 90342; adult male, Liberia, Gola National Forest, 07°27'17"N, 10°41'52"W, 299 m a.s.l.; ZMB 90350, adult female, Guinea, Mont Béro Classified Forest, 08°08'30.9"N, 08°34'09.6"W, 622 m a.s.l.

A historical specimen of the Fishing Cat, *Prionailurus viverrinus* (Bennett, 1833) (Carnivora, Felidae) from Singapore in the zoological collection of the Naturalis Biodiversity Center, Leiden

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<http://zoobank.org/E7A90541-F0BE-45BF-A689-91B8B93F732A>

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Academic editor: Melissa TR Hawkins ♦ Received 23 October 2021 ♦ Accepted 4 January 2022 ♦ Published 21 January 2022

Abstract

The Fishing Cat is not a species known to inhabit Singapore. However, a historical specimen stated to have come from Singapore in 1819 and attributed to Pierre-Médard Diard (RMNH.MAM.59688) is now housed at Naturalis Biodiversity Center, Leiden, the Netherlands. Two hundred years after it was obtained, the mounted skin and skull of this specimen, including specimen labels, were photographed and digitally catalogued. Four sets of annotations from labels and a document detailing records and a receipt of specimens sent by Diard to Leiden are presented to ascertain the specimen's identity, followed by a historical account of Diard based on a reconstruction of the timeline of key events of Singapore's natural history. Subsequently, the specimen is examined to confirm its taxonomic identity using comparative morphometrics with other museum specimens, and data associated with the specimen are analysed to determine the origins of this specimen. We conclude that the current evidence does not allow confirmation of the specimen's status as having been collected in Singapore or being obtained from the pet trade. If the specimen was an imported specimen, it would point towards a trade in rare and large animals in Singapore and the region from as early as 1819. Presently, the specimen remains one of the few extant zoological specimens obtained in Singapore in 1819 and the only one currently known outside of England.

Key Words

digital repatriation, *Felis leucojalamus*, first Singapore expedition, history of mammalogy, Pierre-Médard Diard, Stamford Raffles, zoological collection

Introduction

The Fishing Cat, *Prionailurus viverrinus* (Bennett, 1833) is a medium-sized, nocturnal species of the family Felidae. Its natural distribution is still unclear but current known occurrence records suggest that this species is widely distributed in South and Southeast Asia from Pakistan in the west to Cambodia in the east, and from the Himalayan foothills in the north to Sri Lanka and peninsular Thailand in the south (Mukherjee et al. 2016). Based

on museum records, the species has also been reported from the Malay Peninsula, Java and possibly Sumatra (e.g., Blandford 1888–1891; Jentink 1892; Brongersma 1935). These records from the Malay Peninsula that were based solely on museum specimens have generally been assumed to have been the result of mislabelling, misidentification or material obtained from the pet trade (Duckworth et al. 2009).

The Fishing Cat is absent from present-day Singapore and the origins of a museum specimen from Singapore

housed in the Naturalis Biodiversity Center of the Netherlands (RMNH) need careful investigation. The first mention of the Naturalis specimen was in Jentink (1887: 83), then again by Jentink (1892: 99) and subsequently by Brongersma (1935: 13). This record was mostly overlooked until van Bree and Khan (1992: 80), citing Brongersma (1935) wrote, “[i]n the National Natural History Museum at Leiden, the Netherlands, there is a Fishing Cat collected by P. Diard in Singapore in 1819”. Over a decade later, Duckworth et al. (2009: 7) referred to this same specimen but in reference to Malacca “c.1820s [date inferred from the collector’s identity: Diard], RMNH”. The authors further stated that the specimen is “presumably the Malacca specimen(s) examined by Swinhoe (1862)” which suggests that they may not have known that there were labels associated with the Singapore specimen and were only aware of the collector, leading them to infer the date and locality. The origin of this Singapore specimen of the Fishing Cat in the RMNH therefore, has not been resolved.

As a follow-up study on the historical account of two French naturalists Diard and Duvaucel who collected with Raffles (see below), researchers from Singapore’s Lee Kong Chian Natural History Museum (LKCNHM), and the Netherlands’ Naturalis Biodiversity Center, Leiden (RMNH) searched through zoological collections in RMNH in November–December 2019 to locate and catalogue, amongst others, the Fishing Cat specimen. This work aims to confirm the taxonomic identity of the Fishing Cat specimen and reconstruct the history of this specimen based on a timeline of key events in the early natural history of Singapore to provide a better understanding of the original fauna of Singapore.

The first Singapore Expedition (May–June 1819)

Two French naturalists, Pierre-Médard Diard (1795–1863) and Alfred Duvaucel (1793–1824), who were employed by Sir Thomas Stamford Bingley Raffles (1781–1826), were among the earliest collectors of zoological material from Singapore and first visited Singapore in early 1819 with much of their collecting taking place between 31 May to 28 June 1819 (Pilon and Weiler 2011; Low 2019; Low et al. 2019; Weiler 2019; Low 2021 in press; Low et al. 2021).

During the four-week period in May and June 1819, which may be referred to as the first Singapore expedition, Diard and Duvaucel appear to have collected the majority of zoological specimens (Low 2021 in press; Low et al. 2021). The expedition resulted in many firsts from Singapore (in the scientific sense): the collection of the first mammal to be recorded, the Dugong (*Dugong dugon* (Müller, 1776)), the earliest natural history illustration (of a Spiny Turtle (*Heosemys spinosa* (Gray, 1831))), the first

mammal to be named, the Cream-coloured Giant Squirrel (*Ratufa affinis* (Raffles, 1821)), the first primate to be described, the Banded Leaf Monkey (*Presbytis femoralis* (Martin, 1838)), and the first bird to be named, the Green Broadbill (*Calyptomena viridis* Raffles in Horsfield, 1822) (Low 2019; 2021, in press; Low et al. 2021).

Only two specimens from this 1819 collection are currently known to be extant: the Banded Leaf Monkey, *Presbytis femoralis* (Martin, 1838), and the Green Broadbill, *Calyptomena viridis* Raffles in Horsfield, 1822 (Napier 1985; Wells and Dickinson 2010; Wells 2013; Low and Lim 2015). It is also known that material collected from Singapore in 1819 also resides in other collections (Noltie 2009) and recent research has shown that Raffles gave 21 bird specimens from Sumatra to Lord Edward Smith Stanley (Wilson 2021). There is therefore the possibility that further research may uncover material collected from Singapore in 1819 in other collections in England or elsewhere.

In March 1820, the two French naturalists parted ways with Raffles after a falling out and the vast majority of their collections were seized by Raffles; the French naturalists retained specimens for which there were triplicates (Raffles 1821: 239, 240; Raffles 1830: 713; Burkill 1916; Bastin 2019: 288; Weiler 2019; Low 2021 in press). The duplicates were sent on the *Mary* and used by Raffles in his *Descriptive Catalogue* (Raffles 1821; Raffles 1822) and in Horsfield’s *Zoological Researches in Java* (Horsfield 1821–1824). The primary set of material was retained with Raffles in Bencoolen (Bengkulu) but was lost when the *Fame* burned and sank in 1824 (Noltie 2009: 9–13; Wilson 2021: 40, 41).

Raffles explicitly stated in his *Descriptive Catalogue* (Raffles 1821: 249), that only two species of the genus *Felis* were in his collection (which included material from the first Singapore expedition): “the Royal Tiger, and a species of Tiger-cat” identifying the latter as *Felis bengalensis* (now under *Prionailurus*). Following the death of Raffles in 1826, his wife, Lady Sofia Raffles, published his memoirs with an appendix entitled *Catalogue of Zoological Specimens* by Vigors and Horsfield (Raffles 1830: 633–697) detailing the material collected in the two months following the loss of the *Fame* (Noltie 2009: 9–13; Wilson 2021: 40). No species of cat from Singapore is listed in this appendix or in the *Zoological Researches of Java* (Horsfield 1821–1824; Raffles 1830: 636–637).

The context of the RMNH Fishing Cat specimen

Felis leucojalamus: a nomen nudum

In 1887, Fredericus Anna Jentink (1844–1913) listed the species name, *Felis leucojalamus* in the synonymy of *Felis viverrina* Bennett, 1833 in his first catalogue of

the mammal collections (“Catalogue Ostéologique des Mammifères”) deposited at the National Museum of Natural History (RMNH) in Leiden (i.e., Jentink 1887: 83). Jentink was a curator of vertebrates at RMNH before being appointed as the museum’s director in 1884 (Holthuis 1995: 77).

Jentink (1887: 83) listed Diard’s specimen as specimen “b” of *F. viverrina*: “Crâne d’un individu adulte. Singapore. Diard, 1819. *Felis leucojalamus* Diard, espèce inédite” (Translation: “Skull of an adult individual. Singapore. Diard, 1819. *Felis leucojalamus* Diard, unpublished species”). In his later systematic catalogue, Jentink (1892: 99) retained the same specimen under *F. viverrina* with the following remarks: “Individu adulte monté. Singapore. Des collections de M. Diard, 1819. *Felis leucojalamus* Diard, MS. (Cr. b du Cat. Ost.)” (Translation: “Adult individual mounted. Singapore. From the collections of M. Diard, 1819. *Felis leucojalamus* Diard, MS. (Cr. [= Crâne] b du Cat. Ost. [= *Catalogue Ostéologique des Mammifères*, i.e., Jentink 1887])”). The skull is presently catalogued as RMNH.MAM.59688.a (Fig. 1A–D) while the mounted skin is catalogued as RMNH.MAM.59688.b (Fig. 1E) (also see Table 1).

Table 1. List of specimens originating from Diard with “Singapore” given as the source locality in RMNH. Source of information for donor and/or donation date from Naturalis Biodiversity Center, Leiden, the Netherlands (<https://bioportal.naturalis.nl/>).

Museum Registration Number	Taxon	Date [†]	Identity of material is based on source of material of information [§]
RMNH.AVES.127062	Ave	1858	<i>Chloropsis cyanopogon</i> (Temminck, 1829); <i>Chloropsis cyanopogon</i> Oates
RMNH.AVES.147120	Ave	1858	<i>Malacopteron magnirostre</i> (Moore, 1854); <i>Malacopteron magnirostre</i> (Moore)
RMNH.AVES.170553	Ave	1858	<i>Merula obscura</i> ; <i>Turdus obscurus</i> Gmelin, J.F., 1879
RMNH.AVES.193050	Ave	1858	<i>Micrastur semitorquatus</i> (Vieillot); <i>Micrastur semitorquatus</i>
RMNH.AVES.196738	Ave	1858	<i>Eurystomus orientalis</i> (Linnaeus); <i>Eurystomus orientalis deignani</i>
RMNH.AVES.198076	Ave	1858	<i>Buceros Rhinoceros Rhinoceros</i> Linnaeus; <i>Buceros Rhinoceros Rhinoceros</i>
RMNH.AVES.199028	Ave	1858	<i>Eurostopodus temminckii</i> (Gould); <i>Eurostopodus temminckii</i>
RMNH.AVES.200215	Ave	1858	<i>Calorhamphus fuliginosus hayii</i> (J.E. Gray); <i>Calorhamphus fuliginosus hayii</i>
RMNH.AVES.200551	Ave	1858	<i>Harpactes diardii sumatranus</i> Blasius; <i>Harpactes diardii sumatranus</i>
RMNH.AVES.202561	Ave	1858	<i>Picus mentalis humii</i> (Hargitt); <i>Picus mentalis humii</i>
RMNH.MAM.39155.a	Mammal	1859	<i>Presbytis femoralis</i> (Martin, 1838);
RMNH.MAM.39155.b	Mammal	1869	<i>Semnopithecus neglectus</i> Schlegel, 1876
RMNH.MAM.39156.a	Mammal	1858	<i>Ratufa bicolor</i> (Sparrman, 1778);
RMNH.MAM.53009.a	Mammal	1858	<i>Sciurus albiceps</i> Desmarest
RMNH.MAM.53009.b	Mammal	1819	<i>Felis viverrina</i> ; <i>Felis leucojalamus</i> ;
RMNH.MAM.59688.a	Mammal	1819	<i>Prionailurus viverrinus</i> (Bennett, 1833)
RMNH.MAM.59688.b	Mammal	1819	<i>Prionailurus viverrinus</i> (Bennett, 1833)

[§]Source: Naturalis – Zoology and Geology catalogues. Note that species identification follows original species description, specimen label and/or currently accepted name.

[†]Date is based on collection label date or museum accession record year.

Over four decades later, Leo Daniel Brongersma (1907–1994), a Dutch zoologist and, from 1958, director of RMNH, re-examined the fossil and recent felid material from the Malay Archipelago in RMNH (Brongersma 1935). He referred *F. viverrina* to its currently accepted generic combination of *Prionailurus viverrinus* (Bennett, 1833) and gave the following remarks: “1 ex., ‘Singapore’, leg. Diard, cat. syst.: b, mounted, cat. ost.: b, skull” (i.e., specimen *b* of Jentink’s catalogue, i.e., Jentink 1887) (Brongersma 1935: p. 13). Brongersma (1935: 13) further stated that “[o]ur Museum possesses a specimen labelled ‘Singapore’, but as the species has not been recorded from that island in recent times (it is not mentioned by Chasen (1924, 1925) this locality-record seems extremely doubtful to me”. Brongersma (1935: 13) was referring to Chasen’s preliminary account of mammals in Singapore that were published in two parts. In his conclusion, Chasen (1925: 87–88) made several remarks on species for which he considered “very doubtful records” which was followed by a list of species for which confirmation of local status was needed (while also taking the possibility of some species being imported into Singapore into account), and finally he provided a list of species of what he called “the true Singapore land fauna”. The Fishing Cat (either as *Felis* or *Prionailurus viverrinus*) was not listed or discussed by Chasen (1924; 1925).

RMNH.MAM.59688 is the specimen *b* of Jentink and Brongersma

The following four sets of annotations accompany the specimen. The annotations are based on three labels found in the box labelled “cat. ost. b” (i.e., specimen *b* of the “Catalogue ostéologique des Mammifères”, i.e., Jentink 1887; Fig. 1D) with a skull specimen in it and one label pasted on the pedestal of the mounted skin (Fig. 2A–D):

1. Handwritten label pasted on a piece of wood (Fig. 2A). The French text is presumed to be in Diard’s handwriting because it bears a close resemblance to a letter written by Diard while he was in Singapore (see Tham et al. 2019: 163). While most of the text is easy to read, there are some parts which have become illegible. Also, we suggest that due to variation of spellings, the specific name on the label (the first line) could be interpreted in three different ways. We suggest the following transcription:

Interpretation 1:
Felis leucojalamus
Diard

Interpretation 2:
Felis leucojalamis/ Felis leucopalms
Diard

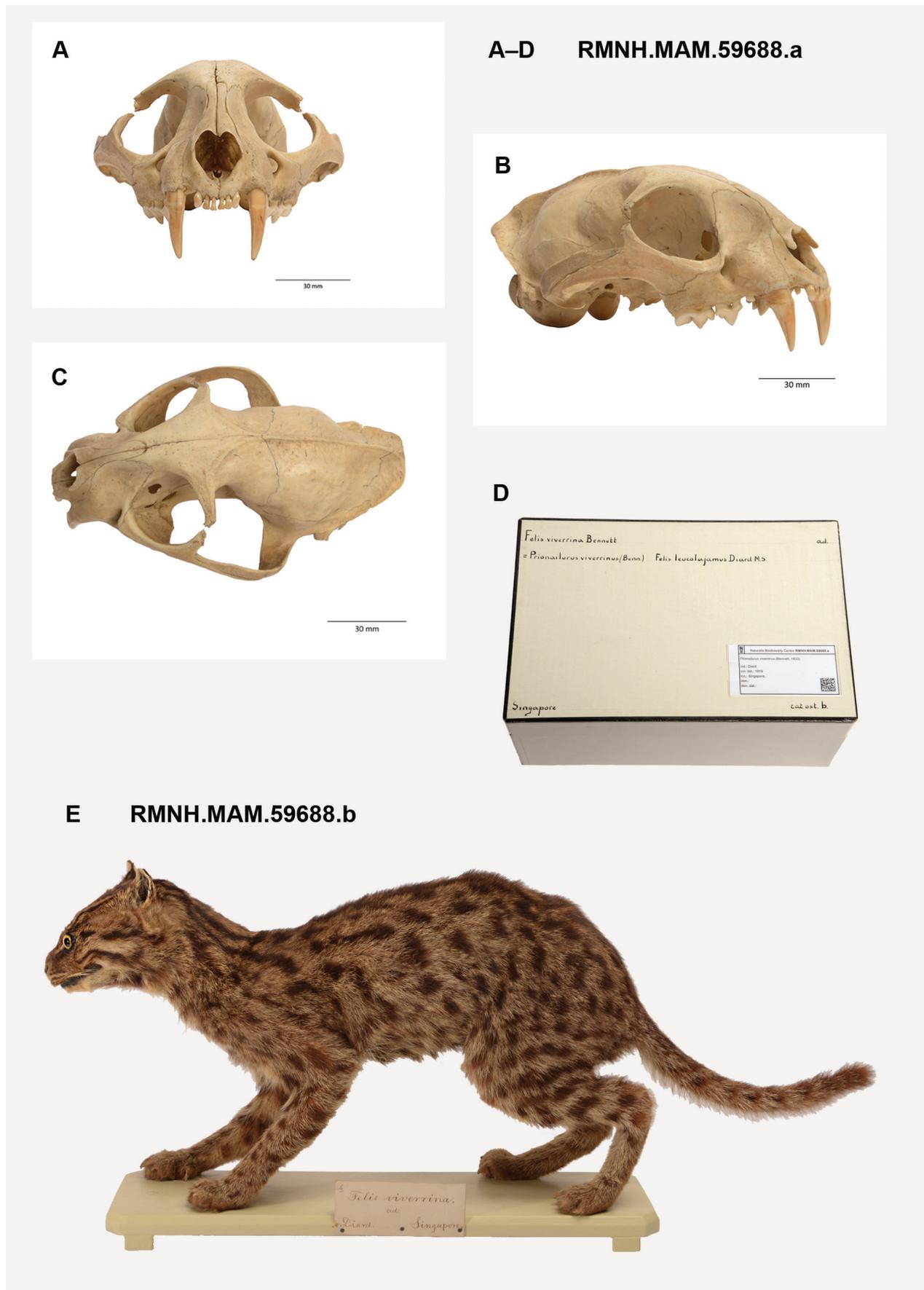


Figure 1. A specimen of *Prionailurus viverrinus* RMNH.MAM.59688, referred to as specimen *b* of *Felis viverrina* in Jentink's two *Catalogues* of 1887 and 1892. **A–C.** The skull from three different angles; **D.** A specimen box containing the skull; **E.** The mounted skin.

Interpretation 3:
Felis bengalensis
Diard

The third and fourth lines are:
[illegible] déterminé en 1819 à Singapour
elle la trouve aussi en Cochinchine et au Cambodge

The phrases “*Felis leucojalampus*”, “Diard”, “1819” and “Singapore” appear in Jentink’s and Brongersma catalogue. It is possible that Jentink may only have glanced at the label and thought that Diard had intended to propose a new name for a “espèce inédite” (translation: unpublished species) from Singapore. As seen from his catalogue entry, Jentink (1887) read “*Felis leucojalampus*”, attributing the name to Diard (interpretation 1). However, after enhancing the image of the label to clarify the handwriting, it clearly shows that the last character of the epithet is an “i”, thus it should read “*leucojalamis*” (interpretation 2). Nevertheless, the specific epithet “*leucojalampus*” is incomprehensible: “*leuco*” is derived from the Greek leukós, meaning “white, bright”, the second part “*jalampus*” or “*jalamis*” is not found in either Latin or Greek. Furthermore, neither Jentink nor Brongersma mentioned the last line of the note (in translation): “it is also found in Cochinchina and Cambodia”.

More details emerged from Natuurkundige Commissie Archives Online (NCO: <https://dh.brill.com/nco>) published by Gasso et al. (2020), which showcases specimens, drawings and illustrations as well as documents, such as field books, notes, shipping lists, and correspondence of members of *Natuurkundige Commissie voor Nederlandsch-Indië*. From NCO, we discovered a two-page document of shipment sent by Diard to Leiden in March 1830 (Fig. 3A, B). The document listed a specimen of “*Felis leucopalmis du détroit de Singapour*” (translated: “*Felis leucopalmis* from the Strait of Singapore”). We assumed that Schlegel interpreted the name based on Diard’s label of the Singapore’s specimen of Fishing Cat that was probably already hard to read at the time. In the same shipment as the Singapore’s Fishing Cat were specimens from Borneo, Java, Cambodia, Malacca and Sumatra, including a *nomen nudum Felis strepsilura* from Java (now *Prionailurus planiceps*; interestingly another wild cat adapted to hunt aquatic prey, but not presently known from Java).

The third interpretation of the specific name is transcribed as “*bengalensis*” because Diard seemed consistent in writing certain characters such as “i” (with a striking dot) and “s” as in “is” which suggests that the second element after the word “*Felis*” should be read as “*bengalensis*” with a cursive “b” (Fig. 2A). Another possible explanation for “*bengalensis*” is perhaps the specimen was part of triplicates that were allowed to be retained by Diard and Duvacel following the separation with Raffles (as discussed above). *Felis bengalensis* in 1819 was still the only cat of similar description known in South and South-East Asia.

The illegible word or phrase before “déterminé” probably consists of five characters, which could read “Crâne” (translation: skull) or “jeune” (translation: young). The elements “Diard”, “1819” and “Singapore” are repeated in Jentink’s and Brongersma’s catalogues.

In addition, there is an inscription in (possibly) Jentink’s handwriting written on the wood: “*b Felis viverrina*,”

2. The second label is in possibly Jentink or Schlegel’s handwriting showing catalogue number, species name, year, and locality (Fig. 2B). Note that locality name “Singapore” is spelt in English:

^b *Felis viverrina*.
ad:
1819 Singapore

3. The third label is the most recent of all available labels written on RMNH’s printed card (’sRijks Museum v. Nat. Hist. LEIDEN) by an unknown writer post-1900 which agrees with Brongersma’s information on in Brongersma (1935: 13) (Fig. 2C):

Prionailurus viverrinus (Benn.) Cat. n°. b
Felis leucojalampus Diard MS. Sex: -
hoort bij opgezette ex.: b (translation: belongs to mounted specimen: b)
Dat: 1819 leg. Diard
Loc: Singapore

4. The fourth label pasted on the pedestal (Fig. 2D) could be one of the earliest preliminary identifications of this specimen, but the information is not captured in any of the catalogues. The label had the following information written in the style Temminck used, but it is not written by him (compared with the other three labels), with *F. viverrina* as a species name and a reference of author was attributed to “Hardw.” (Hardwicke) followed by a publication name, “Ind : Zool:” (= “Illustrations of Indian Zoology”, Gray 1832–1835), “Diard” as collector and “Singapoer”:

Felis viverrina, Hardw.
Ind: Zool.
voy: Diard. Singapoer.

In addition, the pedestal bears the following inscriptions: “849” (written in ink), “314”, and “KOP” (written in pencil, means head or skull) (see Fig. 2D, arrowed), but we were not able to determine the meaning of these numbers or letters.

The reference to “Hardw.” is to Thomas Hardwicke (1756–1835) who figured the “Viverrine Cat” (with the scientific name erroneously spelled as “*Felis vivirinus*”) in the “Illustrations of Indian Zoology” (hereafter the “Illustrations”, Gray 1832–1835) which is sometimes



Figure 2. Three labels found in the box labelled “cat. ost. b” (i.e., specimen *b* of the “*Catalogue ostéologique des Mammifères*”, i.e., Jentink 1887) with a skull specimen in it (A–C) and a label pasted on the pedestal of the mounted skin (D).

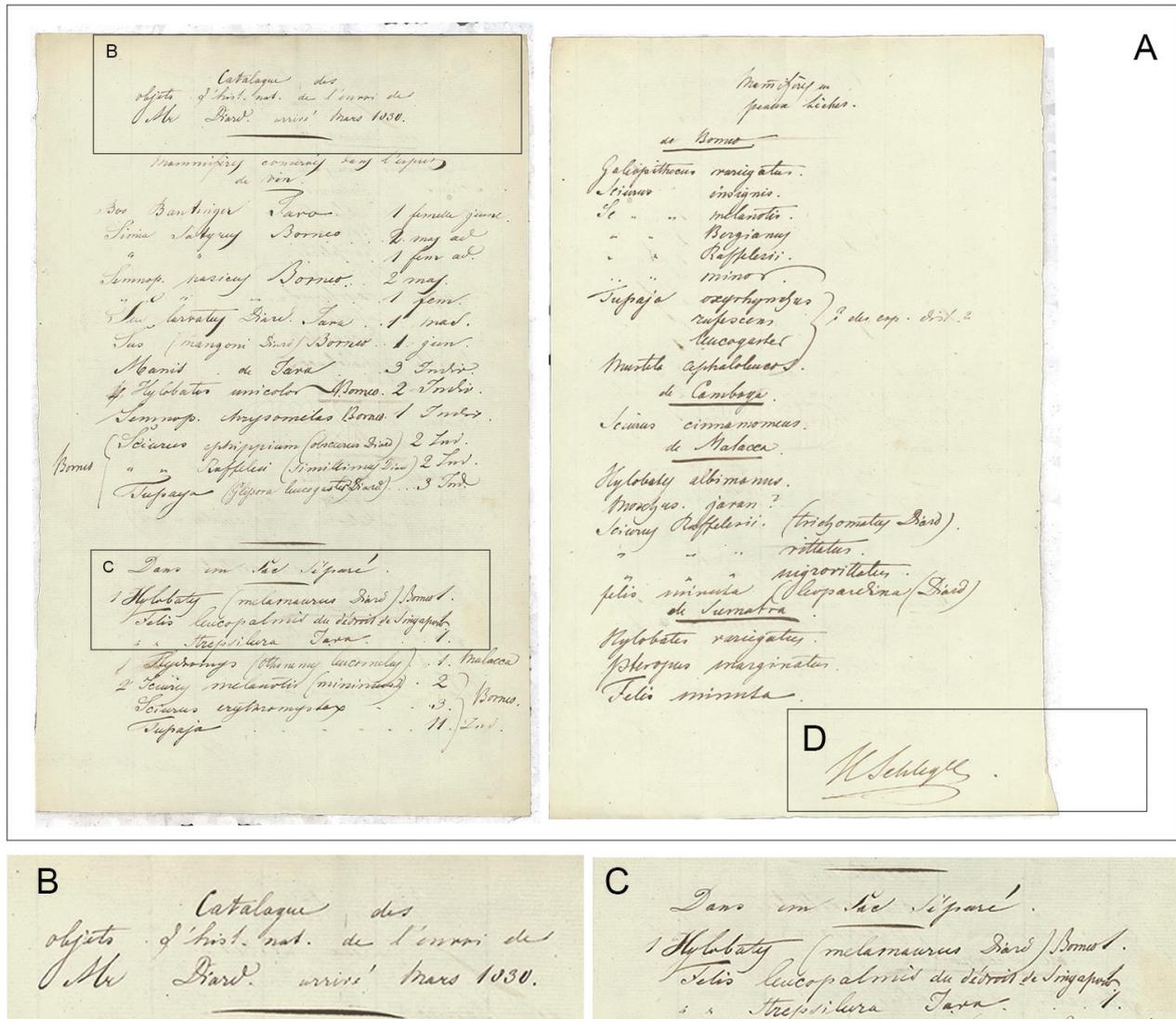


Figure 3. A two-page document listing records of specimens sent by Diard to Leiden in March 1830 (A) with the upper part of the first page (B), shows a title of the document “Catalogue des objets d’hist. nat. de l’envoi de Mr Diard arrivé mars 1830”, while the bottom part of the page (C), shows “*Felis leucopalms* du détroit de Singapour” (translated: *Felis leucopalms* from the Strait of Singapore) was among the specimens sent by Diard; at the bottom part of the second page, the document was signed by H. Schlegel (D). Reproduced from Natuurkundige Commissie Archives Online (Gasso et al. 2020)

attributed to both Hardwicke and John Edward Gray (1800–1875) (see Dawson 1946; Wheeler 1998). The plate (Fig. 4) depicting the “Viverine Cat” is the fourth Mammalia plate in the second part of the “Illustrations” (Gray 1832–1834) which was published between 10 September and 1 October 1834 (Wheeler 1998: 347, 351).

Although some of the material depicted in the “Illustrations” was stated to have come from Singapore (Low and Pocklington 2019: 156–159), the “Viverine Cat” is not one of them. The reference to Hardwicke on the label was probably an ‘update’ to the identity of the specimen when it was later found to be conspecific with the species figured in the “Illustrations”.

But was the specimen RMNH.MAM.59688 that of a Fishing Cat or a misidentified closely related but smaller Leopard Cat, *Prionailurus bengalensis* (Kerr, 1792) that is native to Singapore and Malacca?

RMNH.MAM.59688 is a Fishing Cat

We examined specimens of six Fishing Cats and nine Leopard Cats from the Malay Peninsula from the RMNH and the Zoological Reference Collection of Lee Kong Chian Natural History Museum, National University of Singapore, ZRC; see Suppl. material 1) and compared them with RMNH.MAM.59688 (the Diard specimen). Although no measurements of the live animal are associated with specimen RMNH.MAM.59688 or its labels, the skin and skull provide characters to identify the cat in question (Fig. 1).

We took the following measurements using callipers: i) greatest length of skull from premaxilla to occipital (GL), ii) condylobasal length from back of occipital condyle to premaxilla (CBL), iii) condylocanine length from back of occipital condyle to front of the canine (CCL),



Figure 4. The plate of “Viverine Cat” in the second volume of the “*Illustrations*” (Gray 1832–1834) which was published between 10 September and 1 October 1834. Reproduced from the Biodiversity Heritage Library (<http://biodiversitylibrary.com/>).

iv) maxillary toothrow length (MT), v) zygomatic width (ZW), vi) height of canine from base to tip (C1H), vii) greatest length of bullae (BL), and viii) greatest width of bullae (BW). One missing data field owing to a broken zygoma was filled with the mean for the species. A principal component analysis (PCA) was performed for the eight cranial measurements transformed to a log scale (Meijaard et al. 2017; Jackson et al. 2021). Statistical analyses were conducted in RStudio 1.1.463 (RStudio, Inc.) using the `prcomp` operation.

The greatest length of the skull RMNH.MAM.59688.a at 13.4 cm is within the range of Fishing Cat skulls (Pocock 1939), and far exceeds the mean length of Leopard Cat skulls (9.01 cm; range: 8.19–9.55 cm; $n = 9$) from the Malay Peninsula. The Diard specimen is likely a young adult as the skull’s basilar suture has not fused. The PCA revealed that the Diard specimen grouped with Fishing Cats, which were distinctly separated from Leopard Cats from the Malay Peninsula (Fig. 5). In addition, the specimen grouped more closely with Javan Fishing Cat specimens rather than those of Indochina along the PC2 axis. Components 1 and 2 account for 98.6% of the total variance explained (see Suppl. material 1). In addition, the dark spots on the RMNH.MAM.59688 specimen are large and solid (Fig. 1E), which differs from the “shaded” spots of Leopard Cats on mainland Southeast Asia, which are always lighter anteriorly and darker posteriorly (Groves 1997). Further, the proportion of its tail, which is less than half the head and body length is much shorter than that of

the mainland Leopard Cat (Pocock 1939; Groves 1997). These characters help determine that the specimen is *P. viverrinus* and not a misidentified Leopard Cat.

With the species identity certain, and given that the place and date of acquisition coincide with Diard being in Singapore during his visits to Singapore in 1819 (as discussed above), how then did a specimen collected by Diard end up in RMNH?

The Singapore collections and Diard’s collecting post-1819

To date, apart from the Fishing Cat specimen, all the zoological specimens collected in 1819 that are still extant are currently thought to be in the collections of what is presently known as the Natural History Museum in London (NHMUK) (as discussed above). This material could have entered the collections of the NHMUK from two possible sources. Some material was sent back to the Honourable East India Company’s (HEIC) museum in London and was later transferred to the NHMUK after the HEIC museum disbanded in 1858 (Ratcliff 2016). The other possible route was via the material sent back by Raffles that formed the basis of the museum of the Zoological Society of London (Wheeler 1997).

It is also known that Diard and Duvaucel sent material to the Muséum national d’Histoire naturelle in Paris

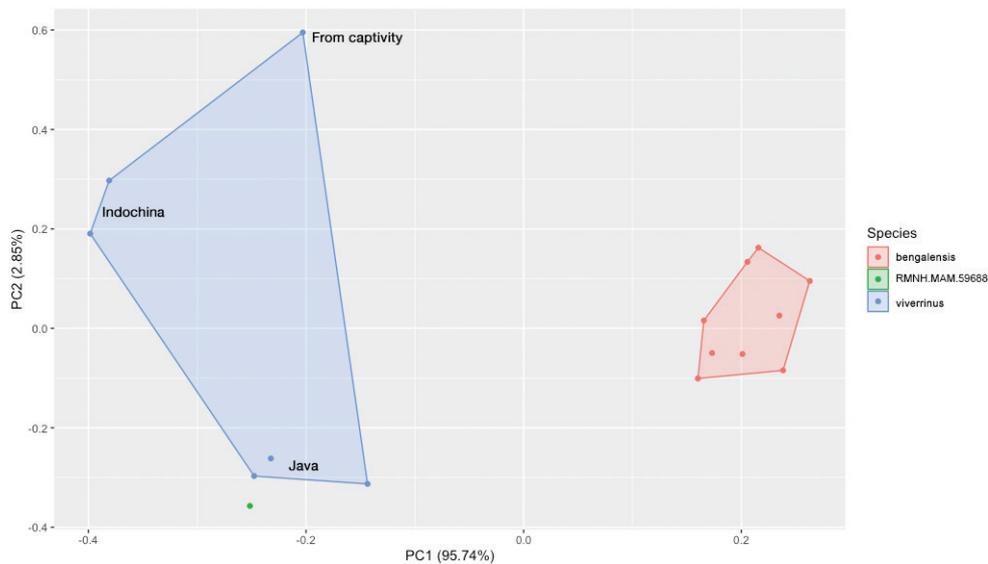


Figure 5. The PCA revealed that the Diard specimen (RMNH.MAM.59688) grouped with Fishing Cats, which were distinctly separated from Leopard Cats from the Malay Peninsula. The specimen grouped more closely with Javan Fishing Cat specimens rather than those of Indochina along the PC2 axis.

(MNHN) in 1820 (Weiler 2019). However, no material from Singapore dating from 1819 is known in the MNHN (Low et al. 2019).

Following the separation with Raffles, Diard and Duvaucel also parted ways and Diard travelled to several places in Southeast Asia (including what is today Vietnam) for 26 years before returning to Europe in 1843, and then once again returning to Southeast Asia in 1848 (Weiler 2019). At this time, Diard was also employed as a member of “Natuurkundige Commissie voor Nederlandsch-Indië” (Committee for Natural History of the Netherlands Indies, hereafter the Committee) (Weber 2019). The Committee’s specimens arrived in Europe in 1830 and at that time RMNH regularly exchanged items from Asia with other natural history museums in Europe (Weber 2019).

From the RMNH’s public database, BioPortal (<https://bioportal.naturalis.nl>, accessed on 13 September 2021) and accession records, over 800 specimens were collected or donated by Diard (while he was still alive and some posthumously) from various places ranging from as early as 1818 to as late as 1869, of which 20% are mammals. For the same period, the database contains 19 specimens (representing 13 species) from Singapore associated with Diard of which the 1819 Fishing Cat specimen appears to be the oldest. Other Diard specimens were acquired by the RMNH in 1858 (viz., eleven specimens of birds and a squirrel) either collected and/or donated by Diard himself for the Committee (Table 1). Three specimens of *Presbytis femoralis* (Martin, 1838) from Singapore (identified as *Semnopithecus neglectus* Schlegel, 1876) with Diard as collector were mentioned in Jentink’s 1892 catalogue (Jentink 1892: 12–13). In the same catalogue, the two specimens of this species that Jentink referred to as specimens *a* and *c*, were dated in 1869 which suggests that they were donated posthumously while specimen *b*

was accessioned in 1859 and could have been donated to RMNH by Diard (Table 1).

In around 1854, Diard was appointed as director of the botanical gardens at Buitenzorg (now Bogor) in the West Java province in which during his tenure, he continued collecting specimens at several places including Ceylon (now Sri Lanka) and sending his collections back to MNHN (Weiler 2019: 40). Diard died on 16 February 1863.

Conclusions

This study concludes that: (1) the Diard specimen is indeed a Fishing Cat, *Prionailurus viverrinus* (Bennett, 1833); (2) that the label was almost certainly written by Diard himself and that the Singapore locality was intended; (3) the 1819 date when it was obtained coincides with Diard’s visits to Singapore. It is interesting to note that the handwritten label indicated that the *Felis leucojalama/leucopalmis/bengalensis* can be found in two other places, Cochinchina (now the southern part of Vietnam) and Cambodia which were part of his later travel routes. Diard’s trip to these two places commenced between 1821 and 1822 which means there was a gap of at least two years after the time spent in Singapore in 1819. The inclusion of Cochinchina and Cambodia on the label suggests that Diard wrote this label after he returned from those places but that the specimen had been obtained in Singapore in 1819. However, we are not able to conclusively determine if Diard collected the cat himself in Singapore or if he purchased a specimen that was transported to Singapore.

RMNH.MAM.59688 was accessioned at the RMNH in March 1830 as evidenced by a document signed by H. Schlegel (Fig. 3) which explains how this specimen

of Fishing Cat was brought to Europe from Singapore. However, further analysis will be required to determine how the 1819 specimen evaded Raffles's seizure of Diard's collections given the value of this animal, although it could be postulated that Diard might have hidden it or that it was part of triplicates of the Singapore first expedition. A study of Diard's correspondence with his brother and relevant papers in the archives of the MNHN or RMNH may help to answer these questions, but this is beyond the scope of our study. It should be noted that if RMNH.MAM.59688 was an imported specimen that Diard acquired in Singapore, it would point towards a trade in rare and large animals in Singapore and the region from as early as 1819. The morphological similarity of RMNH.MAM.59688 to Javan Fishing Cat skulls lends support to the possible Javan origin of the specimen through trade.

An unlikely alternative scenario would be that the specimen was part of a native Singapore population and that the distribution of the Fishing Cat was or is a lot more widespread than hitherto known or accepted. To date, however, there have been no confirmed reports of Fishing Cats in the Malay Peninsula while the possibility of their occurrence in Sumatra is considered speculative in light of insufficient evidence (Duckworth et al. 2019).

This reconstruction of the history of Diard's Fishing Cat specimen from Singapore in RMNH suggests that there may be more Singapore material collected in 1819 yet to be discovered. This is the third species (other than the Green Broadbill and the Banded Leaf Monkey) for which the material collected during the first Singapore expedition is still extant.

Acknowledgements

This pilot study is supported by the Virtual Repatriation of Singapore's Natural History project, Lee Kong Chian Natural History Museum, National University of Singapore. We thank the Editor and two reviewers for their insightful comments and suggestions that greatly improved the manuscript.

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Supplementary material 1

Raw skull measurements and PCA loading matrix

Authors: Wan F. A. Jusoh, Marcus A. H. Chua, Piet A. J. Bakker, Pepijn Kamminga, Danièle Weiler, Kees Rookmaaker, Martyn E. Y. Low

Data type: Raw skull measurements and PCA loading matrix (EXCEL file)

Explanation note: This supplementary file contains data on raw skull measurements and PCA loading matrix.

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Link: <https://doi.org/10.3897/zse.98.76940.suppl1>

New records and a new cave-dwelling species of Agoristenidae (Arachnida, Opiliones) from Colombia

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<http://zoobank.org/F6DA8918-82B8-49C5-BD64-FE152C5D71BE>

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Academic editor: Danilo Harms ♦ Received 19 November 2021 ♦ Accepted 9 February 2022 ♦ Published 16 February 2022

Abstract

Three species of *Avima* are recorded for the first time from Colombia (La Guajira department): *A. venezuelica* Soares & Avram, 1981, *A. troglobia* (Pinto-da-Rocha, 1996), and *A. wayuunaiki* sp. nov. Complementary and new descriptions of the species are offered and scanning electron microscopy photographs of the male genitalia of *A. troglobia* are given.

Key Words

Avima, Caribbean, La Guajira, Laniatores, Leiosteninae

Introduction

The family Agoristenidae is a small and unusual group of Neotropical harvestmen (27 genera and 78 described species), found mainly in leaf litter, under rotten logs and rocks, and over rocky walls (Ahumada-C. et al. 2020; García and Kury 2020). It is currently divided into three subfamilies: Agoristeninae Šilhavý, 1973, Leiosteninae Šilhavý, 1973 and Globibuninae Kury, 2012. Leiosteninae contains 13 genera and 60 species, distributed mainly in northern South America. In Colombia, the subfamily is represented by seven genera and nine species (García and Pastrana 2021, Kury et al. 2021, Villarreal and García 2021), two of them being members of *Avima* Roewer, 1949: *Avima scabra* (Roewer, 1963) and *Avima tuttifrutti* García & Pastrana, 2021, from Cundinamarca and Córdoba departments, respectively (García and Pastrana 2021).

In recent field trips to the Colombian Caribbean, three species of *Avima* were recognized, one new and two of them known previously from Venezuela (*Avima venezuelica* Soares & Avram, 1981 and *Avima troglobia* (Pinto-da-Rocha, 1996)). So, in the present work, we

discuss some aspects of the harvestmen biodiversity of La Guajira and offer complementary and new descriptions, photographs of the general habitus, and SEM images of some of the male genitalia, together with a distributional map of the three species in Northern South America.

Methods

The species were photographed using a Leica M205C stereoscope attached to a Leica DFC450 digital camera and were posteriorly edited in Photoshop CC 2014 software. Color descriptions use the standard names of the 267 Color Centroids of the NBS/IBCC Color System (Jaffer, 2001) as explained in Kury and Orrico (2006). Scanning Electron Microscopy (SEM) was carried out with a JEOL JSM-6390LV belonging to Rudolf Barth Electron Microscopy Platform of the Oswaldo Cruz Institute / Fiocruz (Rio de Janeiro, Brazil).

Geographic coordinates have been transcribed verbatim from the labels and may be in different formats; when there was no indication of coordinates, they were written between square brackets in decimal degrees,

based on Rodríguez and Galán (2008). The distribution map was made with QGIS 3.18 Zurich software (QGIS Development Team 2021). Colored shapes refer to WWF Terrestrial Ecoregions of the World (Olson et al. 2001).

Patterns of description follow García and Villarreal (2020) with modifications. The terminology for dorsal scutum outline types follows Kury and Medrano (2016), with the modifications explained in Villarreal and García (2021), and for chaetotaxy of penis lamina parva and truncus follows Kury and Villarreal (2015). Morphometric abbreviations are: **AL** (maximum abdominal scutum length), **AW** (maximum dorsal scutum width), **BaCh** (basichelicerite length), **ChL** (chelicera length), **CL** (carapace length), **CW** (maximum carapace width), **DS** (dorsal scutum), **DSL** (dorsal scutum length), **Fe** (femur), **LP** (lamina parva), **MS** (macrosetae of penis), **Mt** (metatarsus), **Pa** (patella), **Ta** (tarsus), **Ti** (tibia), **TL** (total length), **Tr** (trochanter). All measurements are in mm unless otherwise noted.

Abbreviations of the cited repositories are: **CBUDC** (Colección de Ejemplares Biológicos de la Universidad de Cartagena. Cartagena de Indias, Colombia); **ICN** (Instituto de Ciencias Naturales, Universidad Nacional de Colombia. Bogotá, Colombia); **MBUZ** (Museo de Biología de La Universidad del Zulia. Maracaibo, Venezuela); **MNRJ** (Museu Nacional, Universidade Federal do Rio de Janeiro. Rio de Janeiro, Brazil); **MUSENUV** (Museo de Entomología de la Universidad del Valle. Cali, Colombia); **MZUSP** (Museu de Zoologia da Universidade de São Paulo. São Paulo, Brazil). The MNRJ material destroyed by the fire in September 2018 is marked with an exclamation mark (!).

Results

Systematics

Order Opiliones Sundevall, 1833

Suborder Laniatores Thorell, 1876

Superfamily Gonyleptoidea Sundevall, 1833

Family Agoristenidae Šilhavý, 1973

Subfamily Leiosteninae Šilhavý, 1973

Genus *Avima* Roewer, 1949

Avima Roewer, 1949: 58, fig. 112; Soares & Avram, 1982: 26 (type SMF 1533/8, male holotype). A complete synonymic list may be found in Villarreal and Kury (2009).

Type species. *Avima leucobunus* Roewer, 1949.

Avima wayuunaiki sp. nov.

<http://zoobank.org/42360820-446E-4054-B0DB-CF52D6DE4317>

Figs 1–3, 6A, C

Type data. COLOMBIA • ♂ holotype: La Guajira, Hatonuevo, Cerro Bañaderos, cueva [Luis Pablo Ojeda]; 11°7'51.5"N, 72°47'23.9"W [11.130972°, -72.789972°];

978 m a.s.l.; 14 July 2015; CarBio Team 17 leg.; ICN-Ao-1976 • 7 ♂ 10 ♀: same data as holotype; ICN-Ao-1718 • 1 ♂ 1 ♀: same data as holotype; MUSENUV-Ar 2102 • 4 ♂ 2 ♀: same locality as holotype, vereda Bañaderos; cueva [Luis Pablo Ojeda]; 11°7'33.3"N, 72°47'06.9"W [11.125899°, -72.785241°]; 785 m a.s.l.; 19 May 2018; Miguel Gutiérrez leg.; MNRJ 283 • 2 ♀: same locality as previous; CBUDC-ARA 335.

Description. Male Holotype (ICN-Ao-1976). Measurements. TL (ChL+DSL): 4.45, DSL: 2.66, CL: 1.07, CW: 1.79, AL: 1.59, AW: 2.21, BaCh: 0.55. Pedipalp: Tr: 0.60, Fe: 1.23, Pa: 0.72, Ti: 0.88, Ta: 0.70, Claw: 0.79. Leg I: Tr: 0.42, Fe: 4.10, Pa: 0.73, Ti: 3.19, Mt: 5.86, Ta: 1.31, TL: 15.61; Leg II: Tr: 0.57, Fe: 9.21, Pa: 0.98, Ti: 7.67, Mt: 12.40, Ta: 5.14, TL: 35.97. Leg III: Tr: 0.62, Fe: 5.92, Pa: 0.97, Ti: 3.65, Mt: 7.52, Ta: 1.58, TL: 20.26. Leg IV: Tr: 0.70, Fe: 8.65, Pa: 1.00, Ti: 4.85, Mt: 11.14, Ta: 1.82, TL: 28.16.

Dorsum. DS Epsilon type 2. Anterior and lateral margins of DS smooth. Ocularium low, smooth, and without median concavity (Fig. 1A, B, E). Mesotergum slightly delimited, divided into four smooth areas: area I divided into two halves; areas II–IV undivided (Fig. 2A). Posterior margin of scutum substraight and with few granules (Figs 1D, 2B). Free tergites I–III with some granules (Fig. 1B, D, E).

Venter. Coxa I with a longitudinal row of tubercles and one large bicapitate tubercle on the anteroproximal margin (Fig. 2C); coxae II–IV with some granules; coxa II longer than coxa I; coxa III longer than coxae I and II; coxa IV backward projected. Stigmata oval, small and transverse (Fig. 1C).

Chelicera. Chelicera swollen. BaCh quadrate in dorsal view, with well-marked bulla, three mesal, five anterior, and three ectal tubercles. Hand with setiferous tubercles of different sizes reaching the medial and posterior region. Fixed finger with the inner surface finely grooved. Movable finger with one trapezoid, small, sub-basal tooth, and with a dentate distal inner surface (Fig. 2D).

Pedipalps. All segments more slender than usual (Fig. 1B). Trochanter with one subapical tubercle on the ventral face. Femur with a ventroectal row of four setiferous tubercles (the two basalmost largest and the two distalmost medium-sized), and one large ventromesal setiferous tubercle in the apical portion (Fig. 2E). Patella with one large mesal setiferous tubercle. Tibia ectal III, mesal III. Tarsus ectal III, mesal III.

Legs. Increasing in thickness from leg I to leg IV, all smooth (Fig. 1A). Leg I filiform; coxae I–III with one dorsoanterior and one dorso-posterior tubercle; Coxa IV with some prolaterodistal tubercles; Fe III–IV darker than I–II. Fe IV length three and a half times DS length (Fig. 1A); Ta I–II each with one smooth claw; Ta III–IV with two subparallel smooth claws and without tarsal process. Tarsal counts: 9(3)–9(3)/19(3)–18(3)/7–6/7–7.

Penis. LP small (width twice the height) and apically depressed, with anterolateral acute corners (crescent-shaped) apically pointed (Fig. 3A–E). Malleus with two

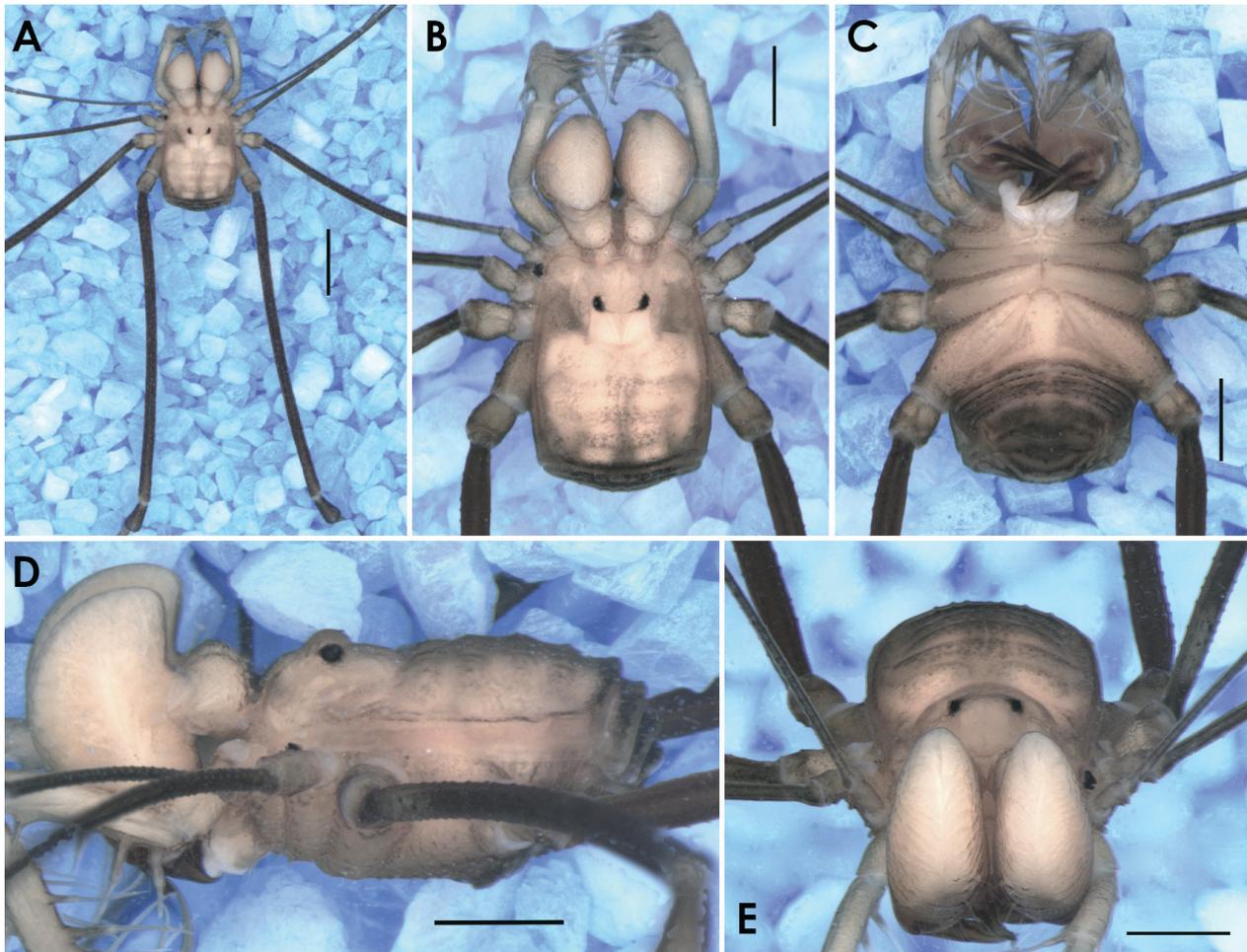


Figure 1. *Avima wayuunaiki* sp. nov. (ICN-Ao-1976), male holotype. Habitus in panoramic (A), dorsal (B), ventral (C), lateral (D), and frontal (E) views. Scale bars: 2 mm (A); 1 mm (B–E).

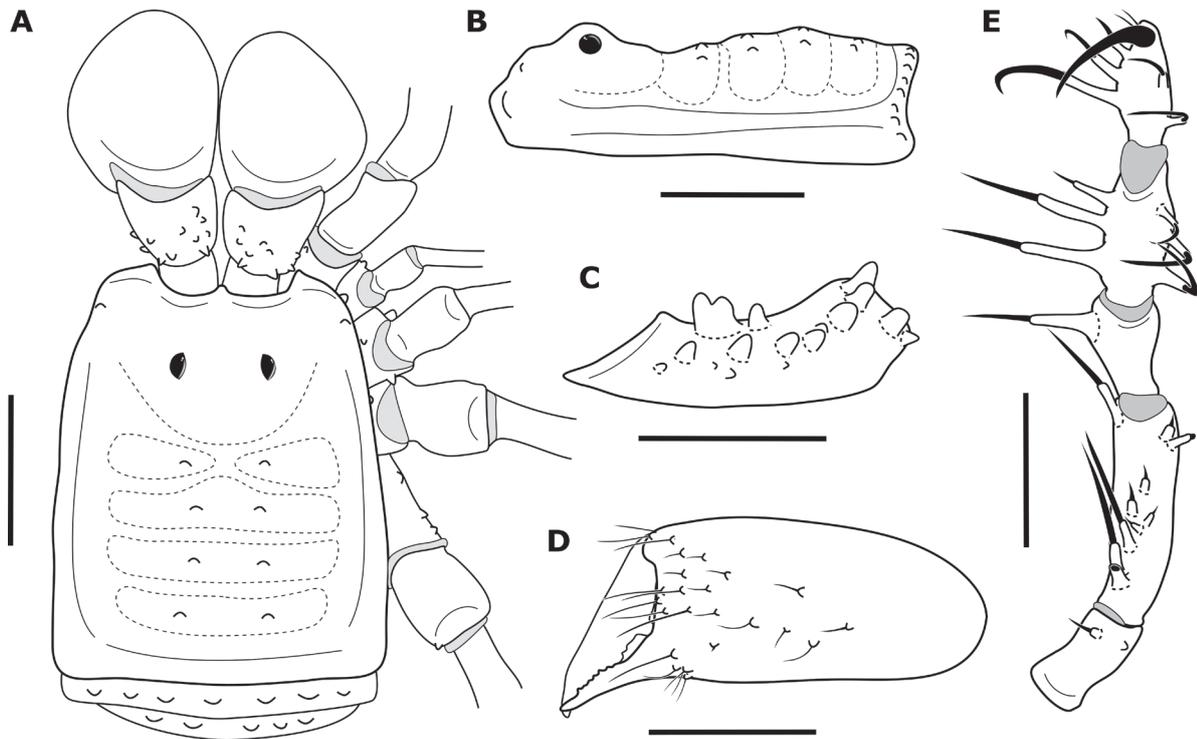


Figure 2. Drawings of *Avima wayuunaiki* sp. nov. (ICN-Ao-1976), male holotype. Habitus in dorsal (A) and lateral (B) views. Left coxa I in ventral view (C). Right chelicera in frontal view (D). Left pedipalp in ventral view (E). Scale bars: 1 mm (A, B, D, E); 0.5 mm (C).

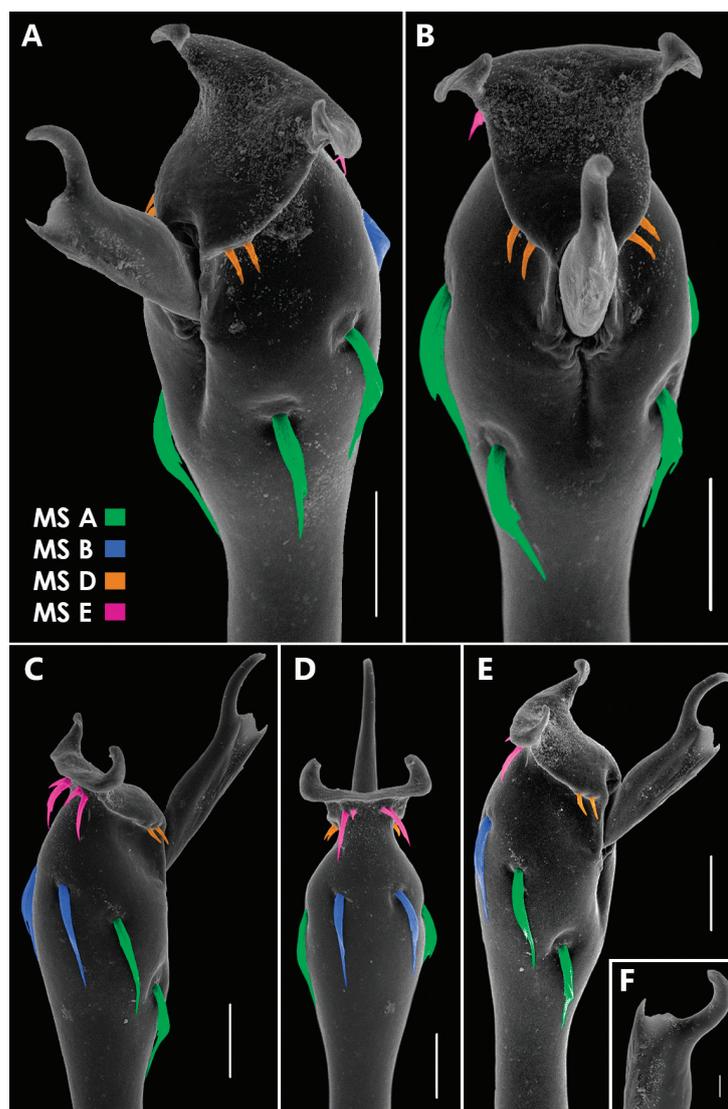


Figure 3. SEM of the male genitalia of *Avima wayuunaiki* sp. nov. (ICN-Ao-1718), paratype. Distal portion of the penis in lateroapical (A), dorsoapical (B), lateral (C), ventral (D), and oblique (E) views; detail of stylus in lateral view (F). Abbreviations: MS = Macrosetae. Scale bars: 50 µm (A–E); 10 µm (F).

pairs of branched MS-A, being MS-A2 far from the base of the stylus; one pair of branched MS-B; MS-C absent; two pairs of medium-sized MS-D located in a keel between the dorsal region of the LP and the base of the stylus (Fig. 3A–C, E); MS-E2 large and branched, MS-E1 short and conical, located slightly distal to MS-E2 on the ventral side of the LP (Fig. 3C, D). Stylus elongated, mostly straight (concave at the apex), and surpassing the LP; dorsal keel present, smooth, elevated at the medial region, with a dorsoapical sharp projection; tip dorsally projected (Fig. 3E, F).

Color (in alcohol). DS and chelicerae Light Yellow (86). Pedipalps, coxae, and trochanters I–IV Pale Yellow (89). Femora to tarsi I–IV Dark Yellowish Brown (78) (Fig. 1).

Female. Paratype (ICN-Ao-1718). Similar to male, except for abdomen wider at areas II–III, chelicerae not swollen, and leg IV slender. Measurements: TL (Ch+DSL): 3.50, DSL: 2.35, CL: 0.96, CW: 1.61, AL:

1.39, AW: 1.96, BaCh: 0.42. Pedipalp: Tr: 0.41, Fe: 1.12, Pa: 0.65, Ti: 0.84, Ta: 0.79, Claw: 0.78. Leg I: Tr: 0.32, Fe: 4.27, Pa: 0.69, Ti: 3.19, Mt: 6.04, Ta: 1.29, TL: 15.8; Leg II: Tr: 0.39, Fe: 9.53, Pa: 0.98, Ti: 8.70, Mt: 12.14, Ta: 5.34, TL: 37.08. Leg III: Tr: 0.39, Fe: 9.36, Pa: 1.04, Ti: 5.77, Mt: 13.25, Ta: 1.51, TL: 31.32. Leg IV: Tr: 0.68, Fe: 8.70, Pa: 0.94, Ti: 4.83, Mt: 11.08, Ta: 1.80, TL: 28.03. Tarsal counts: ?-6(3)? -18(3)/6-6/7-6.

Etymology. Wayuunaiki is the language spoken by the indigenous Wayuu people in northwestern Venezuela and northeastern Colombia on the Guajira Peninsula, where the species was collected. Noun in apposition.

Natural history. Specimens of *A. wayuunaiki* sp. nov. were found inside a cave (Fig. 6B, C) in the northeastern slopes of Sierra Nevada de Santa Marta. This species lives sympatrically with *Loxosceles guajira* Calariqueleme, Gutiérrez-Estrada & Flórez, 2015 (Sicariidae) and *Trichomycterus spectrum* DoNascimento & Prada-Pedrerros, 2020 (Trichomycteridae).

***Avima troglobia* (Pinto-da-Rocha, 1996)**

Figs 4, 6A

Trinella troglobia Pinto-da-Rocha, 1996: 321, figs 4, 8, 15–16; Kury, 2003: 34.

Avima troglobia (Pinto-da-Rocha, 1996): Villarreal & Kury, 2009: 67.

Type material. VENEZUELA• 1 ♂ holotype and 3 ♀ paratypes: Zulia, rio Socuy, cueva de Los Laureles; [10.751 -72.462]; 750 m a.s.l.; 20 Dec. 1990; J. Camacho and A. Viloría leg.; MBUZ• 2 ♀ paratypes; same data as previous [in the original label says Sierra de Perijá]; MZSP-1457 • paratype; same data as previous; 16 Dec.1992; P. Gnaspini and E. Trajano leg.; MZSP-14578• 2 ♀ paratypes; same data as previous, Cueva La Carlotica; 760 m a.s.l., 21 Dec.1990; J. Camacho, A. Viloría and T. Barros leg.; MBUZ• 1 ♀ paratype; same data as previous; MNRJ 5472'.

New records. COLOMBIA• 3 ♀: La Guajira, Barrancas, corregimiento San Pedro, Las Pavas; Cueva, finca La Fortuna; 10°50'04.8"N, 72°40'34.4"W [10.834667 -72.676222]; 1731 m a.s.l.; 8 May 2018; Miguel Gutiérrez leg.; ICN-Ao-1978• 1 ♂ 1 ♀; same data as previous; MNRJ 59052. First records for the country.

Complementary description. **Body.** DS Epsilon type 1. Ocularium low, domed, smooth, and without eyes (Fig. 4D, F) Mesotergum divided into four ill-defined areas, smooth; (Fig. 4A–C). Areas I–IV with a pair of minute paramedian granules; area I divided into two halves; area II–IV undivided (Fig. 4A, D). Posterior border of scutum substraight. Free tergites I–III with some tubercles. **Venter.** Stigmatic area with a few granules. Stigmata large, oval and oblique (Fig. 4E). **Legs.** Increasing in thickness from leg I to leg IV, but legs III and IV at least twice as thick as legs I and II, unarmed. Leg I filiform. Leg IV with darker coloration than the others. Fe IV length four times DS

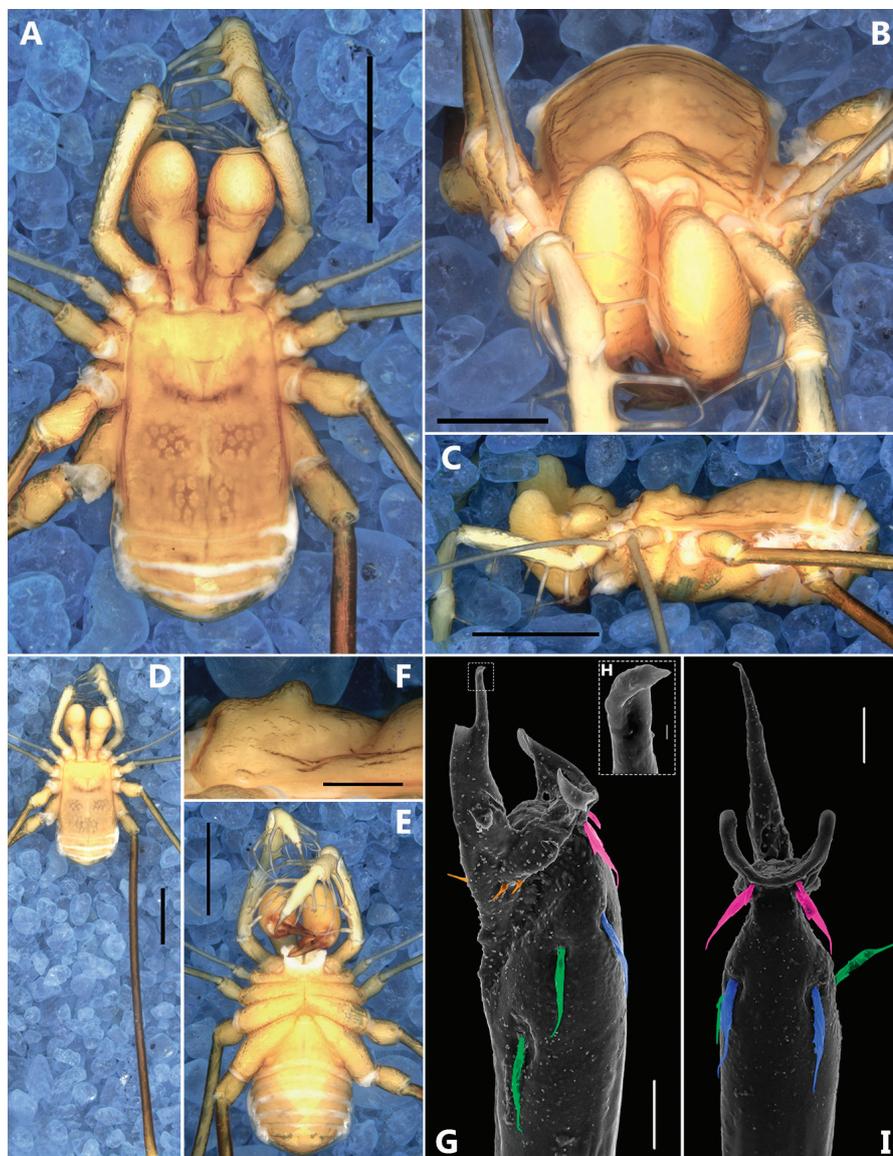


Figure 4. *Avima troglobia* (Pinto-da-Rocha, 1996) (MNRJ 59052), male. Habitus in dorsal (A), frontal (B), lateral (C), panoramic (D), and ventral (E) views; detail of ocularium showing the eye absence (F). SEM of the penis in lateral (G) and ventral (I) views; detail of the tip of the stylus (H). Scale bars: 2 mm (A–F); 50 µm (G, I), 2 µm (H). Macrosetae colors: A. = green, B. = blue, D. = orange, E. = magenta.

length (Fig. 4D). **Penis.** LP small and depressed, crescent-shaped, with anterolateral rounded corners apically pointed (Fig. 4G, I). Hammer (malleus) cylindrical, carrying MS-A-B (two pairs of MS-A and one pair of MS-B, all branched) (Fig. 4G, I); MS-C absent; two pairs of short MS-D located in a keel between the LP and the base of the stylus (Fig. 4G). MS-E1 large and triffid, MS-E2 short and conical, located slightly distal to MS-E1 on the ventral side of the LP (Fig. 4G, I). Stylus straight, elongated, surpassing the LP (Fig. 4G, I), with the tip ventrally curved (Fig. 4H) and a rectangular dorsal keel (Fig. 4G). **Coloration (in alcohol).** Carapace Brilliant Yellow (83) (anterior border) and Brilliant Orange Yellow (67) (posterior border and laterals). Free tergites and chelicerae Brilliant Orange Yellow (67). Pedipalps and legs I-III Brilliant Yellow (83). Leg IV Deep Orange Yellow (69). **Sexual dimorphism.** Chelicerae hypertelic in males.

Natural history. *A. troglobia* was collected exclusively in a cave and shows troglomorphisms (e.g., depigmentation, lack of eyes (Fig. 4F)), in the same way as the individuals from the Venezuelan caves, corroborating the idea that it is an obligatory cave-dwelling species (Pinto-da-Rocha, 1996).

Avima venezuelica Soares & Avram, 1981

Figs 5, 6A

Avima venezuelica Soares & Avram, 1981: 95; Villarreal & Kury, 2009: 67

Vima venezuelica: González-Sponga, 1987: 543, fig. 708–713.

Trinella venezuelica: Pinto-da-Rocha, 1996: 323; Kury, 2003: 34.

Type Locality. VENEZUELA • Zulia, río Guasare, cueva de Cerro Verde; [10.725000 -72.620000]. Remark. It is in Zulia, not Falcón, as in the original description

Records. VENEZUELA • Zulia, Mara, cueva de los Gavilanes [or Mara]; [11.017000 -72.425000]; 200 m a.s.l. • Maracaibo, cueva Francisco Zea; [10.758000, -72.609000]; 360 m a.s.l.

New records. COLOMBIA • 2 ♀: La Guajira, Barrancas, corregimiento San Pedro, Las Pavas, camino a la cueva, finca La Fortuna; 10°50'27.9", N 72°40'23.9", W [10.841083 -72.673306]; 1529 m a.s.l., 4 July 2016; Miguel Gutiérrez leg.; ICN-Ao-1979; 1 ♀: same data as previous, MNRJ 59053. First records for the country.

Complementary description. DS Epsilon type 2. Ocularium low, smooth, and with median concavity

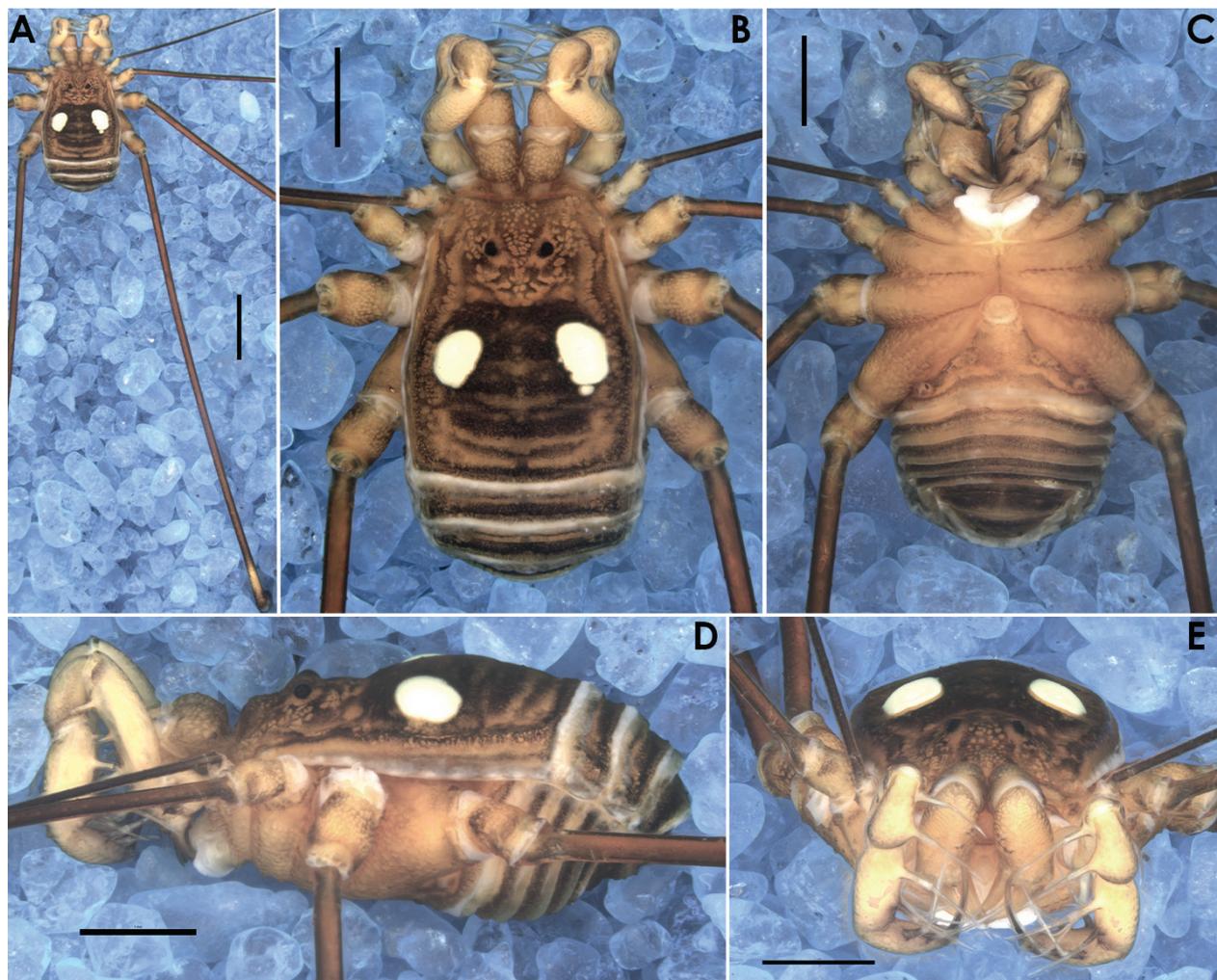


Figure 5. *Avima venezuelica* Soares & Avram, 1981 (MNRJ 59053), female. Habitus in panoramic (A), dorsal (B), ventral (C), lateral (D), and frontal (E) views. Scale bars: 2 mm.

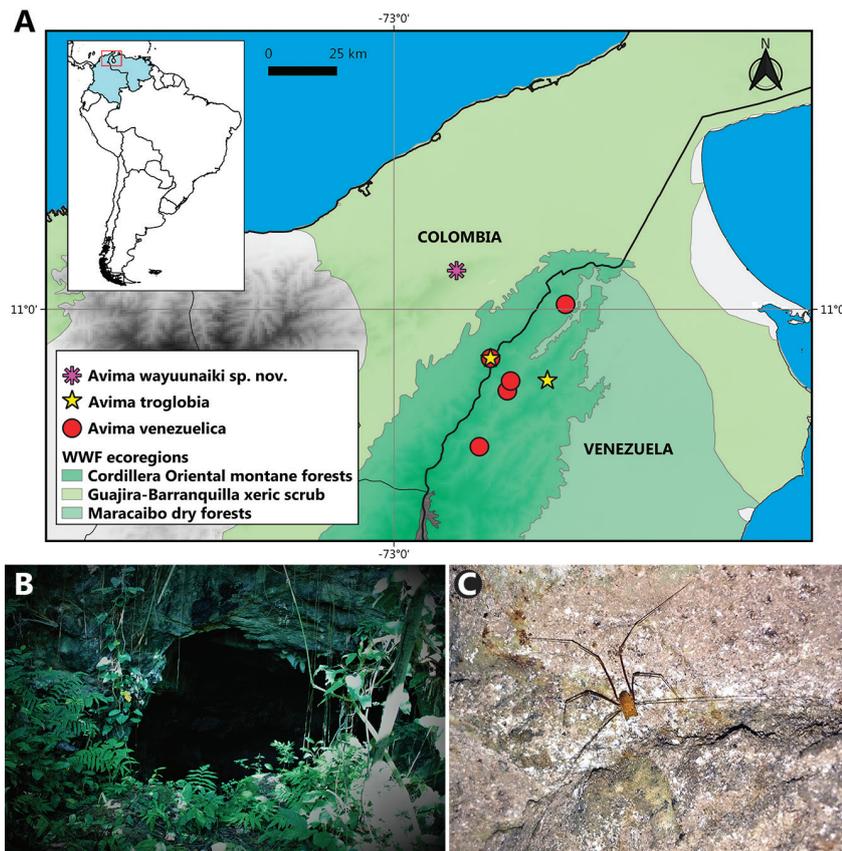


Figure 6. Geographic distribution of *Avima venezuelica*, *Avima troglobia* and *Avima wayuunaiki* sp. nov. in Northern South America. **A.** Map with previous and new records of the species in Colombia and Venezuela (colored areas represent the WWF ecoregions (Olson et al. 2001)); **B.** Bañaderos cave in La Guajira (Colombia), habitat of *A. wayuunaiki* sp. nov.; **C.** Living specimen of *A. wayuunaiki* sp. nov. Photographs by Miguel Gutiérrez Estrada (**B**), and Alex González Vargas (**C**).

(Fig. 5B, E). Mesotergum delimited, divided into four areas: area I divided into two halves; areas II-IV undivided (Fig. 5B). Anterior margin of coxa I with three tubercles, the basalmost bifid, and a medial longitudinal row of small tubercles (Fig. 5C). Pedipalpal segments slender and with long setae (Fig. 5B–D). Legs increasing in thickness from leg I to leg IV, unarmed; leg I filiform. Fe IV four times DS length (Fig. 5A).

Natural history. *A. venezuelica* was found outside the cave where *A. troglobia* was collected, as previously noted by Pinto-da-Rocha (1996) for the same species in Venezuelan caves.

Discussion

Avima is the largest genus of Leiosteninae (34 spp.), representing an entangled miscellany of taxa whose monophyly has not been tested (Villarreal and Kury 2009; García and Villarreal 2020; García and Pastrana 2021). For that reason, we decided to review the harvestmen literature looking for other species of Leiosteninae and *Avima* whose morphology could be related to *A. wayuunaiki* sp. nov., *A. venezuelica* and *A. troglobia*; we found similarities with Andean species like

Avima chiguaraensis (González-Sponga, 1987), and some cave-dwelling species such as *Avima azulitai* (Rambla, 1978), *Avima bordoni* (Muñoz-Cuevas, 1975), *Avima checkeleyi* (Rambla, 1978), *Avima chapmani* (Rambla, 1978), and *Avima falconensis* (González-Sponga, 1987).

According to the original descriptions and redescriptions of the aforementioned species, we found that they are typically (1) large to very large animals (DS + Leg IV length = more than 30 mm); have (2) a low and smooth ocularium (except in *A. chiguaraensis*), without a median concavity; have (3) more or less rounded corners of the LP of the penis and (5) stylus with a longitudinal dorsal expansion (apparently reduced in *A. bordoni*, *A. falconensis*, and *A. troglobia*).

Interestingly, the male genitalia of *A. wayuunaiki* sp. nov. is very similar to that of *A. azulitai* (González-Sponga, 1987: 501, figs 640–641) and *A. chiguaraensis* (González-Sponga, 1987: 513, figs 658–659), sharing the same macrosetae composition and a smooth dorsal keel with an elevated medial region with a dorsoapical sharp projection, reinforcing our idea of some systematic affinities between them. However, further analysis (perhaps including the revision of the type material of *A. azulitai* and *A. chiguaraensis*), will shed more light on this relationship.

About the localities in La Guajira

La Guajira department exhibits a contrasting assemblage of climatic and geographic conditions that make it an interesting region for ecological and biological studies. The majority of its territory is dominated by the Guajira-Barranquilla xeric scrub ecoregion (NT1308), characterized by low precipitation rates and thorn-covered trees and succulent plants as dominant vegetation (World Wide Foundation 2014a). The southeast area exhibits forests of Cordillera Oriental montane forests ecoregion (NT0118), with a predominance of subarborescent and bushy strata, particularly Perijá Mountains sub-ecoregion (World Wide Foundation 2014b) (Fig. 6A). The cave where the new species was collected is located in the latter but is influenced by the humid forests of Sierra Nevada de Santa Marta ecoregion (NT0159), which has many endemic species due to its isolation from the Cordillera Oriental mountain range (World Wide Foundation 2014c). Unfortunately, all of these ecoregions have been changed due to deforestation by extensive pastures and the removal of firewood and timber.

Recent efforts to record the biodiversity of these ecoregions (e.g. particular collections done by the third author of the present work plus rapid faunistic inventories developed by the CarBio team), resulted in the description of new spider taxa (e.g. Cala-Riquelme et al. 2015, 2017, 2018; Cala-Riquelme and Salgado 2021), from both Cesar and La Guajira departments. In the case of harvestmen, material collected from those expeditions led to the description of *A. wayuunaiki* sp. nov. from a cave in NT1308 (Fig. 6B), and the new records of *A. troglobia* and *A. venezuelica* (which are the first published photographs of both species) from NT0118, in La Guajira (Fig. 6). Such findings show that our knowledge about the opiliones fauna of this region is incipient, especially considering that the only recorded harvestman from La Guajira to date was *Cranasus albipustulatus* Roewer, 1943 (Cranidae) (Kury 2003; Ahumada-C. et al. 2020) and no further collection efforts had been made in the region since then. Therefore, we believe that new studies in La Guajira and its ecoregions could reveal hidden diversity and help in conservation initiatives and efforts.

Acknowledgements

We are grateful to Eduardo Flórez (ICN, Bogotá) for the loan of the material here studied, Adriano Kury (MN/UFRJ, Rio de Janeiro) for all the laboratory facilities, and Brittany Damron for the English revision of the text. The photographs were taken with the stereomicroscope (CNPq Universal 14/2013) in the Invertebrate department (MNRJ). The SEM micrographs were taken in the Microscopy Platform Rudolf Barth/IOC-Fiocruz with the kind assistance of Roger Magno Macedo Silva. The OmniPaper Project created and maintained by Adriano Kury was very helpful in providing access to some papers. The suggestions of Willians Porto (MACN) and one

anonymous referee greatly improved the present work. This study has been supported by a scholarship from the Coordenação de aperfeiçoamento de pessoal de nível superior (CAPES) and the scholarship #E-26/204.248/2021 from Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) to AFG.

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Pliocene-Pleistocene dispersal bring along low inter species diversity between *Vimba* species based on multilocus analysis

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<http://zoobank.org/A65F15B9-00BD-4F26-BA1D-4B1EEC4AB30F>

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Academic editor: Nicolas Hubert ♦ Received 23 October 2021 ♦ Accepted 15 February 2022 ♦ Published 25 February 2022

Abstract

This study investigates phylogenetic and phylogeographic relationships of *Vimba* species using mitochondrial cytochrome b (cyt b) (1023 bp) and cytochrome c oxidase subunit I (COI) barcoding region (652 bp) genes. Ninety-one samples from 36 populations for the cyt b gene and 67 samples from 20 populations for the COI were analyzed. We identified 29 haplotypes and calculated overall haplotype diversity as $Hd: 0.907 \pm 0.015$ for cyt b. We also identified 13 COI haplotypes and calculated overall haplotype diversity as 0.826 ± 0.026 for this marker. The phylogenetic analysis of *Vimba* species reveals the presence of four clades, based on concatenated cyt b and COI sequences. The first and second clade consist of *Vimba vimba* Western lineage, and *Vimba vimba* Caspian lineage, while the third and fourth clade consist of *Vimba mirabilis* and *Vimba melanops*. Based on haplotype network analyses and phylogeographic inferences, the *Vimba* genus is monophyletic, and its species dispersed in the Pleistocene era.

Key Words

cyt b, genetic diversity, Phylogeography, *Vimba* bream, *Vimba mirabilis*

Introduction

As a member of the Leuciscidae family, the genus *Vimba* is distributed throughout almost all Eurasia and consists of three species: *Vimba vimba*, *Vimba melanops*, and *Vimba mirabilis*. *V. vimba* was initially described as *Cyprinus vimba* L. from several Swedish lakes in Scandinavia, the North Sea, coastal waters of Baltic Sea basins, and, subsequently, after the description, it was also found in the Caspian, Black Sea, Marmara Sea basins, and the Rhine River. In Anatolia, *V. vimba* is distributed from the Marmara basin up to Büyük Menderes, Eğirdir Lake, Köprüçay and Eşen rivers in the south, and Kızılırmak in the east. *V. melanops* was described initially from the Meriç (Evros) River and its distribution now extends within the borders of Turkey, Greece, Bulgaria, and Macedonia in the North Aegean basin from Meriç to the Pinios River. The Anatolian endemic *V. mirabilis* was detected only in the type locality Büyük Menderes and two individuals in Bafa Lake in Southwest Anatolia (Bogutskaya 1997). According to Crivelli (1996),

V. mirabilis is under threat of extinction due to the water intake from the basin for drinking and irrigation.

Since then, a few taxonomic developments of the genus *Vimba* have occurred, like the subalpine *Vimba* lineage previously identified as *Vimba elongata*, but now considered a synonym of *Vimba vimba*. *Acanthobrama mirabilis* was synonymized with *Vimba vimba tenella* by Durand et al. (2002), as *A. mirabilis* belongs to the *Vimba* clade. But, Perea et al. (2010) found *Acanthobrama mirabilis* is the synonym of *V. mirabilis* based on genetic evidence. Also, in some literature, *V. vimba*, distributed in the Caspian Sea and Caspian bream, has been identified as *Vimba persa* (Hänfling et al. 2009; Naseka and Bogutskaya 2009; Chaichi et al. 2011a, 2011b; Mohamadian et al. 2012).

Globally, *Vimba* genus has been well studied in terms of growth parameters and its parasites, but genetic studies are more limited (Zardoya and Doadrio (1999), Durand et al. (2002), Hänfling et al. (2009), Perea et al. (2010), Triantafyllidis et al. (2011), Geiger et al. (2014), Schönhuth et al. (2018). In particular, Hänfling et al. (2009) discovered

the phylogeographic origin and colonization factor of *V. vimba*, but other studies have quite limited samples.

Phylogeographic and phylogenetic studies have been made on some European freshwater fishes such as *Alburnoides*, *Barbus*, *Capoeta*, *Gobio*, *Squalius*, *Telestes* and typically uncovered distinct patterns according to mitochondrial cytochrome b (cyt b) and cytochrome c oxidase I (COI) genes. In order to study systematics and phylogeny of Leuciscidae and its congeners, cyt b and COI sequence analysis have been demonstrated to be useful DNA markers. (Zardoya and Doadrio 1999; Durand et al. 2000, 2002; Tsigenopoulos et al. 2002; Levin et al. 2012; Bektas et al. 2017; Turan et al. 2018; Aksu and Bektaş 2019).

In European rivers, freshwater fishes have largely colonized the Black Sea upstream, up rivers such as the Dnieper and the Danube. Freshwater fishes contain numerous lineages with genetic divergences representing separation over the past 2 Myr in this region. Several of these have clear geographic distributions and provide evidence of older Black Sea-Caspian Sea divergence. Interglacial and postglacial expansions also indicate colonization of Western Europe from numerous major refugia, particularly the Black Sea, Dnieper-Volga, Danube, Rhine– Rhone, Elbe, and other rivers. The influence of older water bodies such as the Ponto-Caspian Sea and recent great periglacial lakes and floods is also apparent (Hewitt 2004).

While it frequently highlighted that the Pleistocene increased speciation rates, molecular data have recently revealed that species diverged in the Pleistocene and Pliocene in Europe (Hewitt 2000).

In the current study, mtDNA sequences were used (cyt b, COI barcoding) to examine the biogeography of *Vimba* populations, applying a phylogeographic approach. The objective is to reveal the phylogenetic relationships and genetic diversity of *Vimba* species whose populations are currently decreasing.

Materials and methods

Sample collection, DNA extraction, and sequencing

All currently recognized taxa of *Vimba* were included in our dataset. We sequenced 68 *Vimba* specimens collected from their distribution ranges in Turkey and further included sequences of 23 specimens from NCBI GenBank. *Vimba* species of Turkey were collected from 14 sampling sites of drainages of the Black and Aegean and Marmara seas, comprising type localities or type basins (Fig. 1, Table 1). The animals were experimented with as per the Republic of Turkey animal welfare laws, guidelines, and policies, and was approved by the Republic of Turkey Recep Tayyip Erdogan University Local Ethics Committee for Animal Experiments (Decision No: 2014/72). For faunal surveys, fishes were collected, surgical procedures were performed only for excision of fin clips after anesthesia with MS222,

and fin clips were further preserved in 70% ethanol for genetic studies. In our experiments, none of the fishes were distressed by the experimental conditions. Specimens and tissue vouchers from Turkey were kept in DNA Collection and Zoology Museum, Faculty of Fisheries, Recep Tayyip Erdoğan University, Rize. Total DNA was extracted from fin clips via Qiagen automated DNA/RNA purification system by using Qiagen DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). Both the quality and quantity of DNA were checked on a NanoDrop 2000/c spectrophotometer (Thermo Scientific, Rockford, IL, USA) and 0.8% agarose gel electrophoresis. Cyt b (1023 bp) gene was amplified by the newly designed primer set B-cytbF (5'-GAAGAACCACCGTTGTWVTTCAAC-3') and the B-cytbR (5'-CGGATTACAAGACCGATGC-3'), and COI barcoding gene (652 bp) was amplified by the FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGC-CAAAGAATCA-3') (Ward et al. 2005). PCR reactions were performed in a 50 µL reaction volume containing 5 µL 10x PCR buffer, 100 ng template DNA, 0.5 mM dNTPs mix, 3 mM MgCl₂, 0.5 mM of each primer, and 1 µL Taq DNA polymerase (New England Biolabs). The polymerization was carried out under the following conditions: initial denaturation at 95 °C for 30 s, denaturation at 95 °C for 30 s, annealing at 55 °C for 50 s for cyt b and 58 °C for 45 s for COI, extension at 68 °C for 1 min through 35 cycles, and a final extension at 68 °C for 5 min using Biorad T100 (Bio-Rad, Hercules, CA, USA) thermal cycler. The PCR products were run and visualized under UV Quantum-Capt ST4 system (Vilber Lourmat, France) also, purified and sequenced at MacroGen Europa Inc. (Amsterdam, Netherlands) (Turan et al. 2018).

Genetic structure and phylogenetic analysis

Clustal W (Thompson et al. 1994) algorithm was implemented in Bioedit v7.2.5 (Hall 1999) software to align cyt b and COI barcoding regions. Haplotype number (H), polymorphic and variable sites, haplotype diversity (Hd), and nucleotide diversity (π) were computed for each species by DnaSP version 6.12.03 (Rozas et al. 2017) program. Nucleotide frequencies and transition/transversion rate were calculated by MEGA X (Kumar et al. 2018). Molecular Variance (AMOVA) was analyzed using the Arlequin v3.5.1.2 (Excoffier and Lischer 2010) software to calculate genetic variation among and within the groups. The haplotypes were submitted to the NCBI GenBank with accession numbers OK493404–OK493416 for cyt b OK444819–OK444823 for COI barcoding region. Cyt b and COI sequences were concatenated (1675 bp) for further phylogenetic inferences. The TrN+G model: -ln = 2905.1890 was the best-fit nucleotide substitution model for the concatenated dataset according

Table 1. List of sequences analyzed in this study with information on drainage, GenBank no, haplotype no, and country of origin.

Locality no	Species	Locality	Coordinate	cyt b			COI			Reference
				N	Genbank no	Haplotype no	N	Genbank no	Haplotype no	
1	Vimba vimba	Kirmir stream, Ankara, Turkey	40°14'10.5"N, 32°15'41.2"E	7	OK493404	H1	3	OK4444821	H7	This study
2	Vimba vimba	Binkılıç stream, Black sea, Istanbul, Turkey	41°22'48.3"N, 28°17'46.0"E	9	OK493404, OK493407, OK493408	H1,H4,H5	8	OK4444820	H6	This study
3	Vimba vimba	Koca stream, Balıkesir, Turkey	39°46'55.2"N, 27°35'46.2"E	4	OK493404, OK493406, OK493409	H1,H3,H6	4	OK4444820, OK4444821	H6, H7	This study
4	Vimba vimba	Koca çayı stream, Çanakkale, Turkey	39°48'52.9"N, 27°13'46.1"E	3	OK493405, OK493406	H2,H3	3	OK4444820, OK4444821	H6, H7	This study
5	Vimba vimba	Aydınlar stream, Zonguldak, Turkey	41°13'47.1"N, 31°27'11.2"E	2	OK493410	H7				This study
6	Vimba vimba	Aksu stream, Düzce, Turkey	40°45'49.0"N, 30°57'43.0"E	3	OK493411	H8				This study
7	Vimba vimba	Büyük Melen stream, Düzce, Turkey	40°50'08.0"N, 31°06'35.0"E	4	OK493411	H8				This study
8	Vimba vimba	Yenice stream, Zonguldak, Turkey	41°20'27.6"N, 32°04'40.8"E	5	OK493410	H7				This study
9	Vimba vimba	Çayagzı stream, Düzce, Turkey	41°05'27.2"N, 31°13'18.5"E	3	OK493410, OK493411	H7,H8				This study
10	Vimba vimba	Iznik lake, Kocaeli, Turkey	40°26'18.1"N, 29°38'03.5"E	10	OK493406	H3				This study
11	Vimba vimba	Suat Ugurlu Dam lake, Samsun, Turkey	41°01'52.4"N, 36°38'33.4"E	2	OK493412, OK493413	H9,H10				This study
12	Vimba vimba	Curonian Lagoon, Baltic Sea basin, Lithuania	55°42'18.0"N, 20°00'00.0"E	1	GQ279763	H11				Hänfling et al. 2009
13	Vimba vimba	Danube I, Black Sea basin Germany	48°53'24.0"N, 11°48'54.0"E	1	GQ279762	H12				Hänfling et al. 2009
14	Vimba vimba	Elbe, North Sea basin, Germany	51°28'30.0"N, 11°58'01.2"E	1	GQ279761	H13				Hänfling et al. 2009
15	Vimba vimba	Olandsan, Baltic Sea basin, Sweden	60°20'24.0"N, 17°31'19.2"E	1	GQ279756	H14				Hänfling et al. 2009
16	Vimba vimba	Mondsee, Danube, Black Sea basin, Germany	47°49'40.8"N, 13°23'02.4"E	2	GQ279755, AY026405	H18				Hänfling et al. 2009 Durand et al. 2002
17	Vimba vimba	Eder, Weser, North Sea basin, Germany	51°09'18.0"N, 8°54'07.2"E	1	GQ279755	H18				Hänfling et al. 2009
18	Vimba vimba	Tuzlov, Don, Sea of Azov basin, Russia	49°58'58.8"N, 42°01'04.8"E	1	GQ279751	H15				Hänfling et al. 2009
19	Vimba vimba	Seversky, Donetsk, Sea of Azov basin, Russia	47°37'37.2"N, 40°53'16.8"E	1	GQ279751	H15				Hänfling et al. 2009
20	Vimba vimba	Samur, Caspian Sea basin Russia	41°52'26.8"N, 48°33'34.9"E	1	GQ279765	H16				Hänfling et al. 2009
21	Vimba vimba	Sea of Azov, Sea of Azov basin Ukraine	46°03'50.4"N, 36°36'54.0"E	2	GQ279754, GQ279752	H1 H3				Hänfling et al. 2009
22	Vimba vimba	Kuban, Sea of Azov basin, Russia	45°11'56.4"N, 37°42'54.0"E	1	GQ279753	H10				Hänfling et al. 2009
23	Vimba vimba	Tsymlyansk Reservoir, Don, Sea of Azov basin, Russia	47°45'56.2"N, 42°49'18.8"E	1	GQ279751	H15				Hänfling et al. 2009
24	Vimba vimba	Dagomys, northeastern Black Sea basin, Russia	43°40'01.2"N, 39°39'07.2"E	1	GQ279750	H3				Hänfling et al. 2009
25	Vimba vimba	Libechovka river, Elbe basin, Czech Republic	50°28'45.0"N, 14°29'07.5"E, (predict)	1	HM560237	H17	1	HM560383	H6	Perea et al. 2010
26	Vimba vimba	Bashly-chai, Caspian Sea Basin, Russia	42°20'37.0"N, 48°05'30.8"E	1	GQ279765	H16				Hänfling et al. 2009
27	Vimba vimba	Lake Sapanca, Sakarya, Turkey	40°43'14"N, 30°17'41"E				20	KC501853- KC501872	H13	Keskin & Atar 2013
28	Vimba vimba	Gönen drainage, Çanakkale, Turkey	39°56'45.6"N, 27°20'13.2"E	2	KJ554799, KJ554924				H11, H12	Geiger et al. 2014
29	Vimba vimba	Egirdir Lake, Isparta, Turkey	38°02'00.9"N, 30°52'24.3"E (predict)				2	MW940905, MW940906	H10, H6	Eren,H. (unp.)
30	Vimba vimba	Biga drainage, Bursa, Turkey	40°12'18.0"N, 29°05'13.2"E	1	KJ554609				H6	Geiger et al. 2014
31	Vimba vimba	Koeprue drainage, Isparta, Turkey	37°31'40.8"N, 31°16'08.4"E				2	KJ554606, KJ554754	H6	Geiger et al. 2014
32	Vimba vimba	Danube R., Slovakia	48°04'04.2"N, 17°09'53.2"E (predict)	1	AY026404	H1				Durand et al. 2002
33	Vimba melanops	Inece stream, Kirklareli, Turkey	41°41'34.0"N, 27°04'59.0"E	4	OK493415, OK493416	H27 H28	2	OK4444819	H1	This study
34	Vimba melanops	Evros, Aegean Sea basin, Greece	40°50'42.0"N, 26°01'22.8"E	1	GQ279757	H26				Hänfling et al. 2009

Locality no	Species	Locality	Coordinate	cyt b			COI			Reference
				N	Genbank no	Haplotype no	N	Genbank no	Haplotype no	
35	<i>Vimba melanops</i>	Biserska River, Greece	40°55'08.5"N, 26°11'48.0"E (predict)	1	MG806725	H25	1	MG806910	H1	Schonhuth et al. 2018
36	<i>Vimba melanops</i>	River Strymon, Greece	41°43'53.5"N, 23°09'30.9"E (predict)	2	AF090778, HM560236	H24 H21	1	HM560382	H4	Zardoya & Doadrio, 1999 Perea et al 2010
37	<i>Vimba melanops</i>	Pinios, Aegean Sea basin, Greece	39°39'57.6"N, 22°14'02.4"E	2	GQ279758, GQ279759	H23 H22				Hänfling et al. 2009
38	<i>Vimba melanops</i>	Aliakmon R. Kaloneri, Greece	40°17'26.5"N, 21°28'17.9"E (predict)	1	HM560235	H21				Perea et al. 2010
39	<i>Vimba melanops</i>	Volvi lake, Greece	40°39'36.0"N, 23°32'24.0"E	1	AY026403	H29	3	HQ600801- HQ600803	H1	Triantafyllidis et al. 2011 Durand et al. 2002
40	<i>Vimba melanops</i>	Kerkini lake, Greece	41°06'36.0"N, 23°03'00.0"E				3	HQ600804- HQ600806	H1, H3, H4	Triantafyllidis et al. 2011
41	<i>Vimba melanops</i>	Biserska R., Evros drainage, Bulgaria	41°51'18.0"N, 25°55'22.8"E				3	KJ554935, KJ554568, KJ554722	H1	Geiger et al. 2014
42	<i>Vimba melanops</i>	Charmanlijskaja drainage, Bulgaria	41°53'20.4"N, 25°41'13.2"E				1	KJ554876	H2	Geiger et al. 2014
43	<i>Vimba melanops</i>	Vardar drainage, Greece	40°59'16.8"N, 22°33'28.8"E				2	KJ554926, KJ554576	H5	Geiger et al. 2014
44	<i>Vimba mirabilis</i>	Akçay stream, Büyük Menderes, Aydın, Turkey	37°45'34.0"N, 28°20'07.0"E	9	OK493414, AY026410	H20 H19	4	OK444822, OK444823	H8, H9	This study, Durand et al. 2002
45	<i>Vimba mirabilis</i>	Çine stream, Büyük Menderes, Aydın, Turkey	37°45'43.8"N, 27°50'13.1"E	4	OK493414	H20	1	KJ554739	H8	This study, Geiger et al. 2014

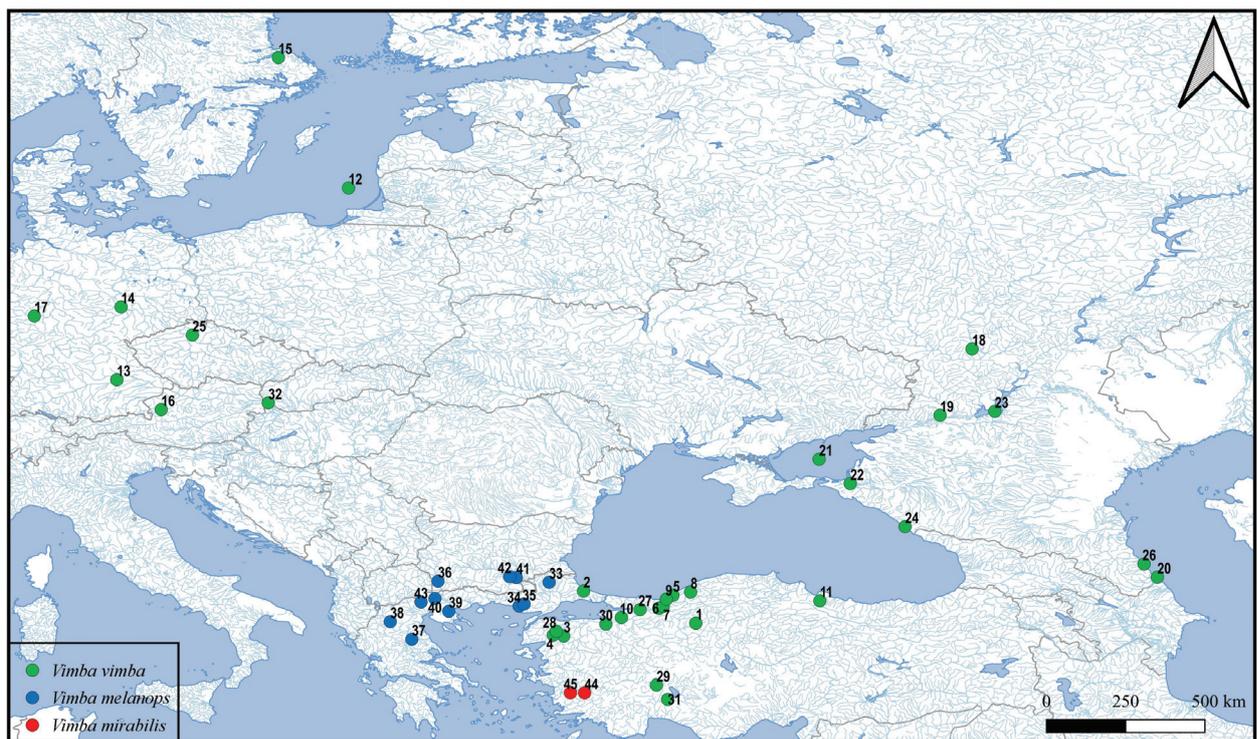


Figure 1. Map showing the analyzed population stations.

to Akaike information criterion (AIC) and Bayesian information criterion (BIC), as implemented in jModeltest v. 0.0.1 (Posada, 2008). Phylogenetic relationships among haplotypes and species were estimated by the maximum likelihood (ML) method using PhyML (Guindon et al. 2010) with 1000 bootstrap. Similarly, the Bayesian inference (BI) analysis was run in the MrBayes 3.1.2 software (Ronquist and Huelsenbeck 2003), using the Metropolis-coupled Markov chain

Monte Carlo (MCMC) algorithm from randomly generated starting trees for five million generations with sampling taking place in every 1000 generations. The initial 25% of saved trees sampled in each MCMC run were discarded as burn-in. In all phylogenetic analyses, *Blicca bjoerkna* (AP009304) was selected as outgroup taxa. Haplotypes' network inference was constructed through a median-joining (MJ) algorithm (Bandelt et al. 1999) implemented in Network 5.0.0.1 software

(www.fluxus-engineering.com). MEGA X (Kumar et al. 2018) software was used to calculate pairwise genetic distance among the species using the Kimura 2-parameter substitution model (Kimura 1980).

We estimated divergence times using StarBEAST (Ogilvie et al. 2017), which was implemented in BEAST 2.6.0 (Bouckaert et al. 2014). Compared with standard BEAST, StarBEAST better accounts for species trees vs. gene trees and intraspecific vs. interspecific events. Species were delimited according to the individual grouping recovered by previous phylogenetic analyses. Thus, the analysis was conducted with 5 groups of individuals consisting of *V. vimba* (Caspian), *V. vimba* (Western), *V. mirabilis*, *V. melanops*, and outgroup *Blicca bjoerkna*. The molecular clock calibration was based on the divergence rate of *cyt b* gene in Leuciscinae of 0.4% per lineage per million years, as determined by Perea et al. (2010), and this rate was used by Buj et al. (2019) and Viñuela Rodríguez et al. (2020). Based on a Yule speciation prior and a strict clock model, branch rate estimates were calculated. Partitioned *cyt b* and COI dataset were used for the molecular clock analysis and the partitions were linked except for substitution models. The substitution models were used the TrN+G:-ln = 1878.5334 (Tamura and Nei 1993) and the HKY:-ln = 1063.2973 (Hasegawa et al. 1985) for *cyt b* and COI barcoding region, respectively, with Gamma site heterogeneity for both markers. The number of MCMC steps (chain length) was ten million, with parameters logged every 1000 generations. The residual parameters were default parameters of the software. Tree results were summarized in TreeAnnotator v.2.6.0 software with 10% burn-in to get a maximum clade credibility tree. Node bars, height median with height 95% HPD, and node labels were mapped on the tree with FigTree v1.4.4 (Rambaut 2018). Geological scale was plotted using the geoscalePhylo function in R package strap (Bell and Lloyd 2015). Effective sample size (ESS) and convergence of parameters was estimated using Tracer v.1.6 (Rambaut and Drummond 2013). The effective sample sizes for all parameters of interest were greater than 200.

Results

Genetic diversity and species divergence

The nucleotide sequences of the *cyt b* gene region (1023 bp) were studied in 91 specimens from three species of *Vimba* (Additional Table 1). The average nucleotide frequencies were estimated as 26.9% A, 29.7% T, 28.0% C, and 15.4% G. The transition/transversion rate $k1 = 115.99$ (purines), $k2 = 34.563$ (pyrimidines) and the overall transition/transversion bias were estimated as $R = 31.458$. The haplotype number was identified as 29: 18 from *V. vimba* (N = 66), nine from *V. melanops* (N = 12), and two from *V. mirabilis* (N = 13) (Additional Table 1). We identified 65 variable sites, of which 36 were parsimony-informative. Species-specific nucleotide (SSN) positions in the mitochondrial DNA *cyt b* gene sequences provide evidence for

genetic differentiation of three *Vimba* species, as in *V. vimba*, *V. mirabilis*, and *V. melanops* are determined: 7, 7, and 1, respectively. The haplotype and nucleotide diversity are $Hd = 0.855 \pm 0.025$ and $\pi = 0.00175 \pm 0.00029$ for *Vimba vimba*, $Hd = 0.939 \pm 0.00333$ and $\pi = 0.00783 \pm 0.00119$ for *V. melanops*, and $Hd = 0.154 \pm 0.01590$ and $\pi = 0.00015 \pm 0.00012$ for *V. mirabilis*. The overall haplotype and nucleotide diversity are $Hd = 0.907 \pm 0.015$ and $\pi = 0.00994 \pm 0.00104$. According to the AMOVA, 88.34% of all genetic variation occurs among three species. In the haplotype network analysis, the 29 distinct haplotypes are separated by one up to nine mutations. The most common haplotype was H1, shared by many populations belonging to *V. vimba*. Many haplotypes were distributed in either one or two populations (Fig. 2). Average pairwise genetic distances between species were estimated as 0.0212 (*V. vimba* and *V. melanops*), 0.0198 (*V. vimba* and *V. mirabilis*), and 0.0171 (*V. melanops* and *V. mirabilis*). Average pairwise genetic distance was estimated as 0.0101 between *V. vimba* Caspian (H16) and West lineages. Average intraspecific variations within *V. vimba*, *V. mirabilis*, and *V. melanops* were estimated as 0.002, 0.000, and 0.008, respectively. Nucleotide sequences of the COI barcoding region (652 bp) were obtained in 67 specimens from three species of *Vimba* (Additional Table 1). The nucleotide frequencies were calculated as 25.28% A, 29.00% T, 27.71% C, and 18.01% G. The transition/transversion rate $k1 = 3.588$ (purines), $k2 = 1.848$ (pyrimidines), and the overall transition/transversion bias was calculated as $R = 1.27$. The haplotype number was identified as 13: six from *V. vimba* (N = 46), five from *V. melanops* (N = 16), and two from *V. mirabilis* (N = 5) (Table 1). A total of 21 variable sites were recognized, and 10 of them were parsimony-informative. The haplotype and nucleotide diversity with the standard deviation are $Hd = 0.680 \pm 0.00156$ and $\pi = 0.00308 \pm 0.00021$ for *V. vimba*, $Hd = 0.608 \pm 0.01695$ and $\pi = 0.00204 \pm 0.00065$ for *V. melanops*, and $Hd = 0.400 \pm 0.237$ and $\pi = 0.00123 \pm 0.00073$ for *V. mirabilis*. The overall haplotype and nucleotide diversity are $Hd: 0.826 \pm 0.026$ and $\pi = 0.00457 \pm 0.00041$. According to the AMOVA, 58.52% of all observed genetic variations in three species are occurring within species. Haplotype network analysis has 13 unique haplotypes with at least one mutational step, and no haplotype is shared between species that are separated from each other by at least one mutational step. The most common haplotypes were H13, represented by 20 specimens sampled by İznik Lake. All haplotypes were distributed in either one or two populations (Fig. 3). Average pairwise genetic distances between species were estimated as 0.0054 (*V. vimba* and *V. melanops*), 0.0093 (*V. vimba* and *V. mirabilis*), and 0.0088 (*V. melanops* and *V. mirabilis*).

Phylogenetic relationships and divergence times

Based on ML and Bayesian phylogenetic inferences, four mitochondrial clades of *Vimba* species are observed. The first and second clades include distinct *V. vimba*

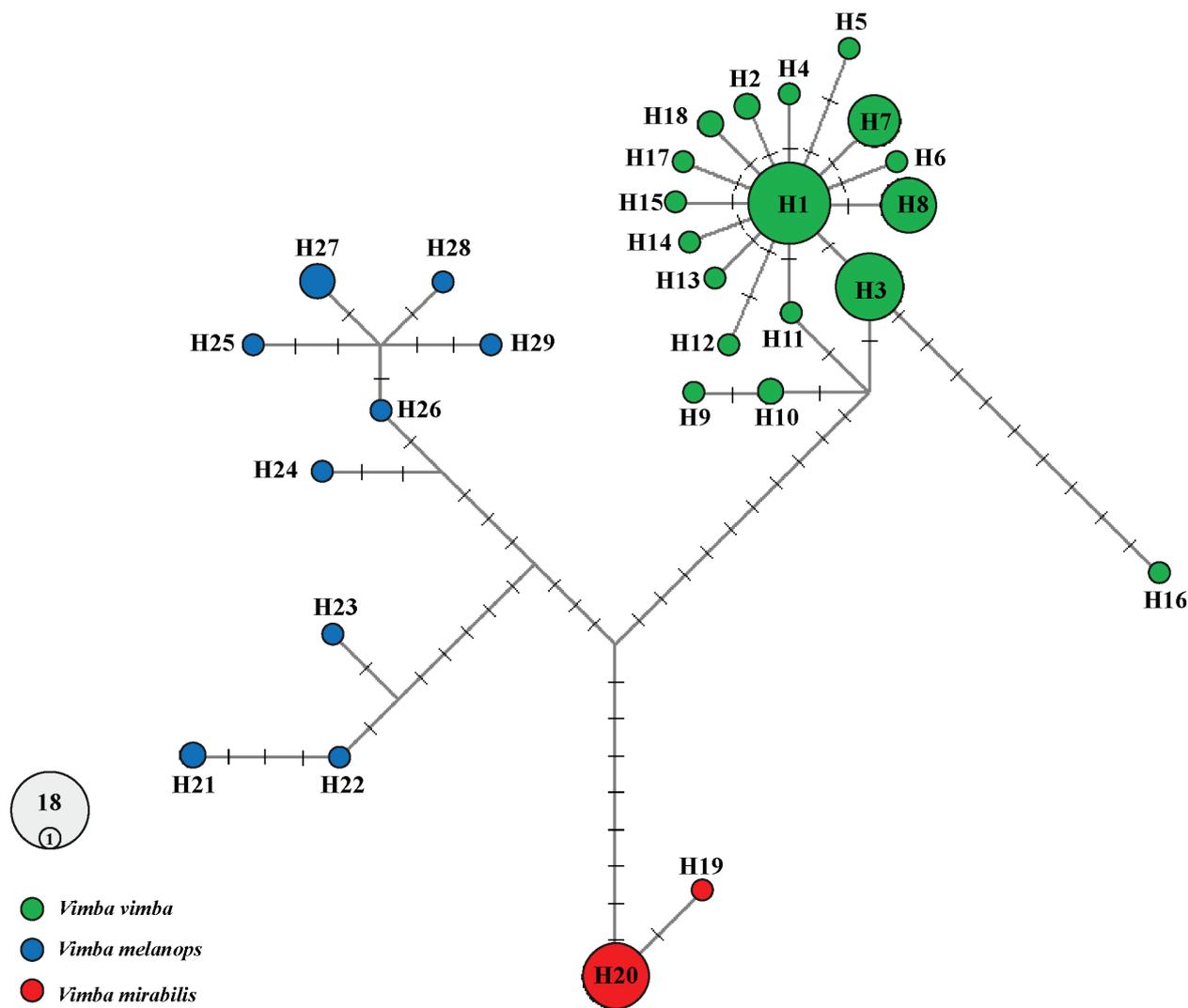


Figure 2. Median-joining network of the cyt b haplotypes. Circle size corresponds to sample size; one bar indicates an additional mutational step. Each small line represents one nucleotide difference.

populations, while the other clades comprise *V. mirabilis* and *V. melanops*. *V. vimba* is not monophyletic because the Western (Pontic) and Caspian basin haplotypes of *V. vimba* are located in two different clades. As shown in Fig. 4, the maximum likelihood and Bayesian inference analyses of concatenated data of mitochondrial cyt b and COI barcoding region trees were supported by high bootstrap values (>75) for the distinction of species and *Vimba vimba* lineages. According to the divergence time between the two main clades, the divergence among the *Vimba* species starts in 2.27 (0.57–4.02) mya, early Pleistocene (Gelasian), and differentiates into four clades in early Pleistocene (Calabrian) based on StarBEAST analysis (Fig. 5).

Discussion

The present study investigates the phylogeny and phylogeography of three *Vimba* species to provide the first comprehensive molecular study on the genus. Although some work has been conducted on *Vimba* species, there

are no comprehensive studies on their phylogeography, which is provided in the present study.

Here, the phylogeny constructed by ML and BI approaches is based on concatenated mtDNA cyt b and COI sequences of three *Vimba* species. The phylogenetic tree topology revealed that species were clustered into four well-supported clades (*V. vimba* (Western), *V. vimba* (Caspian), *V. melanops*, and *V. mirabilis*) with high-reliability BI Posterior probability value (BI = 0.98) although low ML bootstrap value (ML < 50). In *V. vimba*, two lineages are observed, the Caspian and the Western, monophyly of both group supported by high BI (0.98) value. *V. melanops* and *V. mirabilis* belong to different clades with high BP (97) and BI posterior probability (1.00) (Fig. 4). According to tree topology, a clear geographical pattern emerged, which is also supported by the median joining network. However, variability at cyt b and COI markers varied, the AMOVA analysis found almost all (88.34%) of the genetic variations observed for the cyt b gene occurred between species, while this value drops to 58.52% for the COI barcoding region.

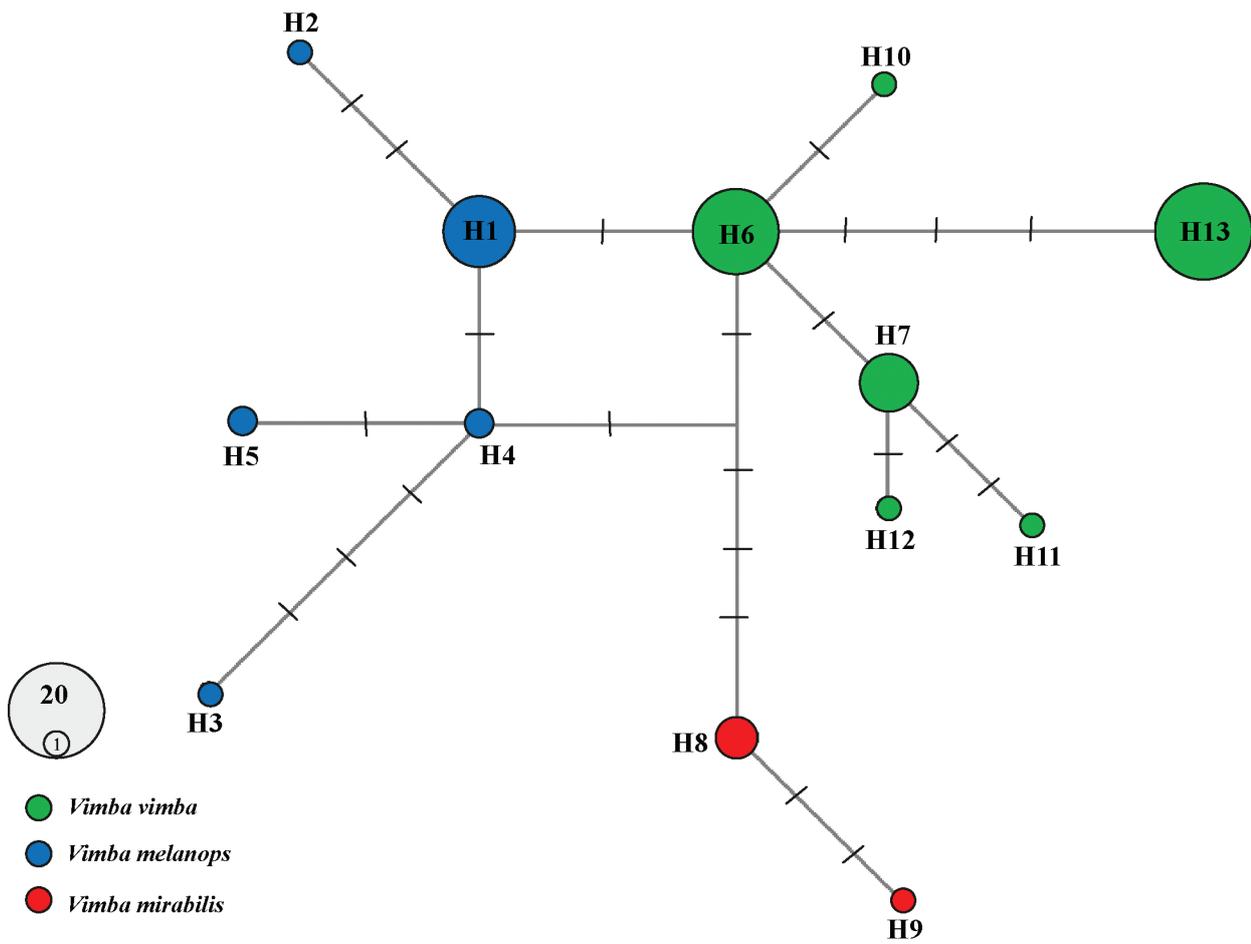


Figure 3. Median-joining network of the COI haplotypes. Circle size corresponds to sample size; one bar indicates an additional mutational step. Each small line represents one nucleotide difference.

Geiger et al. (2014) similarly reported that *V. vimba*, *V. melanops*, *V. mirabilis* are closely related species, with relatively low interspecific genetic distances, but they can be distinguished from each other by morphological characters. Species with high haplotype diversity and low nucleotide diversity are usually thought to originate through small founder populations during the expansion period, in accordance with previous analyses of Anatolian freshwater fish species (Hrbek et al. 2004; Bektas et al. 2017). A small independent evolution of similar origin (originating from the Pliocene/Pleistocene) may account for the low intraspecific diversity and corresponds with tectonic or glacial activity in the area. Anatolia and Central Europe were connected from the late Oligocene to the late Miocene (23–5.33 mya) (Popov et al. 2004). The estimated separation time of *Vimba* from sister genus *Blicca* was calculated as 6.16 (1.42–13.11) mya, a timeframe consistent with a dispersal from the Danube basin to Anatolia via river capture during this period (Levy et al. 2009). Similarly, Hänfling et al. (2009) estimated the time of separation of *Vimba* genus in Pleistocene as well. The divergence time between the two main clades shows that the divergence among the *Vimba* species started in 2.27 (0.57–4.02) mya, early Pleistocene (Gelasian) (Fig. 5).

According to Hänfling et al. (2009), *V. vimba* lineages were defined as Pontic and also Caspian clade, including Caspian Sea haplotype which is highly divergent from the other haplogroup. This suggests *V. vimba* originated from two refugial regions located in the Danubian drainage and the northern Pontic regions. Moreover, Hänfling et al. (2009), Naseka and Bogutskaya (2009), Jouladeh-Roudbar et al. (2015), and Esmacili et al. (2018) stated that the Caspian *Vimba* should be considered as a distinct species corresponding to *V. persa*, which is anadromous and endemic to the Caspian Sea. Similarly, in this study, it is supported that Caspian *Vimba* should be classified as *V. persa*, not *V. vimba* considering intra and interspecies divergence of *Vimba* species. Furthermore, both phylogenetic and StarBEAST analyses support Caspian *Vimba* corresponding to a different lineage from the Western lineage of *V. vimba*. However, advanced research and comprehensive sampling of Caspian and Western populations are required to clarify this. *V. vimba* in other regions has relatively low intraspecific diversity, except for the Caspian haplogroup. Natural distribution records for *V. vimba* in Europe, the former USSR, are most likely due to stock translocations and introductions from other areas due to their economic value (Freyhof 1999).

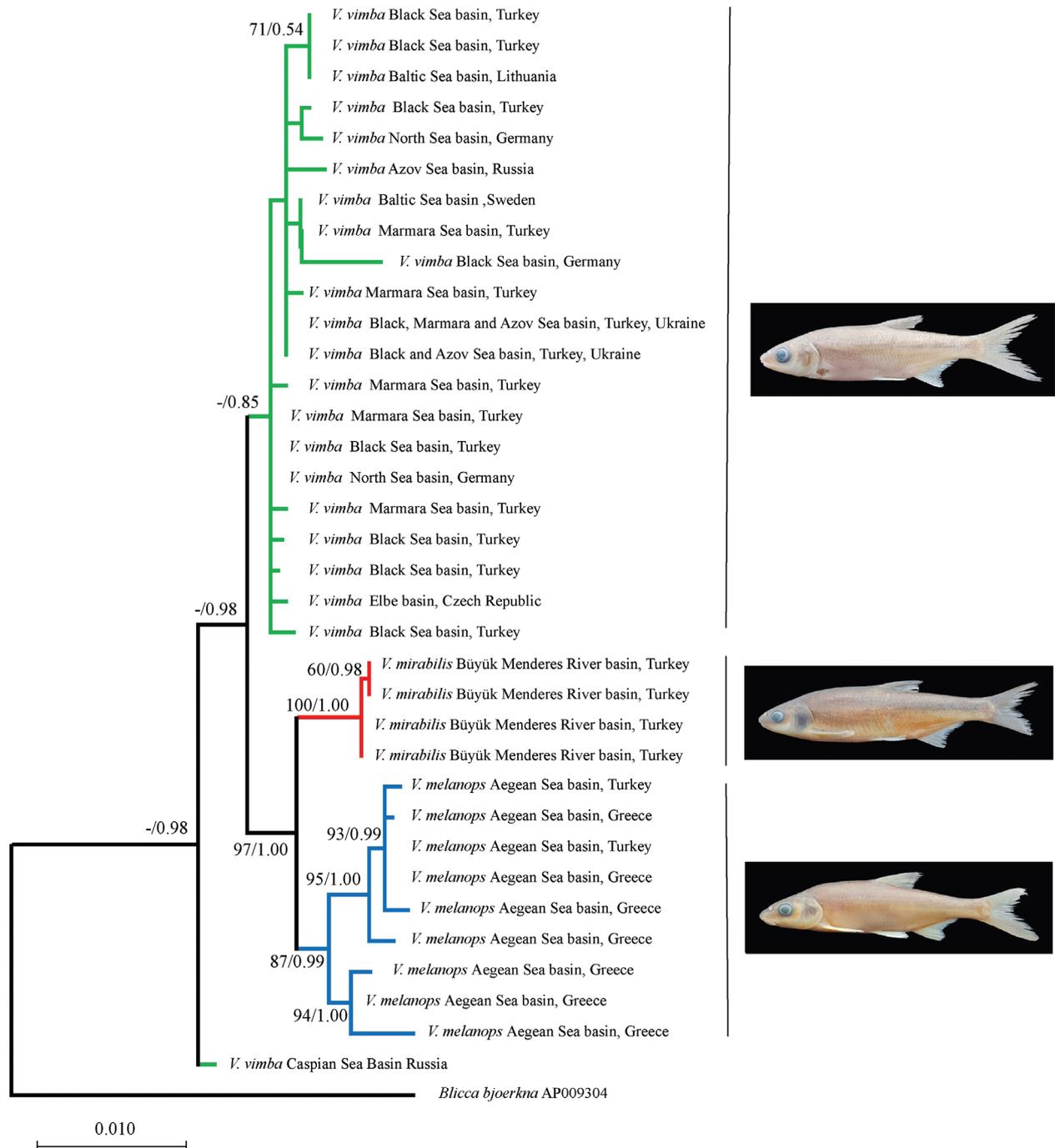


Figure 4. Maximum likelihood tree based on the two concatenated mitochondrial genes (cyt b and COI) (1675 bp) sequences of *Vimba* species. Maximum likelihood and Bayesian inference analyses resulted in congruent trees. Bootstrap and posterior probability values are shown above nodes on a tree if 50% or higher.

Phylogeography of *V. vimba* was investigated by Hänfling et al. (2009), and it reflected the presence of two refugia and recolonization of *V. vimba* as Caspian sea, and Western or Pontid clade, dating back 1–2 mya during the early Pleistocene. According to the present study, the separation of *Vimba*, dated in Pleistocene, occurred at 1.06 (0.2–2.55) mya. Correspondingly, in view of the timing, Pleistocene events must have played a central role in structuring the Balkan's marbled goby populations (Van-hove et al. 2012). In addition, multiples fish groups in

the Black and Caspian Sea basins display similar patterns of Pleistocene divergence such as the Black Sea roach, *Rutilus frisii*, salmon, barbell, and dreissenid mussels (Stepien et al. 2003; Kotlík et al. 2008; Ninua et al. 2018; Bartáková et al. 2019; Levin et al. 2019). Due to the migration of *Vimba* using brackish waters, it spread all over Eurasia also in Northern Germany and Netherlands, using the Danube River and old canal system (Freyhof 1999).

In the Early Pleistocene, the depression of the Marmara Sea and the uplift of the Aegean mountains contributed

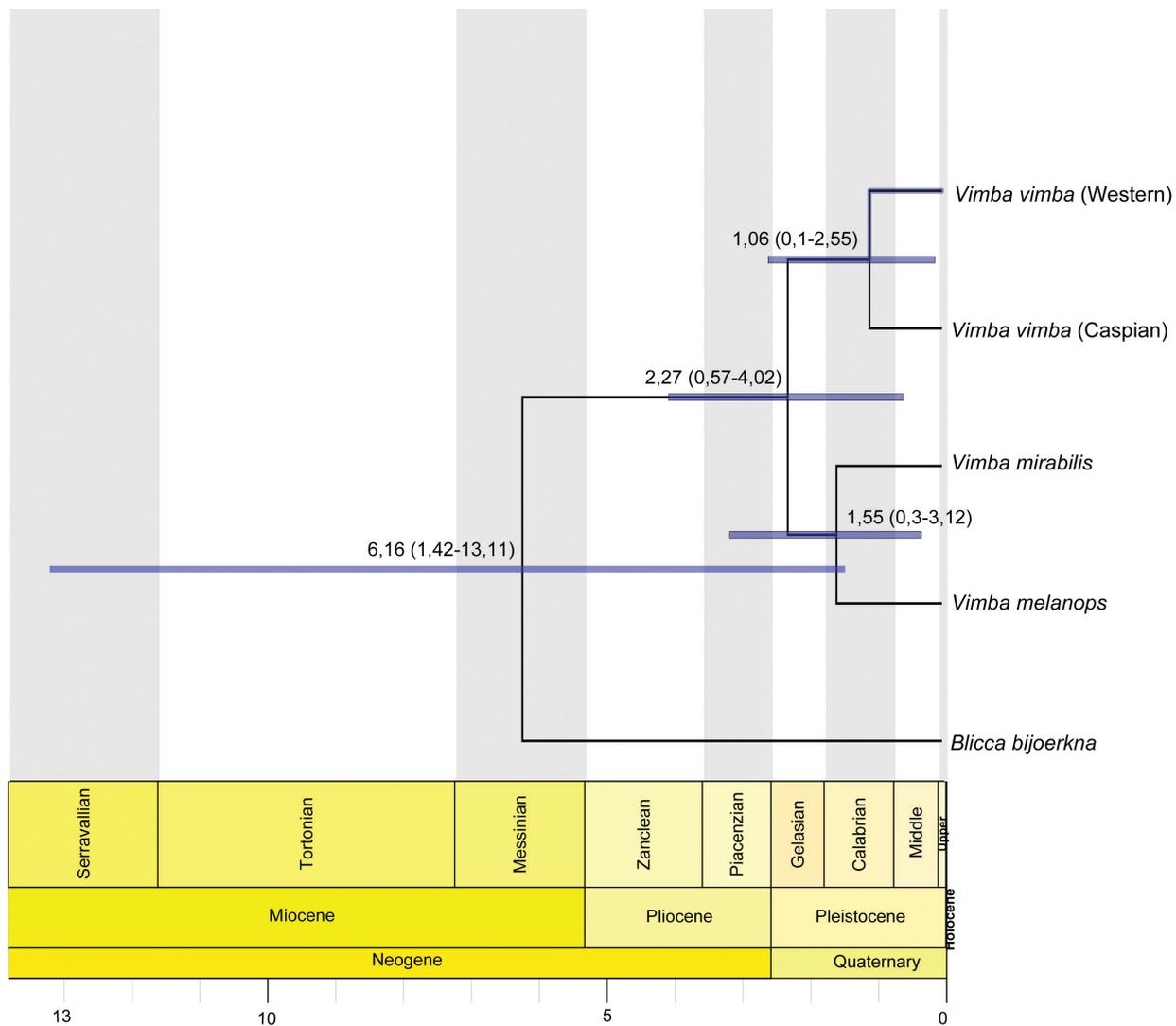


Figure 5. Divergence timescale for the *Vimba* species inferred under Bayesian strict clock method from two concatenated mitochondrial genes (cyt b and COI) (1675 bp) sequences. Numbers in front of the node represent divergence times in million years (Ma) and their HPD 95% credibility intervals.

greatly to the separation of *V. vimba* and *V. mirabilis*. Furthermore, while the water of the Büyük Menderes River was flowing in the north-south direction because of the western Anatolian Mountains barrier, fractures and foldings which occurred in the Early Pleistocene caused the river to turn west and take its present form. *V. mirabilis* is present in Bafa Lake because of alluvium brought in by the Menderes River that blocked the old sea gulf and separated it from the sea, creating Bafa Lake (Akçer-Ön et al. 2020).

Por, (1989) specified that a line drawn from west to east in the middle of Turkey could be considered as a major suture, which leads to distinct species of *Vimba* between the Büyük Menderes and northern Aegean. The differences between the Balkan and Anatolian species can primarily be associated with the formation of the Aegean in the late Pliocene (Kosswig 1955; Bilgin 2011). Also, Bektas et al. (2019) discovered that dispersal of *Alburnoides symrnae* and *Alburnoides economui* was dated in 5.42–2.31 Ma (Early Pliocene), when the former Aegeopotamus River

was a very large river that discharged the waters of Paratethys into the Aegean Sea. Divergence time estimated between *V. melanops* and *V. mirabilis* as 1.69 (1.07–2.38) mya is consistent with this event. Durand et al. (2000), Hewitt (2000), and Hewitt (2004) further highlighted that the Danube was an essential component of the Chub (*Squalius cephalus*) evolutionary history, especially during the Pleistocene glaciations as it acted as a refugium for this species. Our molecular data confirm this influence on the divergence of *V. melanops* and *V. mirabilis*.

Acknowledgements

I am pleased to thank Davut Turan and Cüneyt Kaya for providing samples. Also, I extend my special thanks to the Editor, Nicolas Hubert and two anonymous reviewers for their valuable comments and suggestions that greatly improved the manuscript.

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A new species of *Phalotris* (Serpentes, Colubridae, Elapomorhini) from Paraguay

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Academic editor: Johannes Penner ♦ Received 23 November 2020 ♦ Accepted 21 February 2022 ♦ Published 7 March 2022

Abstract

A new species of *Phalotris* from the *nasutus* group, *Phalotris shawnella* sp. nov., is described. It can be distinguished from the other members of the group by having the following combination of characters: 1) fifth supralabial in contact with parietal, 2) vertebral stripe present, 3) yellowish nuchal collar (2 or 3 dorsal scales long), 4) dull reddish color of head, 5) broad, solidly or near solidly dark, lateral bands, 6) red-orange ventral scales lightly and irregularly spotted with black mainly on the posterior half of the body and 7) a bilobed, extremely asymmetrical hemipenis, with enlarged, curved, lateral spines. The species is only known from a recent specimen collected in the Cerrado zone of northeastern Paraguay at Rancho Laguna Blanca, San Pedro department, and two photographic records of live specimens from this and an additional locality. Limited ecological data based on observations of a captive individual, and a wild record, are provided, and a conservation assessment is performed for this extremely limited range Paraguayan endemic snake.

Key Words

endemic, hemipenis, *Phalotris nasutus*, *Phalotris shawnella* sp. nov.

Resumen

Se presenta la descripción de una nueva especie de *Phalotris* del grupo *nasutus*, *Phalotris shawnella* sp. nov. puede ser diferenciada de otros miembros del grupo por tener la siguiente combinación de caracteres: 1) quinta supralabial en contacto con la parietal, 2) presencia de una estría vertebral oscura, 3) collar nuchal amarillento (de 2 ó 3 escamas de ancho), 4) coloración rojiza en la cabeza, 5) ancha faja lateral muy oscura o bastante oscura, 6) escamas ventrales rojo-anaranjadas e irregularmente salpicadas de negro principalmente en la parte posterior del cuerpo, y 7) hemipenes bilobados, extremadamente asimétricos, con espinas laterales agrandadas y curvas. La especie se la conoce únicamente de un ejemplar colectado en el Cerrado en el noreste de Paraguay en el Rancho Laguna Blanca, departamento de San Pedro, además de dos registros fotográficos de ejemplares vivos, uno de esta misma localidad y otro de una localidad adicional. Se proveen datos ecológicos limitados, en base a observaciones de un ejemplar en cautiverio, y otro en la naturaleza, así como una propuesta de evaluación del estado de conservación para este endemismo de Paraguay de rango extremadamente limitado.

Palabras claves

endémico, hemipene, *Phalotris nasutus*, *Phalotris shawnella* sp. nov.

Introduction

The genus *Phalotris* Cope, 1862 is a group of small to medium-sized, semi-fossorial snakes, distributed largely in open areas of Brazil, Bolivia, Paraguay, Uruguay, and Argentina (Ferrarezzi 1993). Though Cope (1862) described the genus, it was treated as a synonym of *Elapomorphus* Wiegmann, 1843 by most authors for over a century, until its revalidation by Ferrarezzi (1993) who considered it a monophyletic group close to *Apostolepis* Cope, 1862. More recently, Grazziotin et al. (2012) using five mitochondrial and three nuclear gene sequences showed *Phalotris* as the most primitive genus within the Elapomorphini, although this conclusion was only weakly supported.

The genus is poorly represented in museum collections, but 14 species are currently recognized separated into three species groups (Ferrarezzi 1993): the *tricolor* group of five species (Jansen and Köhler 2008), the *bilineatus* group with four species (Puerto and Ferrarezzi 1993; Cacciali and Cabral 2015) and the *nasutus* group which includes five species (Moura et al. 2013). Following Entiauspe-Neto et al. (2021) we do not recognize the recently-described *P. cerradensis* Silveira, 2020 of this latter group as a valid species.

Two synapomorphies distinguish the *nasutus* group: a pointed snout with prominent rostral shield; and fusion between the second and third series of temporal plates (sometimes on only one side of the head) (Ferrarezzi 1993; Moura et al. 2013). The five known species all occur in the Cerrado region of central South America: *Phalotris concolor* Ferrarezzi, 1993, *Phalotris labiomaculatus* Lema, 2002, *Phalotris lativittatus* Ferrarezzi, 1993, *Phalotris nasutus* (Gomes, 1915), and *Phalotris nigrilatus* Ferrarezzi, 1993.

The only species of the *nasutus* group currently recorded from Paraguay is the endangered endemic *P. nigrilatus*. This species was described from a single female specimen (FML 709) collected at “Carumbé”, in the Paraguayan department of San Pedro, in 1973. The redescription of this species, including a description of the hemipenes, by Cacciali et al. (2007), was based on two further specimens (MNHN[M] 89 and 91) from “Primavera”, also San Pedro department (as the holotype), that were collected in 1957. Subsequently, additional specimens of *P. nigrilatus* that had been collected at the latter locality between 1954 and 1959 (NHM 1955.1.5.99, 1955.1.6.2–3, 1956.1.3.48–51, 1956.1.16.39–40, 1958.1.2.31, 1960.1.3.5–8, 1962.110) were also located (Cacciali et al. 2016). Cacciali et al. (2020) summarized the morphological data of these specimens, providing images of the first live specimen and discussing variation in pholidosis of the species.

During field work at Rancho Laguna Blanca (San Pedro department, northeastern Paraguay) (Fig. 1) two specimens of a *Phalotris nasutus* group snake were captured, that exhibited intermediate characters between *P. nigrilatus* and *P. lativittatus* (the latter not previously recorded in Paraguay). With the photographing of



Figure 1. Known localities for *Phalotris shawnella* sp. nov. in San Pedro department, Paraguay Square: type locality. Circle: additional locality at Colonia Volendam.

an additional specimen at a second locality (Colonia Volendam, San Pedro department) we undertook an extensive revision of specimens and the literature, and it became apparent that the differences were consistent and clear enough to indicate species level differentiation. Here we describe this as a new species of *Phalotris* of the *nasutus* group.

Materials and methods

Measurements of cephalic scales were taken on the left side of the body with dial callipers (accurate to 0.1 mm) and body lengths were measured with millimeter tape. Body lengths include snout-vent length (SVL) and tail length (TL). Descriptions of coloration are provided for live and fixed specimens. Ventral scale counts follow Dowling (1951). Dorsal scale counts and terminology follow Peters (1964), recording reduction. For supralabial counts, numbers in parentheses are those scales that contact the orbit and, likewise for infralabial counts, the numbers in parentheses are the scales in contact with the chin shields. First and second rows of temporals were counted. Paired structures are presented in right/left orientation. The right hemipenis of the specimen was everted directly after euthanizing, and hemipenial terminology follows Zaher (1999). We follow Ferrarezzi (1993) for the designation of the groups. Behavioral data were collected from observations on a specimen kept briefly in captivity.

Geographic and morphological data used for comparisons with other species within the *nasutus* group were extracted from Ferrarezzi (1993) for *P. nasutus*, Ferrarezzi (1993) and Silveira Vasconcelos and Gomes dos Santos (2009) for *P. lativittatus*, Moura

et al. (2013) for *P. concolor*, and Hamdan et al. (2013) for *P. labiomaculatus*. A list of examined specimens of *P. nigrilatus* and *P. multipunctatus* is provided in Appendix 1. Museum codes in the appendix are as follows: NHM Natural History Museum, London, UK; CZPLT Colección Zoológica Para La Tierra, Pilar, Paraguay. Given that information on *P. labiomaculatus* is not provided for specific specimens, we used pholidosis traits of this species for comparison of ranges but not for correlations. Morphological data were used to explore the variation in ventral and subcaudal scales among the species in the group.

Geographic baseline data (high resolution elevation maps) were taken from Consortium for Spatial Information (CGIAR-CSI), based on SRTM30 images (30 seconds resolution), available at www.diva-gis.org/gdata (Jarvis et al. 2008). Ecoregion definition was based on Olson et al. (2001). GIS processing was performed in Quantum GIS 3.12.0.

Results

Phalotris shawnella sp. nov.

<http://zoobank.org/253E6610-D61C-49B7-B79F-0625C8FB72A5>

Type locality. Rancho Laguna Blanca, Departamento San Pedro, Paraguay (Fig. 1).

Holotype. CZPLT-H-594; adult male; collected during digging on 3 January 2014 (J-P. Brouard); Rancho Laguna Blanca, 23°48'43"S, 56°17'49"W (WGS 84), 204 masl, San Pedro department. Specimen complete but damaged during collection, being severed approximately at mid-body (Fig. 2).

Diagnosis. *Phalotris shawnella* sp. nov. is assigned to the *nasutus* group on account of the pointed snout with prominent rostral shield and the fusion of the second and third temporal plates (Fig. 3A). The new species can be distinguished from all other members of the *nasutus* group by the combination of the following characters: 1) fifth supralabial in contact with parietal, 2) vertebral stripe present, 3) yellowish nuchal collar (2 or 3 dorsal scales long), 4) dull reddish color of head in adults, 5) broad, solidly or near solidly dark lateral bands, 6) red-orange ventral scales lightly and irregularly spotted with black, mainly on the posterior half of the body, and 7) a bilobed, extremely asymmetrical hemipenis, with enlarged, curved, lateral spines.

Phalotris shawnella sp. nov. is differentiated from the individual members of the *nasutus* group and the only species known to occur sympatrically with it (*P. multipunctatus*) as follows. (The characteristics of *Phalotris shawnella* sp. nov. are given first, followed by the comparison species in parentheses):

Phalotris nasutus Gomes, 1915: 1) broad black lateral bands running the length of the body (absent in *P. nasutus* or present vestigially on the posterior part of the body only); 2) supralabial concolorous with head (supralabials

paler than head); 3) ventral coloration red-orange with blackish smudges on the lateral part of the ventral scales, and scattered larger irregular black blotches (ventral immaculate pink); 4) ventral scales 185 in the male (ventrals in males < 182); 5) hemipenis bilobed and greatly asymmetrical (hemipenis only slightly asymmetrical).

Phalotris lativittatus Ferrarezzi, 1993. Superficially closest to this species within the *nasutus* group, which shows little variation in appearance across the large range and specimen series available (H. Braz in litt.). It can be reliably distinguished from *P. shawnella* sp. nov. with the following characters: 1) Supralabials uniformly brownish red (supralabials pale in *P. lativittatus*); 2) infralabials uniformly grey (infralabials with some dark markings); 3) scattered dark spots along the sides of the ventral scales (uniform pale ventral scales); 4) broad lateral band solid or nearly solid (lateral band with broad pale scale edges along entire length); 5) small spines at the lower part of the hemipenis (larger spines all along the body of the hemipenis).

Phalotris nigrilatus Ferrarezzi, 1993. Geographically, this is the only species that approaches *P. shawnella* sp. nov. within the *nasutus* group and is the only species with which it shares two key characters: a solid dark lateral band and dark markings on the ventral scales. *Phalotris nigrilatus* is otherwise phenotypically strikingly different and the significant specimen series now available indicates that it is morphologically very conservative (Cacciali et al. 2020). 1) Presence of clear pale collar (absence of collar in *P. nigrilatus*); 2) Ventral dark markings irregular, diffuse and widely-spaced, mainly on the posterior half of the body (ventral dark markings dense and typically regular, at the edges of each ventral scale and along the entire underside); 3) Chinshields pale contrasting with dark infralabials (chinshields dark, not contrasting with infralabials); 4) Head brick red dorsally with brownish suffusions in adult (head black in adult); 5) thin spines on the hemipenis (thick spines).

Phalotris concolor Ferrarezzi, 1993: 1) fifth supralabial in contact with parietal (separated in *P. concolor*); 2) presence of broad dark lateral band (lateral coloration uniformly red); 3) indistinct black vertebral line (dorsal coloration uniformly red); 4) ventral scales 185 in the male (212 ventral scales in the only male known).

Phalotris labiomaculatus Lema, 2002. 1) Supralabials uniformly brownish-red, concolorous with rest of head (spotted black and white supralabials in *P. labiomaculatus*); 2) wide dark lateral band (body coloration uniformly orange with no dark lateral band); 3) yellow nuchal collar 2–3 scales wide (white nuchal collar 3–4 scales wide); 4) dark vertebral line present (no vertebral line); 5) irregular dark spots on the ventral scales (uniformly white ventral scales).

Phalotris multipunctatus Puerto & Ferrarezzi, 1993. This is the only species of *Phalotris* known to occur sympatrically with *P. shawnella*. 1) Scales of broad lateral bands lacking white spotted pattern (scales of broad lateral bands with white tips giving spotted pattern



Figure 2. Dorsal (left) and ventral (right) overviews of the holotype of *P. shawnella* sp. nov. The specimen was accidentally severed during collection. Scale bar: 2 cm. (Photograph by Jeremy Dickens).

in *P. multipunctatus*); 2) red-orange ventral scales lightly and irregularly spotted with black, mainly on the posterior half of the body (ventral scales black with broad white posterior edges forming banded pattern); 3) head brick red (in adult) or black (in juvenile) lacking any white spotting (head black with profuse white spotting); 4) infralabials uniform (each infralabial with a single large white medial spot); 5) longitudinal dark mid-dorsal stripe present (longitudinal dark mid-dorsal stripe absent).

Description of holotype. An adult male in two pieces, SVL 260 + 140 (=400) mm; TL 65 mm (16.25% of SVL); one preocular, two postoculars; temporals 0+1/0+1; loreal absent; supralabials 6(2–3)/6(2–3), fifth supralabial broadly contacts parietal; infralabials 7(1–5)/7(1–5), 1st to 4th contacting the anterior pair of chinshields and 4th to 5th contacting the posterior pair of chinshields;

posterior chinshields longer and thinner than anterior chinshields; dorsal scale rows 15-15-15; scales smooth lacking apical pits; 185 ventrals; anal plate divided; 35 paired subcaudals. Yellow nuchal collar two to three scales wide and posterior black collar one to two scales wide. Slight trace of incomplete anterior black collar, most evident laterally. Rostral prominent and wider than it is long (1.9 × 2.6 mm); nasal complete, twice as long as greatest width (2.2 × 1.1 mm), contacting the rostral anteriorly, the 1st and 2nd supralabial ventrally, the preocular posteriorly, and the internasal and frontal dorsally; paired internasals slightly wider than they are long (1.4 × 2.2 mm); the second temporal longer than wide (3.1 × 1.4 mm); preocular longer than wide (1.3 × 0.9 mm), contacting 2nd supralabial; two postoculars as long as wide (approximately 0.6 × 0.6 mm), the lower



Figure 3. Coloration in life of *P. shawnella* sp. nov. **A.** Detail of the head of the holotype (CZPLT-H-594); **B.** Dorsolateral view of the holotype; **C.** Juvenile topotype specimen, kept in captivity and which later escaped; **D.** Live specimen photographed at Colonia Volendam. (A–C photographed by Jean-Paul Brouard, D photographed by Marko Fast).

postocular contacting the 3rd to 5th supralabials and only slightly smaller than the upper; single prefrontal twice as wide as long (2.3 × 4.2 mm); supraocular twice as long as wide (2.2 × 1.2 mm); frontals slightly longer than they are wide (3.6 × 2.8 mm); paired parietals twice as long as wide (6.0 × 3.1 mm). No differences in shape in right/left sides. Eye diameter 1.1 mm.

Hemipenis morphology. Semicapitate and semicapitate (Fig. 4). Long, slender and bilobed, distinctly asymmetrical (right lobe -from asulcate view- 2/3 shorter), with enlarged, curved, lateral spines (13 to 15 large spines on each side). Sulcus furcation located in basal third and branches centrolinear. Note that right lobe (from asulcate view) is not fully everted, missing ca. 1 mm.

Color in life. Head brick red dorsally, with slightly darker suffusions, and uniformly brownish red on supralabials; red-orange ventrally with a greyish tinge to the first three infralabials, and whitish chin shields. A single row of scales on the posterior part of the dorsal surface of the head shows traces of a faint black anterior collar, mainly laterally. A broad yellow collar fades laterally and is followed by a thinner black collar (Fig. 3B). Neither collar is visible ventrally. Body coloration brick red dorsally, with a faint trace of a thin black vertebral line formed by a small dark spot anteriorly on each vertebral scale connected by a thinner black streak along the center of the scale that is variably conspicuous along the length of the body and fades out upon reaching the tail. Broad

black (to brownish-black) lateral lines running the length of the body from the posterior black collar to the tip of the tail. Lateral bands uniformly 3–3.5 scales wide over the entire length of the body, narrowing only slightly to 2 scales width on the tail. Ventrally red-orange, the edges of some of the ventral scales with small black, diffuse blotches (more prominent on the posterior ventral scales). Medial parts of the ventral scales are largely uniform apart from a very small number of often large black smudges, irregularly dispersed on the midbody region.

Color in preservative. Head fades to dull brownish (Fig. 2). Yellow collar to pinkish white. Black lateral and vertebral lines dark blackish-brown. Brick red of dorsum fades to brownish. Ventrally, the coloration is creamy-white, slightly yellower medially on the ventral scales and towards the tail, whiter laterally on the ventral scales and towards the head.

Variation. Described on the basis of an individual captured (on 9 December 2013) close to the collection locality of the holotype, which was photographed (Fig. 3C) and which later escaped. This individual was smaller and presumed to be juvenile female (weight 7 g), SVL 250 mm, TL 20 mm. It initially showed extensive black coloration on the head, broadly suffused with brick red dorsally, but the red areas became more prominent over its few months in captivity – a possible ontogenic change. The yellow nuchal collar was broader (3 to 4 scales wide) and paler, being creamy yellow as opposed to orange-yellow. The



Figure 4. Hemipenis of *P. shawnella* showing the asulcate (left) and sulcate (right) views. Gray bar = 3 mm. (Photograph by Paul Smith).

posterior black nuchal collar was absent dorsally, with the black mid-dorsal line forming a broad, smudgy spot covering an entire scale where it contacted the yellow nuchal collar, and bordered either side by a single orange scale between it and the black lateral bands. Dorsally, it was a deeper red, and ventrally it was a deeper orange-red, than the holotype. Dorsals 15-15-15, ventrals 197, subcaudals 26 (divided), temporals 0+1/0+1. Additional images of the known specimens are stored in FigShare (<https://doi.org/10.6084/m9.figshare.c.5804246.v1>).

Etymology. The species name is a combination of the first names of two remarkable young people who were born around the same time as Fundación Para La Tierra, and who inspired its founders to work towards the study and conservation of the Paraguayan fauna, so that one day they might inherit a better world: Shawn Ariel Smith Fernández and Ella Bethany Atkinson. The epithet is not Latin, is invariable (word in apposition) and is made up of elements of both of their names.

Ecology. The holotype was collected by day in Cerradón forest on a sandy substrate, close to disturbed bushy Cerrado and within 500 m of a lake shore (Fig. 5). The topotype (juvenile female) was kept in captivity for ecological observations, but unfortunately later escaped. Nine days after capture (on 18 December 2013) it was offered a Gymnophthalmid lizard (*Vanzosaura rubricauda*) with 0.7 g mass and 55 mm total length, which it had consumed by the following day. On 22 December 2013 it was offered another *V. rubricauda*, of similar size, and by the next day the tail of the lizard was missing; it later consumed the rest of the lizard on 25 December 2013. Two days later, the snake drank water.

A third individual showing the clear diagnostic characters of this species (Fig. 3D) was photographed in leaf litter 500 m outside of the settlement of Colonia Volendam (24°16'28.6"S, 57°01'25.3"W), San Pedro department (Fig. 1) by Marko Fast on 10 March 2019, at 13:36 h, in a small patch (0.29 km²) of degraded Cerradón forest. When encountered, the



Figure 5. Collection locality of holotype, Rancho Laguna Blanca, San Pedro department, Paraguay. (Photograph by Para La Tierra).

individual had both the head and tail hidden under the leaf litter. The specimen did not show any aggressive behavior, and was released after being photographed.

Discussion

Phalotris shawnella sp. nov. is a distinctive new species of *Phalotris* and is only the second member of the genus to show dark mottling on the ventral side – a character previously considered to be an autapomorphy of *P. nigrilatus* (Cacciali et al. 2007). In fact, with the discovery that the rostral and prefrontal scale contact is a variable character (Cacciali et al. 2020), the only

remaining autapomorphies of *P. nigrilatus* are the black head and the lack of a nuchal collar. *Phalotris shawnella* sp. nov. has less dark and less regular black pigmentation on the belly than *P. nigrilatus*, suggesting that it is perhaps a transitional form between this last species and the other members of the *nasutus* group. Indeed, *P. shawnella* sp. nov. is, in many ways, morphologically intermediate between *P. nigrilatus* and *P. lativittatus*. All three species (*P. lativittatus*, *P. shawnella* sp. nov., and *P. nigrilatus*) also have a low number of ventral scales when compared to other *Phalotris*, with only *P. nasutus* having less. Both *P. lativittatus* and *P. nigrilatus* are known from respectable specimen series that show they are both morphologically conservative and show minimal

variation (H. Braz pers. com.; Cacciali et al. 2020), whilst the three known examples of *P. shawnella* sp. nov. also share the same consistent diagnostic characteristics at both localities at which they have been recorded.

The *nasutus* group has not been the subject of any recent phylogenetic assessment, perhaps because of the relative scarcity of available specimens. The closest approach using molecular datasets, rendered a monophyletic clade that included *P. nasutus* and *P. lativittatus*, as separate from sampled members of the *tricolor* and *bilineatus* groups (Grazziotin et al. 2012; Figueroa et al. 2016). Ferrarezzi (1993) proposed a phylogenetic hypothesis based on morphology (mostly coloration patterns), and presented a cladogram, with *P. nigrilatus* as the most derived taxon, and *P. concolor* as the basal member of the *nasutus* group. Using the same traits, and including the new species described after Ferrarezzi (1993), we are able to complement that hypothesis (Fig. 6). Thus, we consider *P. labiomaculatus* to be a sister clade to the group of species exhibiting loss of the 1st temporal scale. *P. shawnella* sp. nov., together with *P. nigrilatus*, are the most derived members of this phylogeny.

P. shawnella sp. nov. is somewhat morphologically intermediate between *P. nigrilatus* and *P. lativittatus*, however hybridization between these two species can be conclusively ruled out. *P. nigrilatus* and *P. lativittatus* exhibit widely allopatric ranges, while the occurrence of three individuals showing characters of the new species at two different localities at which neither of these putative “parent species” has ever been recorded stretches plausibility. It is important to add that the coloration of *P. nigrilatus* and *P. shawnella* sp. nov. is apparently rather constant (Cacciali et al. 2020). Molecular genetic analyses will be required to reveal whether these two Paraguayan taxa are relicts of a wider ancestral distribution of the group. Currently GenBank has genetic sequences for only two species in the *nasutus* group (*Phalotris nasutus* and *P. lativittatus*), thus efforts still need to be made to collect tissue samples from the remaining species. Unfortunately, snakes are among the least sampled animals, and data on genetics is missing not only within the genus *Phalotris*, but also for most of the Paraguayan snakes (Cacciali et al. 2019).

The two known localities for *P. shawnella* sp. nov. (Laguna Blanca and Colonia Volendam) are separated by just 90.5 km, indicating an extremely restricted global range within a single Paraguayan department. Given that this is potentially a forest species and that the area in which it is known to occur is an agricultural matrix undergoing constant alteration, we suggest that this Paraguayan endemic snake is in need of urgent conservation action. A designation of Endangered (B1a,biii) fits the available data, this being a species with an estimated occurrence of less than 5000 km² and with a severely fragmented range that is known to exist at less than 5 localities and with a continuing decline inferred from the extent and quality of the habitat. Rancho Laguna Blanca, where the holotype was collected, was formerly officially protected (for a period of five years) as a Reserva Natural (Natural

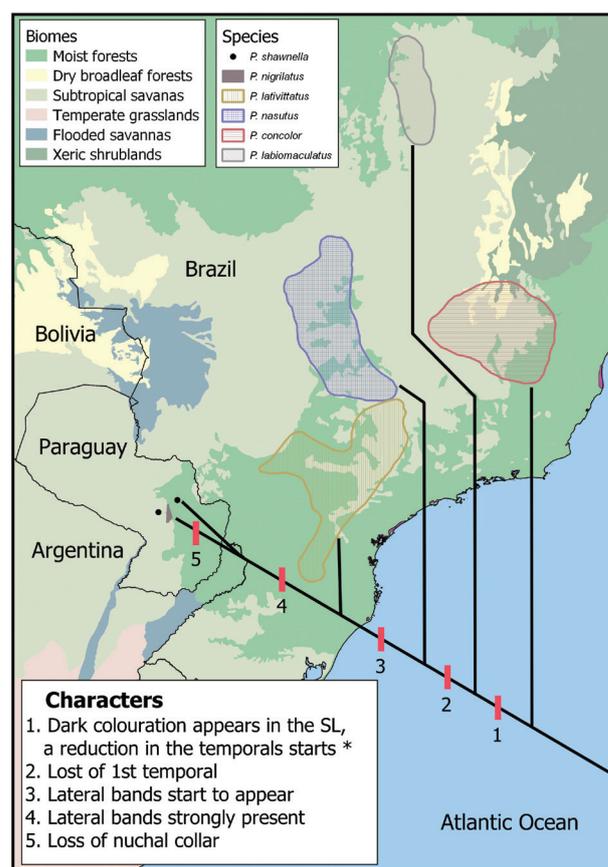


Figure 6. Suggested hypothesis of phylogenetic relationships among species within the *Phalotris nasutus* group, based on a first proposal by Ferrarezzi (1993). This hypothesis shows a probable change in characters during evolution. *Note that the reduction of 1st temporals is visible in *P. labiomaculatus* where the 1st temporal is rather small or absent in some specimens (Hamdan et al. 2013). Base map: biomes of the world, according to Olson et al. (2001)

Reserve) and given its high herpetological diversity it was recognized as the first Paraguayan Important Area for the Conservation of Amphibians and Reptiles (Smith et al. 2016). However, the property no longer counts with official protection and is currently for sale.

Phalotris nigrilatus and *P. shawnella* sp. nov. are two endangered (Cacciali et al. 2020), endemic Paraguayan snakes with extremely isolated ranges within San Pedro department. Additional field surveys are urgently required to improve our understanding of the ecological requirements of both taxa so that effective conservation measures can be implemented.

Acknowledgements

We thank Karina Atkinson, Para La Tierra and Malvina Duarte for their support for research activities and their commitment to biodiversity conservation in Paraguay. Henrique Braz and Hugo Cabral provided valuable comments that helped to improve the manuscript. PS and PC are grateful to Consejo Nacional de Ciencia y Tecnología through the PRONII

programme for financial support. Marko Fast generously shared his photographs and observations to help confirm the validity of this new taxon. We thank Jeremy Dickens for the photographs of the preserved specimen, and Martha Motte, Nicolás Martínez, and Frederick Bauer, from the Herpetology section of the Museo Nacional de Historia Natural del Paraguay (MNHN) for their stewardship of an important part of the Paraguayan heritage, and collaborations on scientific research. Particular thanks to Normand David for his expert advice on the presentation of the scientific name. Collection permit issued by Ministerio del Ambiente (MADES), N° 02/13.

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Appendix 1

Examined specimens

Phalotris nigrilatus (11): PARAGUAY, SAN PEDRO DEPARTMENT: Colonia Primavera (NHM 1955.1.5.99, 1955.1.6.2–3, 1956.1.3.48, 1956.1.16.39–40, 1958.1.3.30, 1960.1.3.5–8).

Phalotris multipunctatus (2): Rancho Laguna Blanca (CZPLT 1138, 1145).

Phalotris shawnella sp. nov. (3): Rancho Laguna Blanca (CZPLT 594), one live topotype from Rancho Laguna Blanca and photographs of a third individual from Colonia Volendam.

A new genus name for pygmy lorises, *Xanthonycticebus* gen. nov. (Mammalia, Primates)

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<http://zoobank.org/AF9D1D54-F1DA-44BD-9F57-47058914BEA7>

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Academic editor: Melissa T.R. Hawkins ♦ Received 10 April 2021 ♦ Accepted 7 March 2022 ♦ Published 23 March 2022

Abstract

Lorisiformes are nocturnal primates from Africa and Asia with four genera, with two (*Arctocebus* and *Loris*), three (*Perodicticus*) and nine (*Nycticebus*) recognised species. Their cryptic lifestyle and lack of study have resulted in an underappreciation of the variation at the species and genus level. There are marked differences between the pygmy slow loris *Nycticebus pygmaeus* and the other *Nycticebus* species and, in the past, several authors have suggested that these may warrant recognition at the generic level. We here combine morphological, behavioural, karyotypical and genetic data to show that these contrasts are, indeed, significantly large and consistent. We propose *Xanthonycticebus* gen. nov. as a new genus name for the pygmy slow lorises and suggest a common name of pygmy lorises. Based on analysis of complete mitochondrial DNA sequences, we calculate the divergence of pygmy from slow lorises at 9.9–10.0%. The median date, calculated for the divergence between *Xanthonycticebus* and *Nycticebus*, is 10.5 Mya (range 4.9–21.0 Mya). *Xanthonycticebus* differs from *Nycticebus* by showing sympatry with other slow loris species, by habitually giving birth to twins, by showing seasonal body mass and whole body coat colour changes (absent in other species living at similar latitudes) and a multi-male, multi-female social system. Pygmy lorises are easily recognisable by the absence of hair on their ears and more protruding premaxilla. *Xanthonycticebus* is threatened by habitat loss and illegal trade despite legal protection across their range and all slow lorises are listed on appendix 1 of CITES. The suggested nomenclatural changes should not affect their legal status.

Key Words

conservation, cytotaxonomy, Lorisidae, Lorisiformes, primate taxonomy, Strepsirrhini

Background

Lorisiformes are a group of nocturnal primates with two genera, *Perodicticus* Bennett, 1831 (three species) and *Arctocebus* Gray, 1863 (two species) occurring in west and equatorial Africa and two, *Loris* É. Geoffroy, 1796 (two species) and *Nycticebus*, É. Geoffroy, 1812 (nine species) occurring in south, east and southeast Asia (Groves 2001; Nekaris 2013; Rowe and Meyers 2016). In west-central Africa, *Perodicticus edwardsi* Bouvier, 1979 and *Arctocebus* occur in sympatry and in eastern Indochina, *Nycticebus bengalensis* (Lacépède, 1800) and *N. pygmaeus* Bonhote, 1907 occur in sympatry. All other species have allopatric distributions.

At a major international conference on nocturnal primates in 1993, Schwartz and Beutel (1995: 189), at a time when only two species of *Nycticebus* were recognised, commented that “*N. coucang* and *N. pygmaeus* are species that are remarkable for their variability”. Since then, a considerable amount of comparative research has been conducted on the slow lorises, including morphologically (e.g. Ravosa 1998; Groves 2001; Nekaris and Jaffe 2007; Munds et al. 2013; Xie et al. 2013 7), behaviourally (e.g. Fitch-Snyder and Ehrlich 2003; Nekaris et al. 2008; Nekaris et al. 2010; Streicher et al. 2012; Ni et al. 2020; Poindexter and Nekaris 2020) and genetically (e.g. Chen et al. 2006; Perelman et al. 2011; Pozzi et al. 2015; Munds et al. 2018; Munds et al. 2021). Combined, these studies

allow us to gain a better understanding of the species and higher-level taxonomy. Recent molecular phylogenetic research has revealed the divergence between genera and between species and, from this, it is evident that one species, the pygmy slow loris *N. pygmaeus* is anomalous. In combination with karyotypical, behavioural and morphological data, this supports the conclusion that this species is best placed in its own genus.

Although under the Code (International Commission on Zoological Nomenclature 1999), Art. 13.1, we are not obliged to provide a description of a new taxon (it would suffice to provide a bibliographic reference to earlier descriptions), we feel that, in this instance, it may be opportune to give a generic diagnosis.

Order Primates Linnaeus, 1758

Suborder Strepsirhini É. Geoffroy Saint-Hilaire, 1812
Family Lorisidae Gray, 1821

Xanthonycticebus gen. nov.

http://zoobank.org/16F2DB84-82CD-44B9-B9A8-30A8BA64BD20

Diagnosis. Morphological synapomorphies to *Xanthonycticebus* include: (i) skull length consistently less than 55 mm, (ii) diastema between P² and P³, (iii) long black ears, hairless at the tips (iv) relatively narrow interorbital distance compared to *Nycticebus* and (v) full seasonal coat colour change including almost complete loss of dorsal stripe (Fig. 1). The species is furthermore distinguished from *Nycticebus* species by giving birth habitually to

twins, frequent sympatry with *N. bengalensis* (sensu lato) and more rapid locomotion. Regarding multiple births, this trait occurs with varying frequency in primates. Most of the marmosets and tamarins are polyovulatory and twins are the dominant litter size in the wild and most twins are considered dizygotic (Ward et al. 2014; Wahab et al. 2015). Old World monkeys, apes and humans are monovulatory species and while single births are the rule, multiple births do occasionally occur in various species, typically at a rate at, or below one percent (Geissmann 1990). Around two-thirds to three-quarters of these twins are estimated to be monozygotic (Geissmann 1990). This contrasts with twinning in strepsirrhines, as here all, or practically all, are dizygotic (Pasztor and Van Horn 1979). No other species of slow lorises are known to be sympatric, with their distribution similar to gibbons Hylobatidae Gray, 1870 and langurs Presbytina Gray, 1825, which, even though having more recent evolutionary histories, contain multiple genera (Rowe and Meyers 2016). Where nocturnal primate genera or species are sympatric, different locomotor strategies have evolved, allowing reduced competition (Charles-Dominique 1977). Additional differences, as well as those from *Loris*, are summarised in Table 1.

Etymology. The genus name *Xanthonycticebus*, masculine, refers to the species orange/ish overall colouration and their nocturnal activity pattern; Xanto, Gr. Yellowish-orange; nykt-, Gr., night; kêbos, Gr., monkey (Gainsford 2020). Currently, the most frequently used common name of this genus is pygmy slow loris, followed by the rarely used lesser slow loris or intermediary slow loris. For the common English name, we suggest pygmy loris in order to differentiate the new genus from

Table 1. Summary of key similarities and differences amongst the three Asian lorisiform genera.

	<i>Loris</i>	<i>Nycticebus</i>	<i>Xanthonycticebus</i>	Reference
Latitudinal range	6°N–20°N	8°S–28°N	10°N–25°N	Ravosa (1998)
Altitudinal range (asl)	0–2,000 m	0–2,400 m	50–1,500 m	Nekaris (2013)
Twins	Rare but occasional	Absent or very rare	Habitually	Fitch-Snyder and Ehrlich (2003)
Torpor	Absent	Present	Present	Streicher and Reinhardt (2020)
Venomous	Absent	Present, 68 volatile and semi-volatile components	Present, 200 volatile and semi-volatile components	Hagey et al. (2007)
Seasonal body mass change	Absent	Absent	Present	Streicher (2004)
Seasonal coat colour change	Absent	Dorsal stripe shortens in some species	Full coat and dorsal stripe change	Streicher (2004); Nekaris, unpubl. data.
Species	Two	Nine	One, possibly two	Rowe and Meyers (2016)
Body size, range	120–330 g	265–2200 g	360–580 g	Nekaris (2013)
Ears	Haired, larger than in <i>Nycticebus</i> or <i>Xanthonycticebus</i>	Haired and small often with tufts	Ear length intermediate and naked at tips	Osman Hill (1953)
Multi-male, multi-female social system	Present	Absent	Present	Poindexter and Nekaris (2020)
Gestation	160–170 d	184–197 d	184–200 d	Fitch-Snyder (2020)
Molar size	M ² larger than M ¹	M ¹ larger than M ²	M ² larger than M ¹	Osman Hill (1953)
Karyotype and nucleolus organiser regions (NORs)	2n = 62	2n = 50; NORs on chromosome 1, 6, 9, 15 and 23	2n = 50; NORs on chromosome 6, 9 and 15	Chen et al. (1993); Goonan et al. (1995)
Third hand pad	Smallest	Intermediate or small	Largest	Osman Hill (1953); Nekaris, unpubl. data.
Snout	Narrow and pointy	Broader and more rounded	Broader than <i>Loris</i> , but longer premaxilla than <i>Nycticebus</i>	Osman Hill (1953)
Interorbit	Narrowest	Widest	Intermediate	Ravosa (1998)
Ocular axial and corneal diameter	AD – 14.0 mm; CD – 12.0 mm	AD – 15.7 mm; CD – 12.1 mm	AD – 15.5 mm; CD – 12.3 mm	Ross and Kirk (2007)

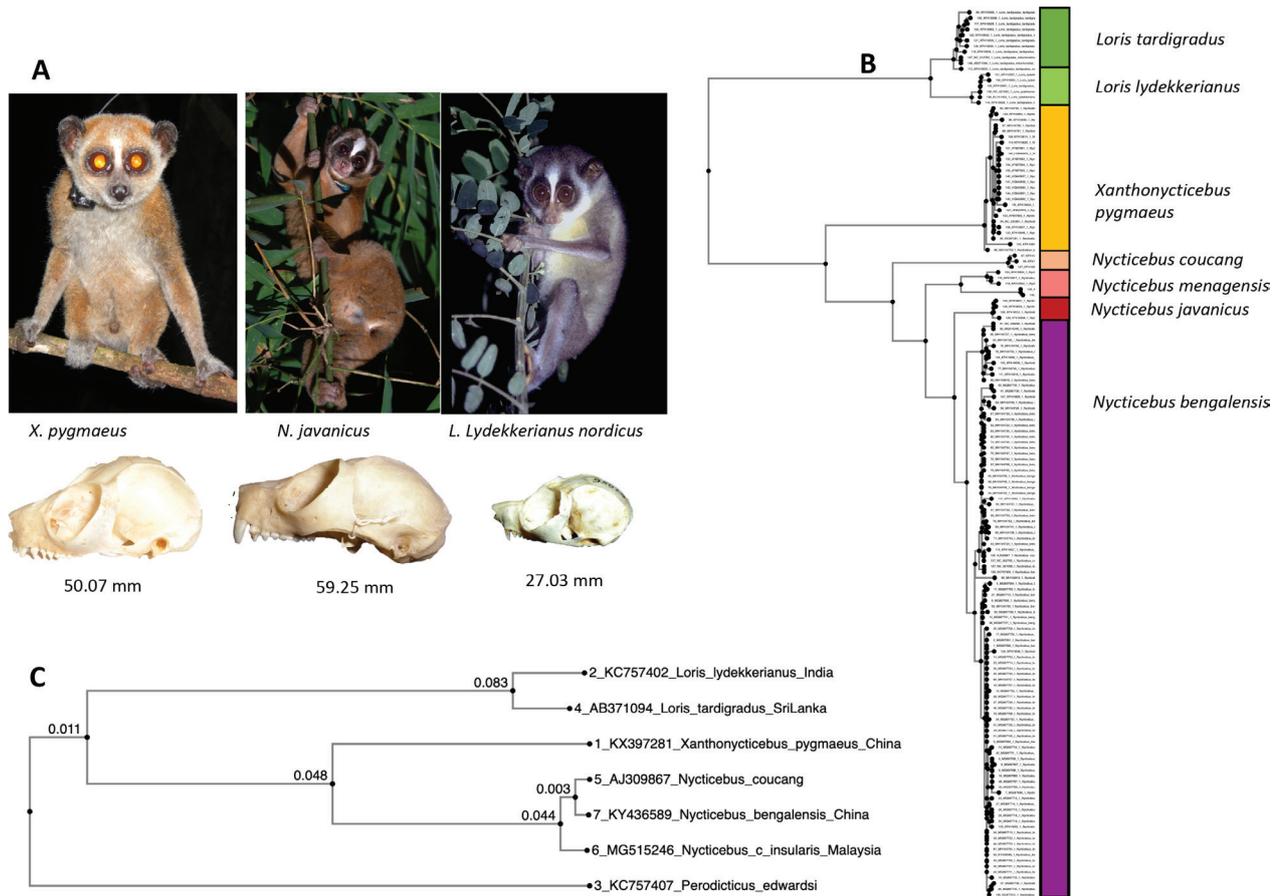


Figure 1. Characteristics of pygmy loris *Xanthonycticebus pygmaeus* gen. nov. **A.** Photograph of wild adult male *X. pygmaeus* from Mondulkiri District, Cambodia and skull from Li Chau, Vietnam (FMNH 32499), compared with *Nycticebus javanicus* from Garut Regency, Indonesia and skull (RMNH14563) from South Java, Indonesia; and with *Loris lydekkerianus nordicus* from Trincomalee District, Sri Lanka and skull (FMNH95029) from Jaffna District, Sri Lanka. Features distinctive to *Xanthonycticebus* include yellowish-orange colour, mid-broad snout with long premaxilla, M² larger than M¹ and ears hairless at the tips; **B.** Neighbour-joining tree of 175 cytochrome b sequences (alignment 1,068 bp) of *Nycticebus*, *Xanthonycticebus* and *Loris*; **C.** Neighbour-joining tree of complete mtDNA sequences of *Nycticebus*, *Xanthonycticebus* and *Loris*, with *Perodicticus* as outgroup, showing considerable divergence of *Xanthonycticebus* from *Nycticebus*. All photographs courtesy of K.A.I. Nekaris.

the two other loris genera (slow and slender lorises). We acknowledge, however, that with the recognition of *N. menagensis* Munds, Nekaris and Ford 2013, from Borneo, with a minimum adult body mass of 265 g, the small size is no longer a unique feature of the pygmy loris. The most commonly-used name for pygmy lorises in Vietnamese is Cu li nhỏ, in Mnong, it is Tau kless, in Lao, it is Linh lom and in Chinese, it is 小懶猴 / Xiǎo lǎn hóu (Nijman and Nekaris 2016; Thach et al. 2018).

Contents. a single species, *Xanthonycticebus pygmaeus* (Bonhote, 1907) is currently recognised and *Nycticebus intermedius* Dao Van Tien, 1960 and the not formally described *N. chinensis* are treated as synonyms. There is clear clinal latitudinal variation in body size and craniofacial size (smaller in the north) (Ravosa 1998). Variation in pelage colourations, coupled with a considerable amount of genetic divergence between available sequences deposited in GenBank (e.g. up to 2.0% in *cytb*; Fig. 1), largely from specimens without exact geographic locality data, may lead to the recognition of additional species in the future. Pozzi et al. (2020), based on monophyletic northern and southern populations of pygmy lorises from

Laos PDR, Cambodia and Vietnam, advocate more research to confirm if these are, indeed, two species.

Mein and Ginsburg (1997) tentatively described a single third upper molar M³ (T Li 41) from Li Mae Long in Lamphum Province, Thailand dated to the early Miocene, 17–18 Mya, as *?Nycticebus linglom* Mein & Ginsburg, 1997. The small size (1.29 × 1.82 mm) shows affinities with *X. pygmaeus*, but absence of a hypocone and a metaconule on M³ on T Li 41 aligns it closer to *N. bengalensis* than to *X. pygmaeus* and Li Mae Long is situated west of the Mekong River, outside the current distribution range of *X. pygmaeus*. We suggest to retain *?Nycticebus linglom* within the genus *Nycticebus*.

The holotype of *X. pygmaeus* is a juvenile male collected by J. Vassal on 13 November 1905 in Nha Thrang Vietnam [12.24, 109.19], that is currently stored in the Natural History Museum London under registration number 1906.11.6.2. It is described in detail by Bonhote (1907).

Divergence and molecular clock dates. Several molecular phylogenetic studies have been conducted that included samples of *X. pygmaeus* and two or more other *Nycticebus* species; in all analyses, *X. pygmaeus* is the first

Table 2. Estimates of the timing of the split between *Nycticebus* and *Xanthonycticebus* (in Million years ago, range is expressed as the 95% highest posterior density of divergence time estimates).

Type (bp)	<i>Nycticebus</i> species included in calculation	Split (mean, range), Mya	Reference
Mitochondrial genes			
Cytochrome b (1140)	<i>javanicus</i> / <i>bengalensis</i> / <i>cougang</i> / <i>menagensis</i>	10.9 (7.6–14.5)	Pozzi et al. (2015)
Cytochrome b + cytochrome oxidase subunit 1 (536)	<i>cougang</i>	26.4 (13.1–39.7)	Munds et al. (2018)
Nuclear genes			
18 gene regions (9,500)	<i>cougang</i>	6.4 (3.5–10.1)	Horvath et al. (2008)
54 gene regions (34,927)	<i>bengalensis</i> / <i>cougang</i>	10.2 (5.4–15.1)	Perelman et al. (2011)
Melanocortin 1 receptor (729)	<i>bengalensis</i> / <i>cougang</i>	12.0	Munds et al. (2021)
Recombinant activation gene 2 intron (716)	<i>cougang</i>	14.5 (6.0–24.9)	Munds et al. (2018)
Mitochondrial and nuclear genes			
4 genes (<i>cytb</i> , <i>col1</i> , <i>rag2</i> , <i>MC1R</i>) (1983)	<i>cougang</i>	18.4 (10.2–26.9)	Munds et al. (2018)

group to split, thus forming two distinct reciprocal monophyletic groups. Our own analysis, based on the complete mitochondrial genome sequences of *Xanthonycticebus* (*X. pygmaeus* GenBank Accession #: KX397281), two species of *Loris* (*L. lydekkerianus* KC757402 from India and *L. tardigradus* AB371094 from Sri Lanka), three *Nycticebus* (*N. bengalensis* KY436589 from China, *N. c. insularis* MG515246 from Malaysia and *N. cougang* AJ309867 from an unknown location) with *P. edwardsi* KC757407 from Cameroon as an outgroup, likewise shows a genetic distance of 9.9–10.0% between *X. pygmaeus* and the three other *Nycticebus* species (Fig. 1).

The divergence time between *X. pygmaeus* and the other *Nycticebus* species was estimated at between 6.4 Mya and 26.4 Mya (Table 2). Pozzi et al. (2015) commented that the gap of around six million years between the divergence of *X. pygmaeus* and the radiation of the other *Nycticebus* species may lend support to the distinction of *X. pygmaeus* at the generic level. Using data from Perelman et al. (2011), it is evident that the split between *Nycticebus* and *Xanthonycticebus*, by them estimated at 10.2 Mya (95% CI range 5.4–15.1 Mya), considerably predates several widely acknowledged generic splits within the Order Primates, including those between geladas *Theropithecus gelada* and baboons *Papio* spp. / mangabays *Lophocebus* (4.1 Mya; 3.4–4.7 Mya), between *Semnopithecus* and *Trachypithecus* langurs (4.1 Mya; 2.9–5.4 Mya), between *Mico* and *Cebuella* marmosets (4.8 Mya; 2.9–7.2 Mya) and, indeed, between humans *Homo* and chimpanzees / bonobos *Pan* spp. (6.6 Mya; 3.4–7.7 Mya). Estimates of the split between the two genera, based on other genes, nuclear, mitochondrial or both, are generally between 10 and 20 Mya, with some estimates exceeding 25 Mya (Table 2). Thus, in all likelihood, the *Nycticebus* and *Xanthonycticebus* split occurred by the Late to Middle Miocene and possibly as early as the Oligocene.

Even the lower estimates of the divergence between *Nycticebus* and *Xanthonycticebus*, of around 8 Mya predate many acknowledged generic splits in a wide range of mammalian taxa. This includes, for instance, those within the Muridae (e.g. *Beamys-Cricetomys*, *Parotomys-Arvicanthis*, *Microtus-Clethrionomys*, *Phyllotis-Calomys* and *Rhipidomys-Phyllotis/Calomys*) (Steppan et al. 2004) and the Cetartiodactyla (e.g. *Capra-Pseudois*, *Ovis-Nilgiritragus*, *Oryx-Addax*, *Bos-Bison*, *Cervus-Rusa* and *Mazama-Odocoileus*) (Zurano et al. 2019). The same is true for other vertebrates, such as birds (e.g. babblers, white-eyes

and bee hummingbirds: Cai et al. 2017; Licona-Vera and Ornelas 2017).

The marked difference between *Nycticebus* and *Xanthonycticebus* is also supported by hybridisation events. In captivity, hybrids (confirmed and suspected) have been recorded between *N. bengalensis* and *N. cougang* and *N. cougang* and *N. hilleri* (other *Nycticebus* species are rarely kept in zoological facilities). Despite being the most common of the slow lorises in captive settings – the Zoological Information Management System lists globally 191 *Xanthonycticebus* and 220 of four other slow loris species combined – there are no records of hybrids between *Xanthonycticebus* and any of the other species. Both *Nycticebus* and *Xanthonycticebus* have $n = 50$ chromosomes, but karyotypically, the former differs from the latter by having a secondary construction in the short arm of chromosome 1 and the additional presence of nucleolus organiser regions on chromosome pair 1 and 23 (Stanyon et al. 1987; Chen et al. 1993).

Status. *Xanthonycticebus pygmaeus* occurs naturally in Vietnam (historically south to the vicinity of Ho Chi Minh City [10.75, 106.66]), Laos PDR (west to Phongsali [21.59, 102.25]), Cambodia (east of the Mekong River), China (historically north to Lüchun County [23.00, 104.67]) (Nekaris 2013). The species may have established itself in Thailand, west of the Mekong River, as a result of poorly planned release efforts (Osterberg and Nekaris 2015). *Xanthonycticebus pygmaeus* has been assessed as Endangered according to IUCN Red List Criteria, with the trade for medicinal purposes and as pets and habitat loss recognised as the main threats (Starr et al. 2011; Blair et al. 2020). The species is protected in all four of its range countries, but active enforcement of these laws is far from optimal (Nekaris and Starr 2015; Thach et al. 2018; Ni et al. 2020). The species is the most common loris kept in accredited zoological collections (i.e. 43 in N America, 86 in Europe, 62 in Asia; Species360 2021). In addition, rescue centres in Vietnam, Laos, Thailand, China, United Arab Emirates and Japan have at least 79 individuals under their care (e.g. Kenyon et al. 2014; Khudamrongsawat et al. 2018; Yamanashi et al. 2021). The species is part of both American Zoo Association and European Association of Zoos and Aquariums breeding programmes, but birth rates are low.

The genus *Nycticebus* is listed in appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), precluding all commercial international trade (Nekaris and Nijman 2007).

In the proposal, submitted by Cambodia in 2007, which was accepted by consensus, three species names were singled out, including *Nycticebus pygmaeus* (with *N. intermedius* and *N. chinensis* listed as synonyms). As such, with respect to international trade, there is no doubt that *Xanthonycticebus* gen. nov. continues to receive the same level of protection and regulation as other slow lorises.

Acknowledgements

We thank the following museums and staff for access to specimens under their care: Colombo Natural History Museum, Field Museum of Natural History-Chicago, Naturalis Leiden, Zoological Museum Amsterdam (now merged with Naturalis), Natural History Museum London and Natural History Museum Oxford. Funding was received from the Systematics Research Fund of the Linnean Society, The Royal Society and SYNTHESIS Project, financed by the European Community Research Infrastructure Action under the FP6 Structuring the European Research Area programme (NL-TAF 3491). Our long-term field projects on slow and slender lorises in Sri Lanka, Cambodia and Indonesia has been supported by People's Trust for Endangered Species, Cleveland Zoological Society and Cleveland Metroparks Zoo and Disney Worldwide Conservation Fund. We thank Aconk Ahmad, Penelope Goodman, Zak Showell, Carly Starr and Ariana Weldon for support and three reviewers and the editor for constructive comments and suggestions for improvement.

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Gobiobotia lii, a new species of gudgeon (Teleostei, Gobionidae) from the middle Chang-Jiang Basin, central China, with notes on the validity of *G. nicholsi* Bănărescu & Nalbant, 1966

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Academic editor: Nicolas Hubert ♦ Received 17 January 2022 ♦ Accepted 11 March 2022 ♦ Published 29 March 2022

Abstract

Gobiobotia lii is described from the Qi-Shui, a stream tributary on the northern bank of the middle Chang-Jiang mainstem in Hubei Province and Lake Dongting in Hunan Province, central China. The new species is distinguished from all other congeneric species by possessing a combination of the following characters: a naked region of the abdomen adjacent to the ventral mid-line extending to the vent and the vertebral count (4+31–32). The validity of *G. lii* is confirmed by its monophyletic nature recovered in a phylogenetic analysis, based on the *cyt b* gene and its significant sequence divergence with sampled congeneric species. Critical notes were given on the species recognition of historically documented eight-barbel gudgeons co-existing in Lake Dongting. *Gobiobotia nicholsi* Bănărescu & Nalbant, 1966 should be a valid species distinct from *G. filifer* (Garman, 1912) and both *G. pappenheimi* Kreyenberg, 1911 and *G. boulengeri* (= *Xenophysogobio boulengeri* (Tchang, 1929)) have an erroneous record from the Lake.

Key Words

cyt b gene, new taxon, morphology, species identification, taxonomy

Introduction

The gudgeon genus *Gobiobotia* Kreyenberg, 1911 (type species *G. pappenheimi*) comprises about 19 species (Fricke et al. 2021), characterised by the combination of four pairs of barbels; a gas bladder with a transversely widened or oval encapsulated anterior chamber and a minute free posterior chamber, but without pneumatic duct; and large scales, with five to six scale rows above the lateral line (He and Chen 1998). They are typically small-sized bottom dwellers hitherto recorded from the major river basins of the Korean Peninsula, Taiwan and Hainan Island, continental China and northern Vietnam (He and Chen 1998; Kottelat 2001a; Kim and Park 2002). The taxonomic history of *Gobiobotia* has been uneven, with the enigmatic genus often placed in a separate subfamily or family of its own (Kottelat 2001a). Species of

this genus and *Xenophysogobio* Chen & Cao, 1977, an endemic Chinese genus currently known from the upper Yangtze River (= Chang-Jiang in Chinese) Basin, were referred to the subfamily Gobiobotinae of the Cyprinidae (He and Chen 1998). All eight-barbel gudgeons were recently referred to the family Gobionidae (Tan and Armbruster 2018). Nonetheless, the monophyletic nature of the either Gobionidae or *Gobiobotia* has been confirmed in many molecular phylogenetic studies of the order Cypriniformes (Yang et al. 2006; Tang et al. 2012; Zhao et al. 2016; Li et al. 2018).

Gobiobotia, especially from China, has a taxonomic inertia despite He and Chen's (1998) taxonomic revision of the genus. For example, the eight-barbel gudgeons from the mid-lower Chang-Jiang Basin are traditionally classified as four species: *G. brevirostris* Chen & Cao, 1977, *G. filifer* (Garman, 1912), *G. meridionalis* Chen & Cao, 1977

and *G. tungi* Fang, 1933 (Zhang and Zhao 2016). However, *G. jiangxiensis*, originally described by Zhang and Liu (1995) from the Poyang Lake Basin, was not included in *Gobiobotia* by He and Chen (1998). *Gobiobotia nicholsi*, initially described by Bănărescu and Nalbant (1966) from Lake Dongting, was regarded as a synonym of *G. ichangensis* Fang, 1930 (Chen and Cao 1977), a species subsequently synonymised with *G. filifer* (Ding 1994). Although this synonym is widely accepted by succeeding Chinese workers, the validity of *G. nicholsi* requires re-evaluation, based on examination on its type specimens.

Fish surveys were conducted during 2011–2015 and 2017–2018 in Lake Dongting in Hunan Province and during 2021 in some tributaries on the northern bank of the middle Chang-Jiang in Hubei Province, central China. These surveys yielded eighty-five specimens referred to as *Gobiobotia*, conforming to *G. filifer* and *G. meridionalis*, as well as a morphologically and genetically distinct subsample which represents an undescribed species. The present study aims to provide a description of this unnamed species. Some notes on the validity of *G. nicholsi* and the identification of other historically documented co-existing eight-barbel gudgeons in Lake Dongting are also provided.

Material and methods

Specimen sampling and preservation.

Specimens utilised for this study were sampled in accordance with the Chinese Laboratory Animal Welfare and Ethics animal welfare laws (GB/T 35892–2018). After being anaesthetised, all captured individuals were fixed by immersion in ethanol or formalin. Specimens were collected using gill nets, trap nets and electrofishing. Caught specimens of *Gobiobotia* were stored in 10% formalin for morphological examination or 95% ethyl alcohol for DNA extraction. All sequences amplified in this study were submitted to GenBank. Their voucher specimens are deposited in the collection of the Museum of Aquatic Organisms at the Institute of Hydrobiology (IHB), Chinese Academy of Sciences (CAS).

Morphological analysis

Measurements were taken point to point with a digital caliper connected directly to a data-recording computer and data recorded to the nearest 0.1 mm. Measurements were taken on the left side of specimens whenever possible, following methods used by Kottelat (2001b) and Song et al. (2018). The head length and measurements of other parts of the body are given as percentages of standard length (SL). Measurements of parts of the head are given as proportions of the head length (HL) (Tables 1, 2). The counts of vertebrae were taken from radiographs of Micro-CT or X-rays. The specimens examined in this study are deposited in the collections of:

AMNH	American Museum of Natural History, New York;
BMNH	Natural History Museum, London;
IHB	Institute of Hydrobiology, Wuhan;
MNHN	Muséum National d'Histoire Naturelle, Paris;
ZMB	Museum für Naturkunde-Leibniz Institute for Evolution and Biodiversity Science, Berlin.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from fin clips stored in ethanol using the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing) with the recommended protocol.. The *cyt b* gene was amplified by using primers L14724 (GACTTGAAAAACCACCGTTG) and H15915 (CTCCGATCTCCGGATTACAAGAC) adopted from Xiao et al. (2001). The mitochondrial gene was performed in 25 µl volumes with 12.5 µl Master mix Taq (Beijing TsingKe Biotech Co., Ltd.), 1 µl of each primer, 1 µl template DNA, adding double distilled water (dd H₂O) to supply the volume. The thermocycling conditions were as follows: initial denaturation for 4 min at 94 °C, denaturation for 50 s at 94 °C, annealing for 50 s at 55 °C and extension for 1 min, in terms of the product length at 72 °C. After 34–35 cycles, the final extension was done at 72 °C for 10 min; the product was then stored at 4 °C. The sequencing was finished by Tianyihuiyuan Biotechnology Company.

Phylogenetic analyses

The *cyt b* gene of around 1100 bp base was chosen for phylogenetic analysis. The amplified 48 gene sequences were utilised for molecular phylogenetic analysis along with another 11 GenBank-retrieved sequences of the same gene from six congeneric species and two outgroups (*Microphysogobio xianyouensis* and *M. fukiensis*). Detailed information about samples, used here for molecular analysis, is given in Table 3. The sequences were aligned using MAFFT 7.0 (Katoh and Standley 2013) and ends trimmed. The genetic distance was calculated with MEGA 7.0, based on the uncorrected p-distance model (Kumar et al. 2016).

PhyloSuite (Zhang et al. 2020) was used for phylogenetic analyses. The selection of the best-fit model of nucleotide evolution, based on Akaike's Information Criterion was performed in ModelFinder (Kalyaanamoorthy et al. 2017). MrBayes 3.2.6 (Ronquist et al. 2012) was utilised for Bayesian analysis with the selected model: GTR+I+G+F, applying the optimal nucleotide evolution model and the MCMC method with four chains (three hot chains and one cold chain) running simultaneously for 20,000,000 generations to calculate posterior probability. Trees were sampled for every 1000 cycles. The initial 25% of sampled data were discarded as burn-in. Sufficient mixing of the chains was considered to be reached when the average standard deviation of split frequencies was below 0.01.

Table 1. Morphometry of *Gobiobotia lii*. Measurements are in percentage of standard length, except for standard length (SL). SD, standard deviation; r, Pearson’s correlation coefficient; p, significance; linear regression parameters calculated from measurements. HT = Holotype.

Measurements	N	HT	Min	Max	Mean	SD	slope (b)	intercept (a)	r (SL)	p
SL (mm)	14	48.6	42.1	51.2	46.8	2.7				
Body depth	14	22.6	15.6	22.3	19.6	2	0.5461	5.7924	0.701	0.005
Head length	14	25.1	22.5	25.1	23.5	0.8	0.0124	23.043	0.037	0.9
Pre-dorsal length	14	49.5	44.7	51.2	47.4	1.7	-0.029	48.906	-0.04	0.883
Pre-pectoral length	14	21.3	20.4	23.2	21.9	0.9	-0.154	29.126	-0.47	0.091
Pre-pelvic length	14	48.7	44.9	52.9	48.1	1.9	-0.1722	56.232	-0.25	0.398
Pre-anal length	14	74.8	70.4	78.4	74	2.4	-0.0537	76.607	-0.06	0.837
Dorsal length	14	25.2	21.1	26	24	1.6	0.3178	9.2449	0.541	0.046
Pectoral length	14	22.8	18.1	23.4	21.5	1.6	-0.1957	30.756	-0.34	0.24
Pelvic length	14	19.6	15.9	19.6	18	1.2	-0.1486	24.994	-0.33	0.257
Anal length	14	20.1	16.2	21.4	18.4	1.4	-0.0407	20.437	-0.08	0.795
Pectoral-pelvic distance	14	29.2	26	30.3	28.4	1.3	-0.0534	30.95	-0.11	0.705
Pelvic-anal distance	14	27.2	24.9	30	27.5	1.5	-0.2996	41.551	-0.54	0.046
Pelvic-anus distance	14	13.2	9.6	13.1	11.6	1.1	-0.0514	14.082	-0.12	0.682
Anus-anal distance	14	16.4	12.7	18.1	15.4	1.7	0.1019	10.664	0.164	0.575
Head depth	14	61.6	52	69.1	61.5	4.9	0.7576	39.821	0.54	0.046
Head width	14	73.1	65.4	80.8	75.4	3.8	-0.066	64.639	-0.04	0.901
Snout length	14	40.1	38.9	43.2	41.8	1.1	0.007	41.347	0.017	0.955
Interorbital width	14	20.8	21.1	28.6	24.6	2	0.0054	24.079	0.007	0.982
Eye diameter	14	19.1	20	25.8	22.5	1.7	-0.2117	32.172	-0.3	0.29
Maxillary barbel length	14	28.7	23.9	67.9	33	10.5	0.6906	0.2997	0.18	0.539
Post-orbital length	14	33.8	31.5	46.6	39.5	5	-0.355	55.727	-0.19	0.526

Table 2. Morphometric measurements for four species of *Gobiobotia*: *G. jiangxiensis*, *G. pappenheimi*, *G. meridionalis* and *G. filifer*.

Character	<i>G. jiangxiensis</i> (n = 6)		<i>G. pappenheimi</i> (n = 14)		<i>G. meridionalis</i> (n = 19)		<i>G. filifer</i> (n = 21)	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
SL (mm)	35.2–52.5	40.2±5.8	27.9–56.1	43.7±8.7	54.0–99.5	80.4±10.8	52.2–96.5	75.5±11.7
Morphometric data								
% of SL								
Body depth	12.2–22.5	17.1±3.3	27.9–56.1	43.7±8.7	14.9–20.8	17.9±1.5	15.4–22.5	18.2±1.9
Head length	22.0–25.9	24.4±1.3	12.5–14.5	13.6±0.7	24.4–28.1	26.7±1.0	17.9–28.2	25.7±2.1
Pre-dorsal length	46.8–50.5	48.6±1.6	46.2–51.8	49.2±1.6	43.8–51.9	46.5±2.0	41.2–52.7	46.8±2.4
Pre-pectoral length	19.8–24.1	22.9±1.6	18.8–28.1	23.6±2.8	24.6–27.3	26.0±0.9	23.2–28.5	25.6±1.3
Pre-pelvic length	44.6–50.8	48.3±2.3	44.7–50.7	47.7±1.9	45.0–52.4	48.3±1.7	43.5–51.1	47.8±2.0
Pre-anal length	71.0–78.1	74.7±2.2	69.4–79.0	75.2±2.4	72.7–77.5	75.0±1.4	69.0–77.3	73.3±2.1
Dorsal length	27.1–31.2	28.8±1.6	22.8–28.1	24.7±1.5	23.4–27.5	25.6±1.1	19.8–25.6	23.1±1.6
Pectoral length	25.1–28.2	26.5±1.0	17.0–22.5	20.1±1.6	21.9–29.1	25.0±1.5	22.9–31.8	26.1±2.1
Pelvic length	18.1–23.4	20.6±1.7	16.0–21.4	19.2±1.4	18.0–22.1	20.5±1.2	15.2–20.0	17.8±1.2
Anal length	18.1–21.3	19.1±1.0	16.0–21.4	19.2±1.4	17.5–20.9	19.0±0.9	15.5–19.2	17.0±0.9
Pectoral-pelvic distance	26.3–28.7	27.3±0.8	22.9–27.1	25.1±1.4	21.0–28.1	24.2±1.7	21.9–26.0	24.1±1.1
Pelvic-anal distance	24.4–26.1	25.5±0.6	25.7–31.9	28.1±1.9	26.9–31.1	28.5±1.2	23.9–29.9	26.2±1.6
Pelvic-anus distance	11.7–14.9	12.9±1.1	8.2–15.4	11.0±1.7	9.0–14.5	11.8±1.2	7.6–13.1	10.8±1.3
Anus-anal distance	11.3–14.3	12.7±0.9	14.0–18.1	16.0±1.2	14.2–19.6	16.8±1.4	14.4–19.9	16.4±1.6
% of HL								
Head depth	54.4–60.3	57.1±1.9	47.5–59.0	53.6±3.7	51.3–59.0	55.4±2.2	47.1–76.1	53.9±6.1
Head width	60.9–82.1	70.4±6.2	51.3–73.2	67.4±5.3	55.4–70.9	63.0±4.0	49.8–79.4	59.4±7.2
Snout length	40.5–46.4	42.6±2.0	33.7–41.8	38.0±2.0	41.6–62.7	46.0±4.8	34.7–55.1	40.2±4.2
Interorbital width	21.5–29.3	24.8±2.6	17.0–29.1	21.8±3.6	16.6–29.1	21.0±3.8	24.9–40.7	28.8±3.2
Eye diameter	19.3–25.2	22.5±2.0	16.1–27.4	21.3±3.7	22.1–35.8	26.0±3.7	12.1–23.2	16.1±2.6
Maxillary barbel length	15.4–19.0	16.9±1.3	20.1–45.4	31.6±6.9	20.3–39.5	35.2±2.7	25.6–37.4	31.1±3.8
Post-orbital length	35.7–38.2	37.1±1.0	33.5–44.4	39.1±2.9	32.1–50.4	37.2±4.7	42.0–60.5	46.7±3.7
Meristic counts								
Lateral-line scales	38–39	38.4±0.5	37–39	38.1±0.7	41–43	41.8±0.6	40–42	40.8±0.7
Scale rows above lateral line	5–6	5.5±0.5	5–6	5.5±0.5	5–6	5.3±0.4	5–6	5.6±0.5
Pre-dorsal scales	12	12	13	13	12–13	12.6±0.5	12–13	12.8±0.4
Circumpeduncular scales	12	12	12–13	12.4±0.5	12	12	12	12

Table 3. Detailed information on specimens used in this study. The species with * means the samples available in GenBank.

Species	Specimen voucher	Sampling location	GenBank no.
<i>G. lii</i>	IHB201711015435	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275368
<i>G. lii</i>	IHB2017101929	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275369
<i>G. lii</i>	IHB202103050300	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275370
<i>G. lii</i>	IHB202103050303	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275371
<i>G. lii</i>	IHB201801016013	Yuanjiang, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275372
<i>G. lii</i>	IHB2017097256	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275373
<i>G. lii</i>	IHB2017097257	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275374
<i>G. lii</i>	IHB2017097258	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275375
<i>G. lii</i>	IHB202103050858	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275376
<i>G. lii</i>	IHB202103050859	Qichun, Hubei Prov. (Qi-Shui, Chang-Jiang)	OM275377
<i>G. filifer</i>	IHB201711015758	Maocaojie, Hunan Prov. (Lake Dongting, Chang-Jiang)	OM275378
<i>G. guilingensis</i>	IHB20161062A	Rongshui, Guangxi Prov. (Zhu-Jiang)	OM275379
<i>G. tungi</i>	IHB202108056037	Shangrao, Jiangxi Prov. (Chang-Jiang)	OM275380
<i>G. tungi</i>	IHB202108056038	Shangrao, Jiangxi Prov. (Chang-Jiang)	OM275381
<i>G. meridionalis</i>	IHB2017040446	Hengdong, Hunan Prov. (Xiang-Jiang, Chang-Jiang)	OM275382
<i>G. meridionalis</i>	IHB2017040447	Hengdong, Hunan Prov. (Xiang-Jiang, Chang-Jiang)	OM275383
<i>G. meridionalis</i>	IHB2016106403	Chenxi, Hunan Prov. (Yuan-Jiang, Chang-Jiang)	OM275384
<i>G. meridionalis</i>	IHB2016106404	Chenxi, Hunan Prov. (Yuan-Jiang, Chang-Jiang)	OM275385
<i>G. meridionalis</i>	IHB2017104358	Kaili, Guizhou Prov. (Yuan-Jiang, Chang-Jiang)	OM275386
<i>G. meridionalis</i>	IHB2017104362	Kaili, Guizhou Prov. (Yuan-Jiang, Chang-Jiang)	OM275387
<i>G. meridionalis</i>	IHB2017040817	Nanxiong, Guangdong Prov. (Zhu-Jiang)	OM275388
<i>G. meridionalis</i>	IHB2017040832	Shixing, Guangdong Prov. (Zhu-Jiang)	OM275389
<i>G. meridionalis</i>	IHB2017013891	Suichuan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275390
<i>G. meridionalis</i>	IHB2017013893	Suichuan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275391
<i>G. meridionalis</i>	IHB201804014151	Fuzhou, Jiangxi Prov. (Fu-He, Chang-Jiang)	OM275392
<i>G. meridionalis</i>	IHB201804014152	Fuzhou, Jiangxi Prov. (Fu-He, Chang-Jiang)	OM275393
<i>G. meridionalis</i>	IHB201707014638	Ningdu, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275394
<i>G. meridionalis</i>	IHB201707012675	Ningdu, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275395
<i>G. meridionalis</i>	IHB201707018995	Taihe, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275396
<i>G. meridionalis</i>	IHB2017075488	Longnan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275397
<i>G. meridionalis</i>	IHB201707017921	Shicheng, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275398
<i>G. meridionalis</i>	IHB201707017918	Shicheng, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275399
<i>G. meridionalis</i>	IHB201707013207	Huichang, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275400
<i>G. meridionalis</i>	IHB201707012757	Pingshan, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275401
<i>G. meridionalis</i>	IHB2016104697	Ji'an, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275402
<i>G. meridionalis</i>	IHB201707018980	Taihe, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275403
<i>G. meridionalis</i>	IHB201707018994	Taihe, Jiangxi Prov. (Gan-Jiang, Chang-Jiang)	OM275404
<i>G. pappenheimi</i> *	Uncatalogued	Tieling, Liaoning Prov. (Liao-He)	NC032293.1
<i>G. naktongensis</i> *	Uncatalogued	Korean Peninsula	JX104484.1
<i>G. naktongensis</i> *	Uncatalogued	Korean Peninsula	JX104485.1
<i>G. macrocephala</i> *	Uncatalogued	Korean Peninsula	JX046845.1
<i>G. macrocephala</i> *	Uncatalogued	Korean Peninsula	JX046846.1
<i>G. brevibarba</i> *	Uncatalogued	Korean Peninsula	JX094047.1
<i>G. brevibarba</i> *	Uncatalogued	Korean Peninsula	JX094048.1
<i>Xenophysogobio boulengeri</i> *	Uncatalogued	Upper Chang-Jiang Basin	AF375868.1
<i>Xenophysogobio nudicorpa</i> *	Uncatalogued	Upper Chang-Jiang Basin	NC_025300.1
<i>Microphysogobio xianyouensis</i> *	NTOUP 201011-534	Mulan-Xi, Fujian Prov.	KM999931.1
<i>Microphysogobio fukiensis</i> *	NTOUP 2015-10-001	Shaowu City, Fujian Prov.	KT877353.1

Results

Gobiobotia lii sp. nov.

<http://zoobank.org/0061399E-1D8E-4764-A475-9FFC29A6490F>

Figs 1, 2

Gobiobotia pappenheimi Chen & Cao, 1977: 556 (Lake Dongting),
Synonym

Holotype. IHB 202103051401, 48.6 mm SL. P. R. China: Hubei Province: Qichun County: Xiangqiao Town: Chang-Jiang Basin, Qi-Shui stream, 30°20'31"N,

115°43'43"E; D. M. Guo, X. Gong and Y. Liu; 5 March 2021.

Paratypes. IHB 202103051399-1400, 202103050858-0859, 4 specimens, 45.1–48.0 mm SL, other data same as holotype. IHB 2014070560439-0447, 9 specimens, 42.1–51.2 mm SL. P. R. China: Hunan Province: Hanshou City: Potou Town: Chang-Jiang Basin, Lake Dongting, 29°00'05"N, 111°58'31"E; L. Cao, Z. G. Jiang, S. J. Ren, R. X. Xie and X. Wang; 5 July 2014. IHB 2017101929, 201711015435, 201801015997; 6012–6015; 6143–6146, 11 specimens, 35.0–40.1 mm SL. P. R. China: Hunan Province: Yuanjiang City: Liaodaokou: Chang-Jiang



Figure 1. *Gobiobotia lii*, holotype, IHB 202103051401, 48.6 mm SL, photographed alive immediately upon capture. P. R. China: Hubei Province: Qichun County: Xiangqiao Town: Chang-Jiang Basin, Qi-Shui.

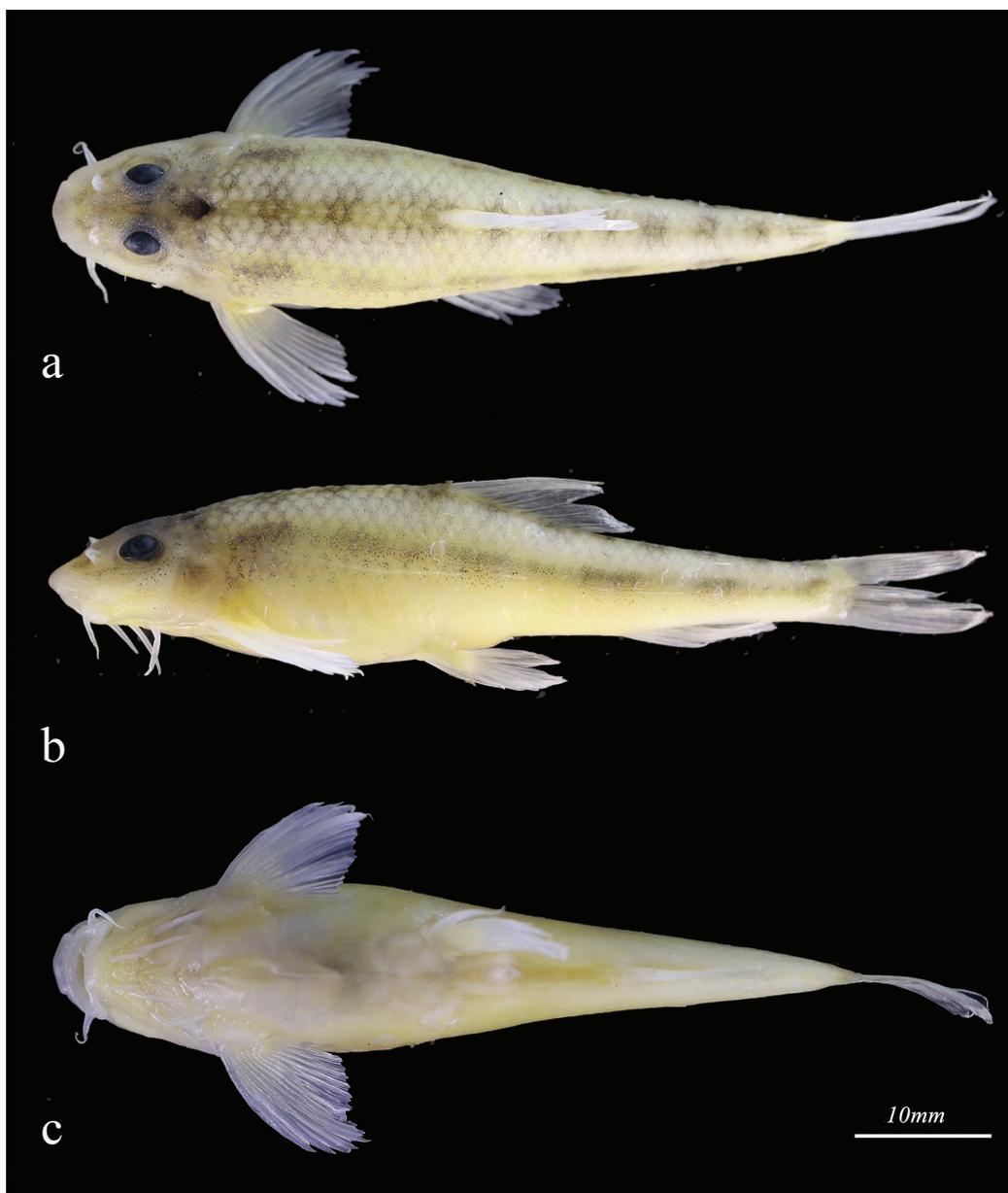


Figure 2. Dorsal (a), lateral (b) and ventral (c) views of *Gobiobotia lii*, holotype, IHB 202103051401, 48.6 mm SL. P. R. China: Hubei Province: Qichun County: Xiangqiao Town: Chang-Jiang Basin, Qi-Shui.

Basin, Lake Dongting, 28°51'39.02"N, 112°33'37.87"E; X. Chen, C. T. An, W. H. Shao and Z. T. Wang; 19 October 2017 and 1 January 2018.

Diagnosis. *Gobiobotia lii* is distinct from all other congeneric species, except *G. brevirostris* Chen & Cao, 1977, *G. homalopteroidea* Rendahl, 1933, *G. jiangxiensis* Zhang & Liu, 1995 and *G. pappenheimi* Kreyenberg, 1911, in having a naked region of the abdomen adjacent to the ventral mid-line extending to or beyond the vent (vs. to or away from the pelvic-fin base) (Figs 2c, 3). It differs from these four species in having 4+31–32 (vs. 4+33–37) vertebrae. The new species shares with *G. homalopteroidea* and *G. pappenheimi* the presence of smaller

eyes (diameter less than the interorbital width), maxillary barbels longer than the eye diameter and the third pair of longer mental barbels extending to the pectoral-fin insertion, these three characters separating them from *G. brevirostris* and *G. jiangxiensis*. The new species further differs from *G. homalopteroidea* in possessing a smaller (vs. larger) naked region of the abdomen adjacent to the ventral mid-line extending to the anus (vs. to the anal-fin origin) and the eye diameter 20.0–25.8% of HL (vs. 10.8–13.9%); and from *G. pappenheimi* in having pectoral fins extending away from (vs. beyond) the pelvic-fin insertion, the second branched pectoral-fin ray not prolonged (vs. prolonged) and a longer (vs. shorter) snout than the post-orbital length.



Figure 3. Ventral view of body in: (a) *G. guilingensis* IHB 202104053183, 86.7 mm SL. P. R. China: Guangxi Province: Rongshui County: Zhu-Jiang Basin, Rong-Jiang; and (b) *G. meridionalis*, IHB 201609021174, 84.8 mm SL. P. R. China: Hunan Province: Chenxi County: Chang-Jiang Basin: Yuan-Jiang.

Description. Morphometric data for type specimens given in Table 1. General body appearance of holotype shown in Figs 1 and 2. Body small and slender, with maximum depth at dorsal-fin origin. Pre-dorsal body profile depressed. Ventral profile slightly compressed. Abdomen flat or slightly convex from pectoral-fin insertion to anal-fin origin and slightly concave from anal-fin origin to caudal-fin base.

Head depressed and broad. Snout pointed in lateral view and slightly bluntly rounded in dorsal view, longer than post-orbital head. Eyes small and dorsolateral; diameter less than interorbital width. Interorbital space flattened. Some irregular papillae present on the mental region.

Mouth inferior, with opening laterally extending to the vertical line of front edge of nostril. Four pairs of barbels: one pair of maxillary barbels and three pairs of mental barbels. Maxillary barbels extending closely to a vertical line through middle of eye; first pair of mental barbels

short, inserted at the same level passing through maxillary-barbel roots and anterior to roots of second mental barbels and reaching anterior margin of eye; second pair of mental barbels extending beyond bases of third pair of mental barbels to hind margin of pre-opercula; third pair of mental barbels long, reaching pectoral-fin insertion.

Fins rays flexible, dorsal fin with 3 simple and 7 (14 specimens examined) branched rays; pectoral fin with 1 simple and 12 (6) or 13 (8) branched rays, extending about two-thirds of the distance to pelvic-fin insertion. Pelvic fin with 1 simple and 7 (14) branched rays, reaching beyond the midway to anal-fin origin and surpassing anus; inserted closer to anal-fin origin than to anterior end of pectoral-fin base; located opposite to that of dorsal-fin base. Anal fin with 3 simple and 6 (14) branched rays; origin equidistant from pelvic-fin insertion and caudal-fin base. Anus positioned closer to the pelvic-fin

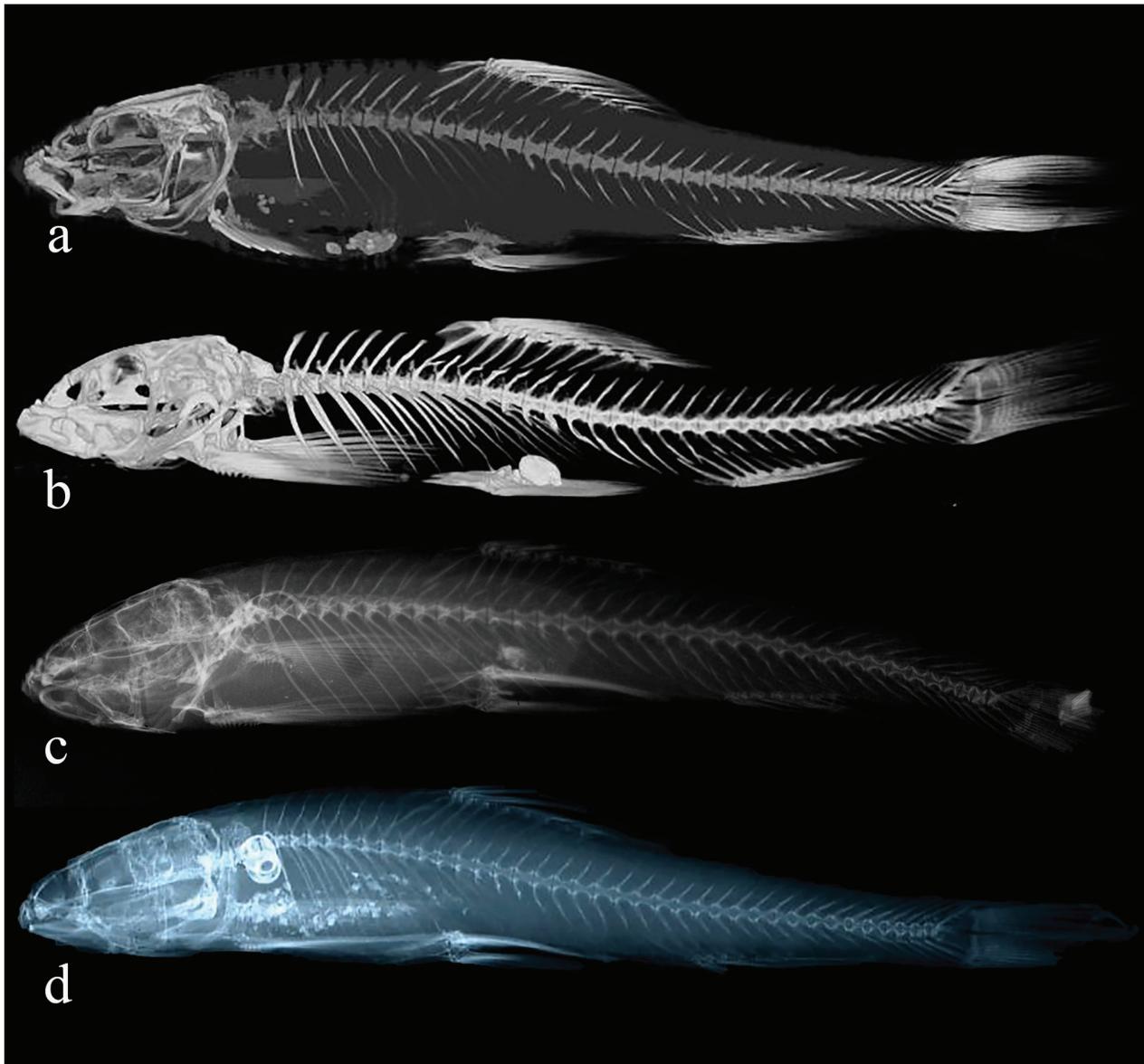


Figure 4. Micro-CT images of lateral body in: (a) *G. lii*, IHB 202103051401, holotype and (b) *G. jiangxiensis*, IHB 90IV1256, holotype. X-rayed photographs of lateral body in: (c) *G. pappenheimi*, ZMB 18466, holotype and (d) *G. nicholsi*, AMNH 20523, holotype.

insertion than to anal-fin origin. Caudal fin moderately forked; lower lobes are longer than upper lobes.

Lateral line complete and almost straight, extending along mid-lateral of body, with 37 (7) or 38 (7) pored scales; scale rows above and below lateral lines 5 and 3; circumpeduncular scales 11 (7) or 12 (7) and pre-dorsal mid-line scales 13 (14). Body covered with moderately-sized scales; no scales on breast and belly in front of vent. Vertebral counts 4+31–32 (modally 4+32, see Fig. 4).

Colouration. In freshly-collected specimens, head and dorsum of body golden yellow, underside and abdomen golden grey; back and lateral head peppered with dark flecks. Back darker and belly lighter, with a dark spot back of the head, 12–13 dorsal dark brown spots extending along mid-line of dorsum from nape to caudal-fin base and 9–10 large dark brown spots along middle of

side from gill opening to caudal-fin base. Fin golden yellow, distal margin hyaline.

In formalin-stored specimens, ground colour slightly faded; body dorsally greyish and ventrally greyish-white; dorsal and lateral dark brown spots not clear and the back of the head becoming yellowish-brown. Dorsal, pectoral, pelvic and anal fins light greyish; caudal fin with numerous narrow dark crossbars (Fig. 2).

Sexual dimorphism. No sexual dimorphism was observed in the specimens examined.

Distribution and habitat. *Gobiobotia lii* is known from the Qi-Shui, a stream tributary to the middle Chang-Jiang mainstem at Qichun County, Hubei (Fig. 5). The new species was found in turbid, slow-running water with mixed substrates including sand and gravel (Fig. 6); and Lake Dongting in Hunan, where it was collected in lotic habitats or estuaries of effluents. Co-existing species

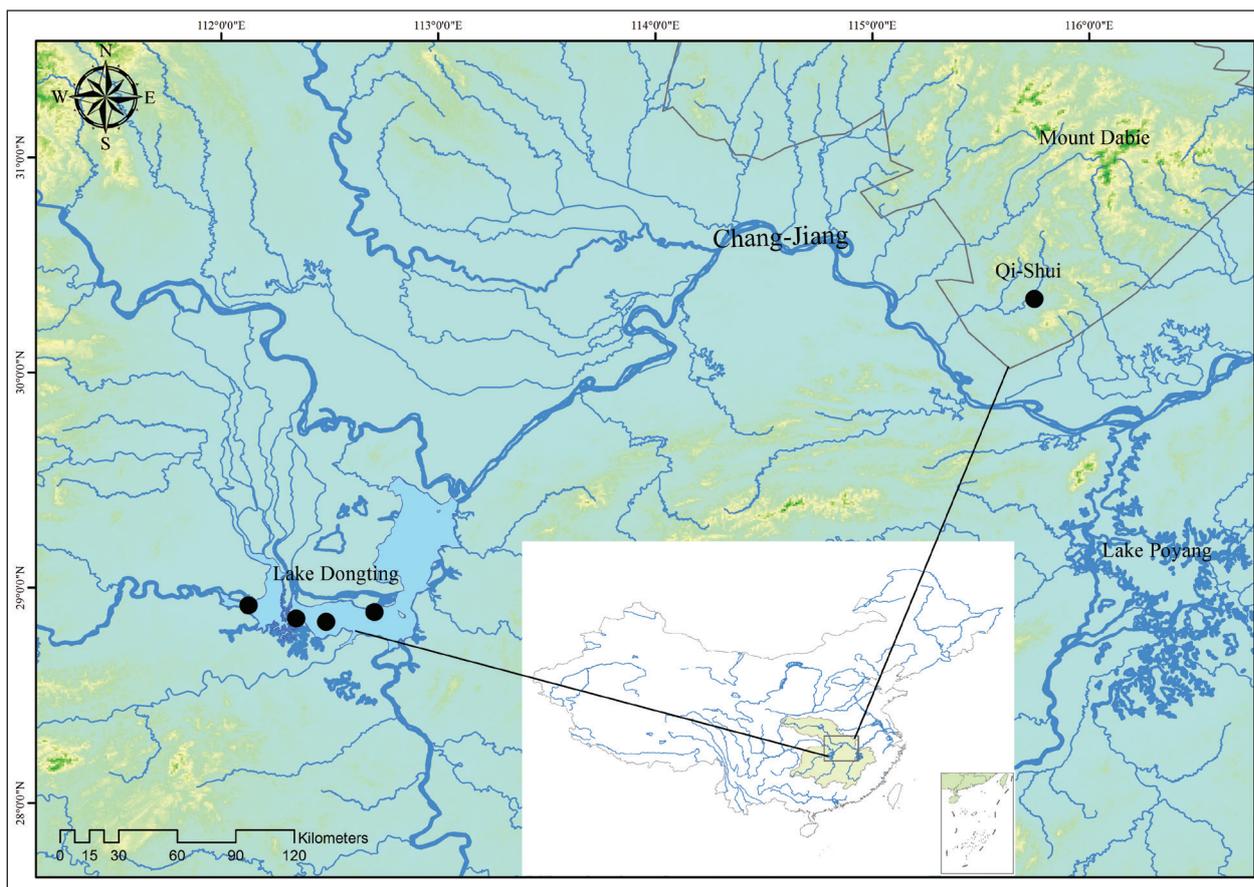


Figure 5. Map showing the distribution of type series of *Gobiobotia lii* in the middle Chang-Jiang Basin.



Figure 6. Sampling locality of the holotype (IHB 202103051401) of *Gobiobotia lii* in the Qi-Shui stream, tributary to the middle Chang-Jiang mainstem in Xiangqiao Town, Qichun County, 5 March 2021.

Table 4. Main diagnostic characters among *G. lii* and closely-related eight-barbel gudgeons. Number of specimens examined for vertebrae are given in parentheses.

	<i>G. lii</i> [^]	<i>G. brevirostris</i> [^]	<i>G. homalopteroidea</i> [°]	<i>G. jiangxiensis</i> [^]	<i>G. pappenheimi</i> [^]
1. Scaleless region of the abdomen adjacent to the ventral mid-line	To anus	To anus	To anal-fin origin	To anus	To anus
2. Snout length/post-orbital head length	> 1	< 1	< 1	> 1	> 1
3. Eye diameter/interorbital width	< 1	> 1	< 1	> 1	< 1
4. Pupil shape	Round	Elliptical	Round	Round	Round
5. Eye diameter % of HL	20.0–25.8	20.0–26.3	10.8–13.9	19.3–25.2	16.1–27.4
6. Maxillary-barbel length/eye diameter	> 1	< 1	> 1	< 1	> 1
7. Extension of third pair of mental barbel	To base of pectoral fin	To middle of opercula	To base of pectoral fin	To front margin of pre-opercula	To base of pectoral fin
8. Pectoral-fin length/distance between pectoral- and pelvic-fin insertions	< 1	< 1	< 1	< 1	> 1
9. Second branched pectoral-fin ray	Not elongated	Elongated	Not elongated	Not elongated	Elongated
10. Vertebral counts	4+31–32 (3)	4+34 (2)	4+36–37	4+33–34 (6)	4+34–35 (5)
11. Lateral-line pored scales	37–38 (14)	38–39 (2)	41–43	38–39 (6)	37–39 (14)

° From He and Chen (1998); ^ from this study.

included *Misgurnus anguillicaudatus*, *Opsariichthys bidens* and *Parabotia fasciata*.

Etymology. The specific epithet is named after Shi-Zhen Li, a native of Qichun County where the holotype and partial paratypes were caught. Li was a well-known medical scientist in the Ming Dynasty, who compiled “Compendium of Materia Medica” (‘本草纲目’ in Chinese) - one of the most valuable pieces of literature of traditional Chinese medicine. He had a typical image as an old man with a long white dense beard, just like the eight-barbel gudgeon. The common Chinese name ‘李氏鳅鲇’ in here proposed for *Gobiobotia lii*.

Sequence variation and molecular phylogeny. A total of 37 *cyt b* gene sequences from five Chinese species of *Gobiobotia*: *G. filifer* (one sequence), *G. guilingensis* (one), *G. lii* (10), *G. meridionalis* (23) and *G. tungi* (two) were amplified in this study. These sequences were used for phylogenetic analysis along with another 11 GenBank-retrieved sequences from six congeneric species and two out-groups (*Microphysogobio fukiensis* and *M. xianyouensis*) (Table 3). A total length of 1038 bp gene sequence was obtained after sequence alignment and trimming, including 643 conserved sites, 396 variable sites, 349 parsimony informative sites and 47 singleton sites. The mean frequency of four nucleotides of the new species was A = 30.7%, T = 27.5%, C = 15.5% and G = 26.4%; the base composition was A-T rich (58.2%). The Bayesian Inference tree, based on the *cyt b* gene for *G. lii* and other 10 congeners, is shown in Fig. 7, with node support values displayed. Intraspecific genetic distance values for here-recognised species of the Gobionidae ranged from 0.0% to 1.8%. The interspecific genetic distance of *G. lii* with all other sampled congeneric species varied from 2.6% to 22.1% (mean 16.8%) and the intraspecific genetic distance value of this species was 0.8% (Table 5). In the Bayesian 50% majority-rule consensus tree, samples of *G. lii* constituted a robustly-supported (100% Bayesian posterior probability, bpp) lineage, strongly supported by 100% bpp to be sister of *G. naktongensis* Mori, 1935.

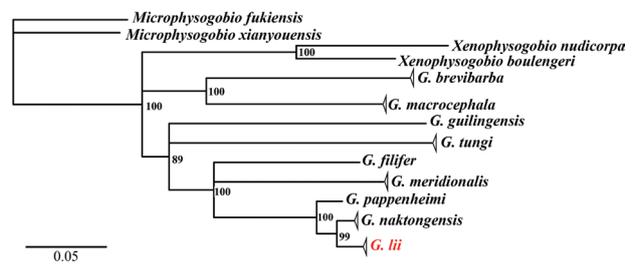


Figure 7. Bayesian Inference tree inferred from the *cyt b* gene for 11 putative species of eight-barbel gudgeons. Nodal numbers are posterior probability values greater than 50%.

Photographic examination on relevant specimens

Some of specimens, utilised in Banărescu & Nalbant’s (1966) taxonomic revision of *Gobiobotia*, are from Huping (now Yueyang City) on Lake Tungting (= Dongting). These specimens were not examined by Chinese workers when they revised species of this genus. In this study, photographic examinations are made on them and also on the holotype of *Xenophysogobio boulengeri* and *Gobiobotia pappenheimi* and their topotypical specimens are examined as well.

Both the holotype (AMNH 20523; Fig. 8) and the paratype (AMNH 20524) of *G. nicholsi*, as stated in its original description, have 44–45 lateral-line pored scales, four scale rows below the lateral line, the eye diameter about half of the interorbital width and the second branched pectoral-fin ray not prolonged. Nevertheless, the naked region of the abdomen adjacent to the ventral mid-line extends away from or to the pelvic-fin insertion rather than to or behind the vent in the original description.

Nineteen specimens (AMNH 10311) of 32.2–41.0 mm SL, under the name of *G. pappenheimi* from Lake Dongting, have a naked region of the abdomen adjacent to the ventral mid-line extending to the pelvic-fin insertion

Table 5. Genetic distances (uncorrected p-distance) of *cyt b* gene computed by MEGA 11 amongst 11 species.

Species	Within Group	1	2	3	4	5	6	7	8	9	10
1. <i>G. lili</i>	0.0076										
2. <i>G. naktongensis</i>	0.0078	0.0260									
3. <i>G. pappenheimi</i>	n/c	0.0387	0.0350								
4. <i>G. filifer</i>	n/c	0.1296	0.1212	0.1395							
5. <i>G. meridionalis</i>	0.0029	0.1358	0.1356	0.1352	0.1424						
6. <i>G. guilingensis</i>	n/c	0.1721	0.1649	0.1824	0.1575	0.1805					
7. <i>G. tungi</i>	0.0029	0.1736	0.1660	0.1757	0.1856	0.1890	0.1925				
8. <i>G. brevibarba</i>	0.0029	0.1826	0.1835	0.1853	0.1926	0.1864	0.2009	0.1927			
9. <i>G. macrocephala</i>	0.0019	0.1856	0.1776	0.1814	0.1684	0.1740	0.1881	0.1993	0.1631		
10. <i>X. boulengeri</i>	n/c	0.1833	0.1849	0.1800	0.1787	0.1804	0.1979	0.1935	0.1914	0.1915	
11. <i>X. nudicorpa</i>	n/c	0.1964	0.1822	0.1847	0.1996	0.1883	0.1912	0.2209	0.1199	0.2070	0.1199

**Figure 8.** Lateral (a) and ventral (b) views of *G. nicholsi*, AMNH 20523, holotype.

and more than 40 lateral-line pored scales (Fig. 9a, b). By contrast, the holotype of *G. pappenheimi* (ZMB 18466) has a naked region of the abdomen adjacent to the ventral mid-line extending to the vent and 38–39 lateral-line pored scales (Fig. 9c, d), as found in specimens of this species from northern China.

Two small specimens (AMNH 20522) of 30.0 mm and 31.2 mm SL, under the name of *G. boulengeri* (= *Xenophysogobio boulengeri*) from Lake Dongting, have larger eyes (diameter about one-fifth head length), the adpressed tip of the pectoral fin reaching or slightly beyond the pelvic-fin insertion, the adpressed tip of the pelvic fin reaching the anal-fin origin and the upper extremity of the gill opening aligned with the upper margin of the eye (Fig. 10a, b). Nevertheless, three available topotypical specimens and the holotype (MNHN-IC-1934-0180; photograph examined) have smaller eyes (diameter about one-tenth head length), the adpressed tip of the pectoral fin not reaching the pelvic-fin insertion, the adpressed tip of the pelvic fin not reaching the anal-fin origin and the upper extremity of the gill opening aligned with the lower margin of the eye (Fig. 10c, d).

Discussion

The generic concept of *Gobiobotia* still remains contentious. All eight-barbel gudgeons were traditionally classified into three subgenera, namely *Gobiobotia*, *Progobiobotia* and *Xenophysogobio* (Chen and Cao 1977). The generic rank was subsequently assigned to *Xenophysogobio* and *Progobiobotia* was rendered subgeneric to *Gobiobotia* (He and Chen 1998), a classification widely accepted by succeeding authors, for example, Zhang and Zhao (2016) and Wu et al. (2021). The generic status of *Gobiobotia* and *Xenophysogobio* was verified in molecular phylogenetic analyses of the gobiobotine fishes (Wang et al. 2002) or gudgeons (Tang et al. 2012; Li et al. 2018). However, the taxonomic status of the subgenus *Progobiobotia* (including two species *G. (P.) abbreviata* Fang & Wang, 1931, type species and *G. (P.) guilingensis* Chen, 1989) remained yet to be evaluated in these analyses as a result of failure to sample its included species. In the BI tree yielded from the *cyt b* gene (Fig. 7), *G. (P.) guilingensis* was distantly related to *G. (G.) brevibarba* Mori, 1935 and *G. (G.) macrocephala* Mori, 1935, but constituted a polytomy with *G. (G.) tungi* and a lineage



Figure 9. Lateral (a) and ventral (b) views of *G. pappenheimi*, AMNH 10311, from Lake Dongting; lateral (c) and ventral (d) views of *G. pappenheimi*, ZMB 18466, holotype.

consisting of *G. (G.) filifer*, *G. (G.) lii*, *G. (G.) meridionalis*, *G. (G.) naktongensis* Mori, 1935 and *G. (G.) pappenheimi*. Evidently, *G. (P.) guilingensis* was intertwined with sampled species of the subgenus *Gobiobotia*. For this reason, the subgeneric status of *Progobiobotia* is not warranted. The generic definition of *Gobiobotia* (*sensu* He and Chen 1998) is followed in this study. The presence of four pairs of barbels can easily distinguish it and *Xenophysogobio* from all other genera of the Gobionidae. The genus *Gobiobotia* includes those eight-barbel gudgeons with a tiny free posterior chamber of the gas bladder without pneumatic duct and large scales, with five or six scale rows above the lateral line.

Species of the gudgeon genus *Gobiobotia* are subdivided into two groups, based on body squamation. One group includes those species with a naked region of the abdomen adjacent to the ventral mid-line, extending to the vent or even the anal-fin origin. Four species, along

with *G. lii*, are placed in the group: *G. brevirostris*, *G. homalopteroidea*, *G. jiangxiensis* and *G. pappenheimi*. The rest of congeneric species are assigned to the other group defined by having an unscaled region of the abdomen adjacent to the ventral mid-line extending to or away from the pelvic-fin base. There are marked variations of the new species with the most similar species, *G. homalopteroidea* and *G. pappenheimi*, which are provided in the diagnosis. *Gobiobotia brevirostris* is presently known only from the upper Han-Jiang of the middle Chang-Jiang Basin, while *G. jiangxiensis* occurs in the Xin-Jiang, an affluent of Lake Poyang. The new species is further distinct from the two co-existing species in having 4+31–32 (vs. 4+33–35) vertebrae, the smaller eyes (diameter less than the interorbital width), maxillary barbels longer than the eye diameter and the third pair of longer mental barbels extending to the pectoral-fin insertion; from *G. brevirostris* in having a longer (vs. shorter) snout than



Figure 10. Lateral (a) and ventral (b) views of *X. boulengeri*, AMNH 20522, from Lake Dongting; lateral (c) and ventral (d) views of *X. boulengeri*, MNHN-IC-1934-0180, holotype.

post-orbital head and relatively smaller (vs. larger) eye diameter than interorbital width; and from *G. jiangxiensis* in having longer (vs. shorter) maxillary barbels than the eye diameter and the third pair of mental barbels extending to the pectoral-fin insertion (vs. to the front margin of the pre-opercula) (See Table 4 and Fig. 4).

Some of type specimens of *G. lii* were collected from Lake Dongting where five nominal species of eight-barbel gudgeons have been documented: *G. boulengeri* (= *Xenophysogobio boulengeri*), *G. filifer*, *G. meridionalis*, *G. nicholsi* and *G. pappenheimi* (Bănărescu and Nalbant 1966; Chen and Cao 1977; He and Chen 1998; Wu et al. 2021). The identification of all these species, except the third one, indeed requires re-evaluation. From Chen and Cao's (1977) point of view, type specimens of *G. nicholsi* were small and similar to *G. ichangensis* (= *G. filifer*) for the presence of small eyes, the first pair of mental barbels rooted anterior to maxillary-barbel bases and the well-developed keels on pre-dorsal scales of the dorsum; they also had no remarkable differences with small (25.5–32.0 mm SL) topotypes. These

characteristics led them to reach a conclusion that these type specimens were juveniles of *G. filifer*, a species currently known from the Chang-Jiang Basin. *Gobiobotia filifer* is characterised by having an elongated tip of the second branched pectoral-fin ray (He and Chen 1998). This prolonged tip is definitely not exhibited by type specimens of *G. nicholsi* (AMNH 20523; Fig. 8). The observation by Gao et al. (1988) revealed that the second branched pectoral-fin ray of *G. filifer* has an elongated tip at the juvenile stage (16 mm TL). This finding negates Chen and Cao's (1977) hypothesis that the types of *G. nicholsi* were juveniles of *G. filifer*. Other characters diagnostic for *G. filifer* comprise 40–42 lateral-line pored scales, three scale rows below the lateral line and the eye diameter slightly less than the interorbital width (Chen and Cao 1977). They are not shared with the type specimens (photograph examined; Fig. 8) of *G. nicholsi*, with 44–45 lateral-line pored scales, four scale rows below the lateral line and the eye diameter about half of the interorbital width. It is apparent that *G. nicholsi* and *G. filifer* are two distinct species.

Bănărescu and Nalbant (1966) followed Nichols (1928; 1943) to identify specimens from Lake Dongting as *G. pappenheimi*, initially described by Kreyenberg (1911) from Tientsin (now Tianjin) of China, while giving its precise type locality as the Pai-ho (= Bai-He of the Hai-He Basin) in Hopei (now Hebei Province). The original description is vague and, thus, of limited taxonomic use for current species identification. This species, as exhibited in the holotype (ZMB 18466, photograph examined; Fig. 9c, d), has a naked region of the abdomen adjacent to the ventral mid-line up to the vent and a slightly prolonged tip of the second branched pectoral-fin ray and 38 lateral-line pored scales. These characters, however, are not shared with two large specimens (photograph examined; Fig. 9a, b) recognised by Bănărescu and Nalbant (1966) as *G. pappenheimi* from Lake Dongting. Instead, both have a naked region of the abdomen adjacent to the ventral mid-line up to the pelvic-fin base and more than 40 lateral-line scales and lacks a slightly elongated tip of the second branched pectoral-fin ray (Fig. 9a, b), thus not conspecific with either *G. filifer* or *G. lii*, as well as *G. pappenheimi*. Due to the two specimens and other 17 small specimens (photograph examined) not preserved in good condition, the precise count of lateral-line pored scales for them is impossible. All these specimens from Lake Dongting are probably the misidentification of *G. nicholsi*. Nonetheless, the hypothesis needs to be confirmed in a future study. In Chinese literature (Liang and Liu 1966; Chen and Cao 1977; He and Chen 1998; Zhang et al. 2016), the occurrence of *G. pappenheimi* in Lake Dongting is an erroneous record. This species is not found in the Chang-Jiang and river basin south of the river.

One small specimen of 37.5 mm SL caught from Lake Dongting was referred to as a distinct species by Chen and Cao (1977), who stopped short of describing a new species owing to lack of large specimens. It has a naked region of the abdomen adjacent to the ventral mid-line up to the vent, no prolonged tip of the second branched pectoral-fin ray, the first pair of mental barbels inserted anterior to the maxillary-barbel bases, 38 lateral-line pored scales and a gas bladder with an anterior chamber enclosed in a membranous capsule (Chen and Cao 1977, page 556: table 10-1). All these characters, except the last one, are shared with *G. lii*. It is possible that the small specimen from Lake Dongting is conspecific with this species. Size-related change is the plausible explanation for the variation in the anterior chamber of the gas bladder between it and type specimens of *G. lii*.

Bănărescu and Nalbant (1966) was the first to recognise two specimens from Lake Dongting of the middle Chang-Jiang Basin as *G. boulengeri*, a species that was initially described by Tchang (1929) from Szechwan (today's Sichuan Province in the upper Chang-Jiang Basin). Both (AMNH 20522, photograph examined; Fig. 10a, b) have larger eyes (diameter about one-fifth head length), the adpressed tip of the pectoral fin reaching or slightly beyond the pelvic-fin insertion, the adpressed tip of

the pelvic fin reaching the anal-fin origin and the upper extremity of the gill opening aligned with the upper margin of the eye. All these characters are incongruent with the original description of *G. boulengeri* (= *Xenophysogobio boulengeri*). Three available topotypes and the holotype (MNHN-IC-1934-0180, photograph examined; Fig. 10c, d) have small eyes (diameter about one of tenth head length), the adpressed tip of the pectoral fin not reaching the pelvic-fin insertion, the adpressed tip of the pelvic fin not reaching the anal-fin origin and the upper extremity of the gill opening aligned with the lower margin of the eye. Evidently, the aforementioned two specimens from Lake Dongting are not identical to *X. boulengeri*. To which species they belong still remains unclear, given the poor condition of specimens and no examination on the structure of their gas bladders. In Chinese literature (Chen and Cao 1977; He and Chen 1998; Zhang et al. 2016; Guo et al. 2021), *X. boulengeri* has an erroneous record from Lake Dongting. This species is endemic to the upper Chang-Jiang Basin.

Based on the above analysis, it is here concluded that Lake Dongting harbours four eight-barbel gudgeons: *G. filifer*, *G. lii*, *G. meridionalis* and *G. nicholsi*. The new species is separated from all other three co-existing congeneric species by the presence of a naked region of the abdomen adjacent to the ventral mid-line extending to the vent (vs. extending away from or to the pelvic-fin base). It lacks an elongated tip of the second branched pectoral-fin ray typical for *G. filifer* and has 37–38 lateral-line pored scales fewer than 44–45 and 40–43 for *G. nicholsi* and *G. meridionalis*, respectively.

The validity of *G. lii* is further affirmed by its monophyletic nature recovered in *cyt b* gene-based phylogenetic analysis and its significant sequence variation on all sampled congeners. In the BI trees (Fig. 7), *Gobiobotia lii* formed a strongly-supported exclusive lineage, being sister to the endemic South Korean species *G. naktongensis*. The new species differs from it in having, amongst others, a naked region of the abdomen adjacent to the ventral mid-line reaching the pelvic-fin base, the pectoral fin extending beyond the pelvic-fin insertion and the third pair of mental barbels reaching over the hind margin of the opercula. The sequence divergence of *G. lii* with sampled congeneric species varied from 2.6–22.1% (average 12.4%) (Table 5), greater than 2% being utilised as a threshold for vertebrates' species delimitation (Avice and Walker 1999; Hebert et al. 2003).

Comparative material

- G. abbreviata*: IHB2113-2117, 5 specimens, 53.4–74.8 mm SL, Min-Jiang at Leshan City, Sichuan Province, China.
- G. brevibarba*: IHB, uncatalogued, 2 specimens, 61.5–88.6 mm SL; Misan-Ri, Sangnam-Myon Inje-Gun, Kangwon-Do, South Korea.

- G. brevirostris*: IHB 81VIII1310-1311, 2 specimens, 39.0–41.7 mm SL; Han-Jiang at Tanghe County, Henan Province, China.
- G. filifer*: IHB 83IV1438-1439, 83IV1441-1450, 64V2287, 64IV0803-0804; IHB 0305, 53120-53121, 53123, 20 specimens, 52.2–96.5 mm SL; Yichang City, Hubei Province, China. IHB 2017100792, 1 specimen, 78.6 mm SL; Yueyang City, Hunan Province, China. AMNH 79426, 1 specimen, all other data same as Yueyang City, Hunan Province, China (photograph examined).
- G. guilingensis*: IHB 20161062A, 1 specimen, 86.7 mm SL; Zhu-Jiang at Rongshui County, Guangxi Province, China.
- G. jiangxiensis*: IHB 90-IV-1256, holotype, 41.0 mm SL; Xin-Jiang at Shangrao City, Jiangxi Province. IHB 90-IV-1257, IHB 90-IV-1377, IHB 90-IV-1731-1732, 4 paratypes, 37.0–54.0 mm SL; Shangrao City and Guangfeng County. IHB 90-IV-1796, 6 specimens, 35.2–52.5 mm SL; all other data same as holotype.
- G. macrocephala*: IHB, uncatalogued, 1 specimen, 53.8 mm SL; Somaegok-Ri pukpsng-Myon, Hongcheon-Gun, Kangwon-Do, South Korea.
- G. meridionalis*: IHB 201609021340-1344, 201609056055, 6 specimens, 70.5–85.9 mm SL; Yuan-Jiang at Luxi County, Hunan Province, China. IHB 201609025884-5885, 2 specimens, 95.3–99.5 mm SL; Yuan-Jiang at Mayang County, Hunan Province, China. IHB 201609056048, 201609021171-201609021176, 7 specimens, 75.2–93.7 mm SL; Yuan-Jiang at Chenxi County, Hunan Province, China. IHB 201711056049, 201809036686, 201809036690, 3 specimens, 54.0–74.3 mm SL; Xiang-Jiang at Chalin City, Hunan Province, China.
- G. naktongensis*: IHB, uncatalogued, 2 specimens, 44.3–47.5 mm SL; Kyongsangbuk-Do, South Korea.
- G. nicholsi*: AMNH 20523, holotype, 35.7 mm SL; Yueyang City, Hunan Province, China (X-radiograph examined); AMNH 20524, 2 paratypes, 21.9–32.2 mm SL; same locality as holotype (photograph examined).
- G. pappenheimi*: ZMB 18466, holotype, 45.0 mm SL, Tianjin City, China (X-radiograph examined); BMNH 1925.8.6.36, Russia (X-radiograph examined); AMNH 10311, 10308, 10553, 19 specimens, 32.2–41.0 mm SL; Yueyang City, Hunan Province, China (photograph examined). IHB 80-VII-607-609, 611–613, 6 specimens, 45.8–56.1 mm SL; Nen-Jiang at Heilongjiang Province, China. IHB 80-II-1282-1283, 92-V-1609–1611, 5 specimens, 27.9–39.5 mm SL; Liao-He at Liaoning Province, China.
- G. tungi*: IHB 202108056037-6038, 2 specimens, Xin-Jiang, an affluent of Poyang Lake at Shangrao City, Jiangxi Province, China.
- Xenophysogobio boulengeri*: MNHN-IC-1934-0180, holotype, Szechwan (now Sichuan Province), China (X-radiograph and photograph examined); AMNH 20522, 2 specimens, 30.0–31.2 mm SL; Yueyang City, Hunan Province, China (photograph examined); IHB78IV0215-16, 0218, 3 specimens, Min-Jiang, an

effluent of the upper Chang-Jiang at Leshan, Sichuan Province, China.

Ethics approval and consent to participate

All procedures described in this paper were in accordance with Chinese laws and were licensed by the Ministry of Ecology and Environment of the People's Republic of China.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was granted by special fund of Program for Biodiversity Investigation & Assessment Project for Biodiversity Conservation of Lake Dongting (Grant No. 2016HB2096001006) and National Science & Technology Fundamental Resources Investigation Program of Mount Dabie (Grant No. 2019FY101800).

Authors' contributions

Xiao Chen and Man Wang conceived the study and analysed the data. Xiao Chen led the writing. Liang Cao and E Zhang revised the manuscript. All authors contributed to the writing of the paper.

Acknowledgements

Our sincere thanks should be given to Prof. Jian-Zhong Shen (HZAU, Huazhong Agricultural University) and Hong Li (Hunan Fisheries Science Institute) for assisting us in fieldworks and Wei-Han Shao, Zi-Tong Wang, Dong-Ming Guo, Xiong Gong and Yi Liu (IHB) for fieldworks and laboratory analysis. Special thanks go to Chang-Ting An (IHB), who provided constructive suggestions for this manuscript. We thank Radford Arrindell (AMNH) and Peter Bartsch and Edda Aßel (ZMB) for friendly help in providing specimen photographs and X-radiographs. We greatly appreciated Prof. Sven Kullander, Dr. Fan Li and Anonymous Reviewer for constructive comments to improve our manuscript.

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A new neotenous genus and species, *Deltanthura palpus* gen. et sp. nov. (Isopoda, Anthuroidea, Paranthuridae) from Japan, with a revised key to the genera in Paranthuridae

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Academic editor: Sammy De Grave ♦ Received 7 February 2022 ♦ Accepted 15 March 2022 ♦ Published 31 March 2022

Abstract

We describe a new paranthurid isopod genus and species, *Deltanthura palpus* **gen. et sp. nov.**, collected from a depth of 805–852 m off the southern coast of Mie prefecture, Japan. *Deltanthura* is similar to *Pseudanthura* Richardson, 1911 in having a triangular pleotelson, acute mandible with a 3-articulate palp, a maxillipedal endite, and a tapering uropodal exopod, but differs in having eyes and neotenous characters (reduced pereonite 7 and pereopods 7 lacking). *Deltanthura* and four paranthurid genera (*Califanthura* Schultz, 1977, *Colanthura* Richardson, 1902, *Cruranthura* Thomson, 1946, and *Cruregens* Chilton, 1882) share neotenous characters, but in *Deltanthura* the mandible is acute, with a 3-articulate palp and maxillipedal endites are present. *Califanthura minuta* Kensley & Heard, 1991 may belong in *Deltanthura* as they share the triangular pleotelson and tapering uropodal exopod, but we refrain from transferring it to *Deltanthura* as its description lacks the other diagnostic characters of *Deltanthura*. We provide a revised key to all genera in Paranthuridae Menzies & Glynn, 1968.

Key Words

Califanthura, Cymothoidea, deep sea, neoteny, Peracarida, taxonomy

Introduction

Species in the anthuroid isopod family Paranthuridae Menzies & Glynn, 1968 have the mouthparts acutely produced; the palm of pereopod 1 lacks a proximal tooth; and statocysts are absent (Poore 2001). Paranthuridae comprises six genera: *Paranthura* Bate & Westwood, 1866, *Pseudanthura* Richardson, 1911, *Califanthura* Schultz, 1977, *Colanthura* Richardson, 1902, *Cruranthura* Thomson, 1946, and *Cruregens* Chilton, 1882. The latter four are neotenous genera in which non-manca individuals lack pereopod 7, as is the case for manca-stage individuals, and share the character combination of having a blunt mandible lacking a palp and an extremely

reduced pereonite 7. On the basis of morphological observations, Poore (1984) hypothesized that (1) the four neotenous genera comprise a monophyletic group (hereafter, the “neotenous clade”); (2) *Paranthura* is the sister group to the neotenous clade, as species in both groups lack maxillipedal endites; and (3) *Pseudanthura* is the most basal genus in Paranthuridae.

We collected a paranthurid individual lacking pereopod 7 from off the southern coast of Mie, Japan, but unlike the four known neotenous paranthurid genera, its mandible bears an acute incisor and 3-articulate palp, and its maxilliped bears an endite. Here we describe the species as new, establish a new genus for it, and provide a revised key to the genera in Paranthuridae.

Materials and methods

A single specimen was collected in 2008 from Shima Spur off the southern coast of Mie prefecture, Japan, with a 3-m beam trawl at a depth between 805 and 852 m, during the cruise KT 08-3 of RV *Tansei-maru*. The specimen was fixed in 5–10% borate-buffered formalin in seawater and preserved in 70% ethanol. The methods for dissection, observation, drawing, and measurement were as described in Shiraki et al. (2021). Body length was measured from the tip of the anterolateral lobe of the head to the tip of the telson, and body width at the widest portion of pereonite 4. The specimen examined is deposited in the collections of the Seto Marine Biological Laboratory (SMBL-V0645).

Systematics

Superfamily Anthuroidea Leach, 1814 Family Paranthuridae Menzies & Glynn, 1968

Deltanthura gen. nov.

<http://zoobank.org/04EA59DC-C5AD-486B-A3CB-AA7076F5ABE5>

New Japanese name. *Sankaku-ashitarazu-uminanafushi-zoku*

Diagnosis. Eyes present. Pereonite 7 reduced and hidden laterally. Pleonites 1–5 fused but with sutures. Pleonite 6 entirely fused dorsally to telson; pleotelson triangular, without statocyst. Mandible acute, with 3-articulate palp. Maxilliped with 4-articulate palp; endite present. Pereopod 7 absent on non-manca individuals. Uropodal exopod elongate triangular, tapering.

Type species. *Deltanthura palpus* Shiraki, Shimomura & Kakui, by original designation.

Etymology. The generic name is a combination of the Greek letter *delta* (δέλτα), which is triangular in shape, referring to the shape of pleotelson, prefixed to *anthura*, from Greek *anthos* (a flower) and *oura* (a tail). The gender is feminine.

Remarks. Like *Pseudanthura* and *Paranthura*, *Deltanthura* has an acute mandible with a 3-articulate palp, but it differs from the former two genera in having pereonite 7 reduced and in lacking pereopods 7. *Deltanthura* and four paranthurid genera share neotenous characters (reduced pereonite 7 and pereopods 7 lacking), but in *Deltanthura* the mandible is acute, with a 3-articulate palp and maxillipedal endites present, characters that are unique among the neotenous paranthurid genera (Table 1).

Deltanthura gen. nov. does not fit well within the clade (*Paranthura* + neotenous clade) (cf. Poore 2001), as it has a maxillipedal endite. Rather, *Deltanthura* seems to be more closely related to *Pseudanthura*, as these two share multiple character states such as a triangular pleotelson; elongate triangular, tapering uropodal exopod; and maxillipedal endite. If this latter hypothesis is correct, the neotenous condition in Paranthuridae may not have had

a single origin. In addition to the five neotenous genera now known in Paranthuridae, three neotenous genera are known in anthuroid isopods (Poore 2009): the monotypic genera *Exallanthura* Kensley, 1980 and *Leipanthura* Poore, 2009 in Anthuridae Leach, 1814; and the genus *Curassanthura* Kensley, 1981 in Leptanthuridae Poore, 2001. We note that Poore (2009) pointed out that the *Exallanthura* females *sensu* Kensley (1980) may actually have been manca individuals.

The neotenous species *Califanthura minuta* Kensley & Heard, 1991 from the British West Indies may belong in *Deltanthura* gen. nov. It has the triangular pleotelson and tapering uropodal exopod (Kensley and Heard 1991) that occur in *Deltanthura* but not in other *Califanthura* species. However, as the shape of mandible and the presence or absence of the maxillipedal endite (characters diagnostic for *Deltanthura*) are unknown for *C. minuta*, we refrain from transferring it to *Deltanthura*.

Deltanthura palpus sp. nov.

<http://zoobank.org/290406E3-4688-4CC7-8A3F-DC104E7C7BBF>

[New Japanese name: *Sankaku-ashitarazu-uminanafushi*]

Figs 1–4

Material examined. Holotype. JAPAN • 1 female lacking oostegites, body length 7.03 mm, body width 0.91 mm; Mie prefecture, off the southern coast, Shima Spur, Stn. SM-01-(1); 34°00.83'N, 136°53.79'E to 34°01.42'N, 136°51.80'E; depth 805–852 m; 4 Mar. 2008; Tadashi Akiyama leg.; SMBL-V0645, 12 slides and 1 vial.

Etymology. The specific name, *palpus* (Latin: palp), is a singular noun in the nominative case, referring to the presence of a mandibular palp, a unique trait in paranthurid neotenous genera.

Description of holotype female. Body relatively slender (Figs 1, 2A–C), length 7.69 times width. Head (Fig. 2A) length 1.02 times head width, roughened, irregular dorsally; rostrum protruding as much as anterolateral lobes; eyes dorsolateral, with scattered ommatidia. Pereonites 1–7 (Fig. 2A, B) roughened, irregular dorsally, with length ratio 1.00:1.02:1.26:1.19:1.08:0.78:0.18; pereonite 7 (Fig. 2B, C) reduced, hidden laterally, lacking pereopod 7. Pleonites 1–5 (Fig. 2B, C) fused but with sutures, length 0.06 times body length. Pleonite 6 entirely fused dorsally to telson (Figs 2B, C, 4H); pleotelson triangular, length 1.04 times width, with four dorsal and six apical simple setae.

Antennula (Fig. 2D) with three peduncular and six flagellar articles. Peduncular article 1 with one inner and two outer plumose sensory setae; article 2 with inner simple seta; article 3 with three inner and one outer simple setae. Flagellar articles 1 and 2 naked; article 3 with distal aesthetasc and three distal simple setae; article 4 with distal aesthetasc; article 5 with distal aesthetasc and simple seta; article 6 with four distal simple setae.

Antenna (Fig. 2E) with five peduncular and nine flagellar articles. Peduncular article 1 with outer simple

Table 1. Comparison of selected characters among the seven genera in Paranthuridae.

Characters	<i>Pseudanthura</i> Richardson, 1911	<i>Paranthura</i> Bate & Westwood, 1866	<i>Califanthura</i> Schultz, 1977	<i>Colanthura</i> Richardson, 1902	<i>Cruranthura</i> Thomson, 1946	<i>Cruregens</i> Chilton, 1882	<i>Deltanthura</i> gen. nov.
Eyes	Absent	Present/absent	Present	Present	Present	Absent	Present
Pereonite 7	Not hidden laterally	Not hidden laterally	Hidden laterally	Hidden laterally	Not hidden laterally	Not hidden laterally	Hidden laterally
Pleonites 1–5	Fused	Free or fused	Fused	Indicated by folds	1 free, 2–5 fused	Free	Fused
Pleonite 6 and telson	Fused dorsally	Not fused dorsally	Not fused dorsally	Not fused dorsally	Not fused dorsally	Not fused dorsally	Fused dorsally
Mandible	Acute	Acute	Blunt	Blunt	Blunt	Blunt	Acute
Mandibular palp	3-articulate	3-articulate	Absent	Absent	Absent	Absent	3-articulate
Maxillipedal endite	Present	Absent	Absent	Absent	Absent	Absent	Present
Pereopod 7 in non-manca individuals	Present	Present	Absent	Absent	Absent	Absent	Absent
Uropodal exopod	Tapering	Oval	Oval	Oval	Oval	Oval	Tapering
References	Poore and Lew Ton (1986)	Frutos et al. (2011), Poore (2001)	Poore (2001)	Poore (2001)	Poore (2001)	Poore (2001)	Present study



Figure 1. *Deltanthura palpus* sp. nov., holotype, female, lateral view of fixed specimen. Scale bar: 1 mm.

seta; article 2 with two distal simple setae; article 3 with two inner simple setae; article 4 with two distal plumose sensory setae and three simple setae; article 5 with four distal plumose sensory setae and four inner simple setae. Flagellar articles 1–9 with four, four, seven, four, three, three, two, four and zero distal simple setae, respectively.

Mandible (Fig. 2F) with 3-articulate palp. Palp article 1 naked; article 2 with distal simple seta; article 3 with eleven simple setae. Molar absent. Incisor acute.

Maxilla (Fig. 2G) slender, with twelve teeth and narrow lamella.

Maxilliped (Fig. 2H) with 4-articulate palp. Palp articles 1 and 2 long, 3 and 4 short, with one, four, one, and three simple setae, respectively. Endite present, reaching middle of article 2, with distal simple seta. Epipod oval.

Pereopod 1 (Fig. 3A) subchelate, robust. Basis with three dorsal plumose sensory setae and ventrodistal seta (tip broken). Ischium with one outer and one ventrodistal simple setae. Merus with two dorsal, one

outer, and one ventral simple setae. Carpus strongly protruding ventrodistally, with three inner mid-ventral spiniform setae and four simple setae. Propodus broad, with two inner proximal spiniform setae, and one outer, four dorsal, and one distal simple setae. Palm with five outer spiniform setae and seven simple setae. Dactylus and unguis fused, with two ventral and five middle simple setae.

Pereopod 2 (Fig. 3B) narrow. Basis with two dorsal plumose sensory setae, two simple setae, and dorsal seta (tip broken). Ischium with three simple setae. Merus with two dorsal and two ventrodistal simple setae. Carpus triangular, longer than wide, with three ventrodistal simple setae and ventrodistal seta (tip broken). Propodus with dorsal plumose sensory seta, three ventral spiniform setae, and two dorsal and three ventral simple setae. Dactylus with ventrodistal thick seta and three ventral, three ventrodistal, and four inner distal simple setae. Unguis naked.

Pereopod 3 (Fig. 3C) similar to pereopod 2 except in number of setae.

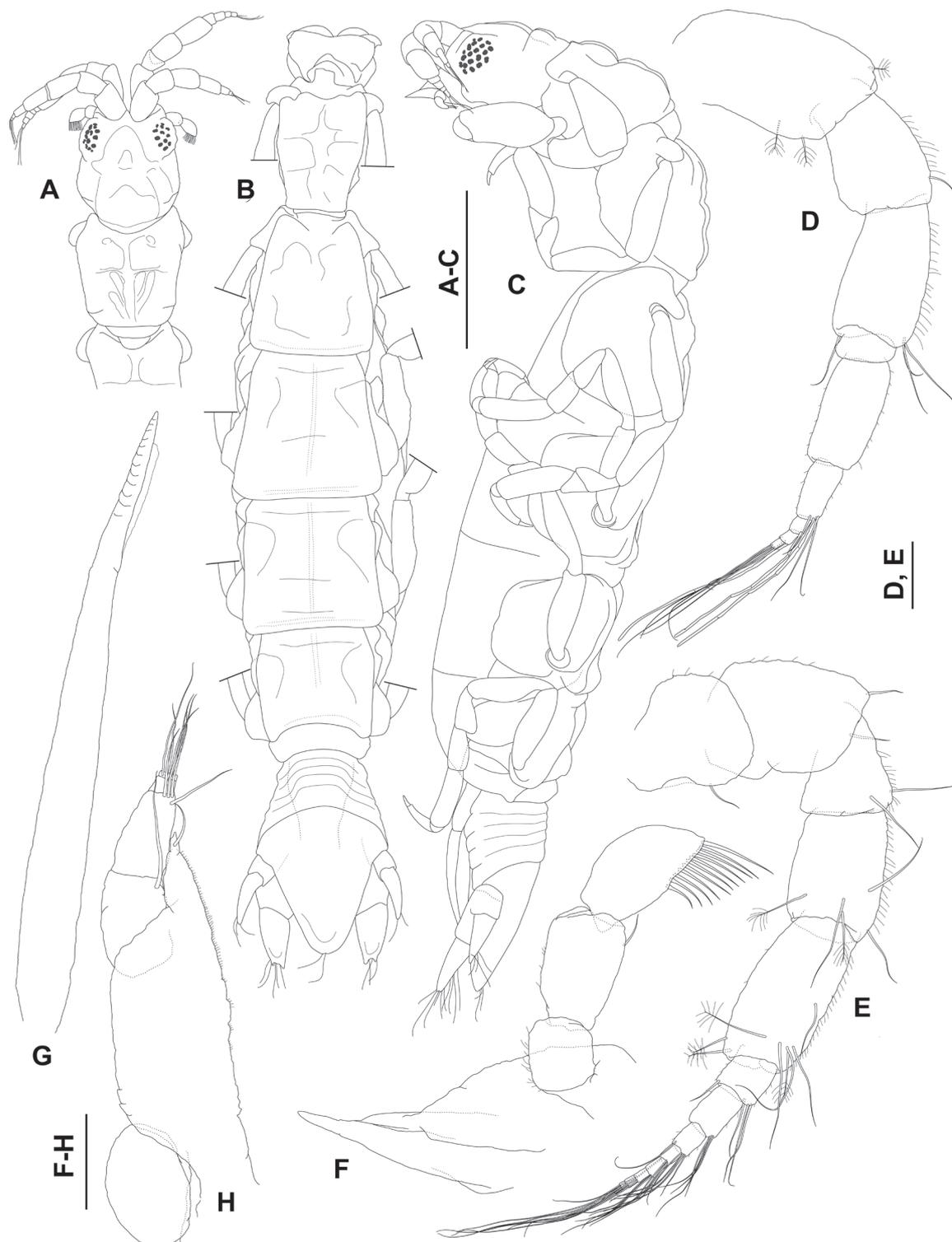


Figure 2. *Deltanthura palpus* sp. nov., holotype, female. **A.** Dorsal view of head and pereonite 1; **B.** Dorsal view from pereonite 2 to telson; **C.** Lateral view; **D.** Left antennula; **E.** Left antenna; **F.** Right mandible; **G.** Right maxilla; **H.** Right maxilliped. Scale bars: 1 mm (A–C); 100 μ m (D–H).

Pereopods 4–6 (Fig. 3D–F) narrower than pereopod 2, but similar to it except in number of setae and shape of carpus. Carpus rectangular, with ventrodistal spiniform seta.

Pleopod 1 (Fig. 4A) protopod with inner simple seta and outer seta (tip broken). Exopod operculiform, distal

margin serrate, with 15 marginal plumose setae and four simple setae on surface. Endopod 0.53 times longer than exopod, with three distal simple setae.

Pleopods 2–5 (Fig. 4B–E) similar to one another. Protopod with one or two simple setae. Exopod with five to seven distal plumose setae and outer simple (pleopods

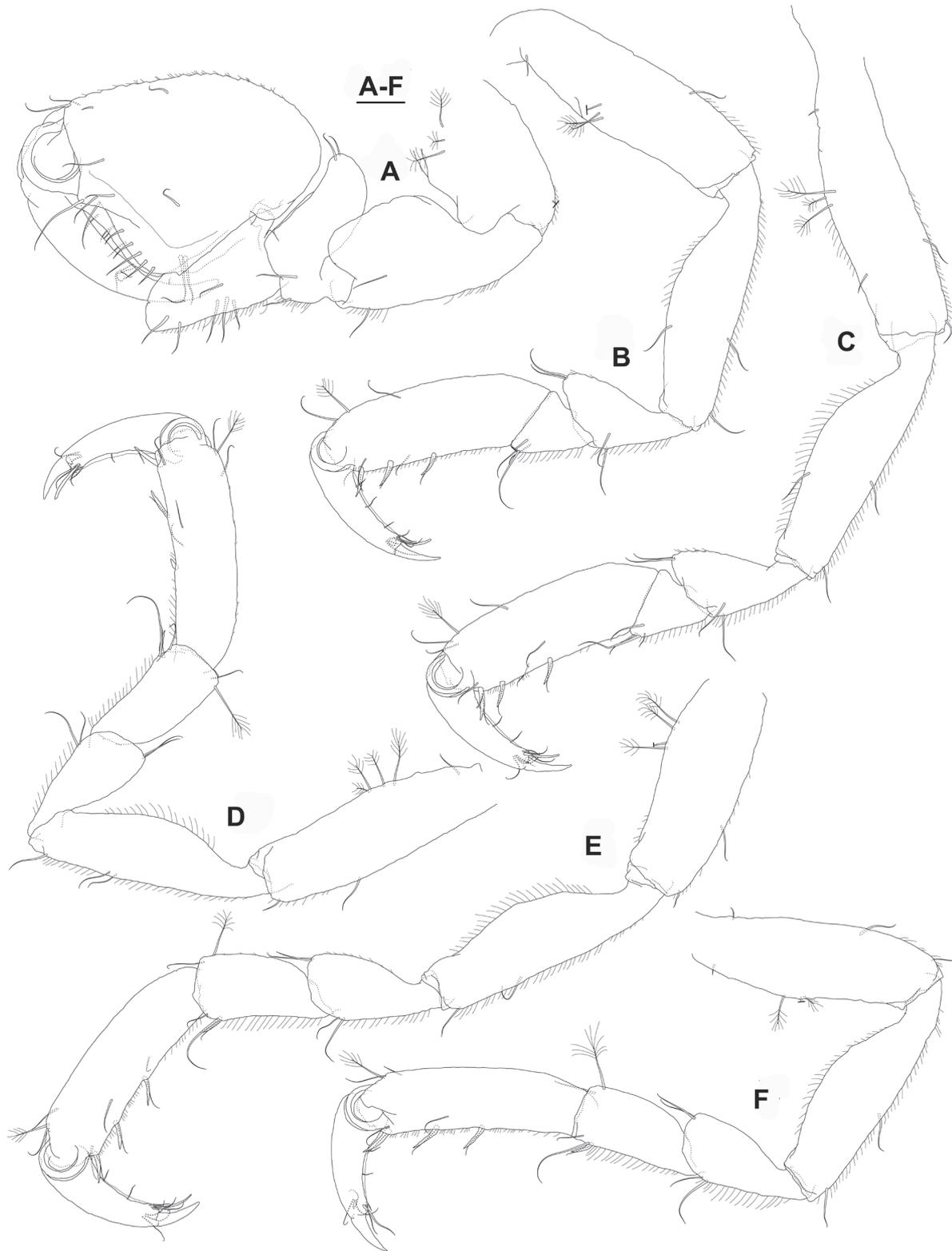


Figure 3. *Deltanthura palpus* sp. nov., holotype, female. A–F. Left pereopods 1–6. Scale bar: 100 μ m.

2–4) or plumose (pleopod 5) seta. Endopod with three distal plumose setae.

Uropod (Fig. 4F, G) with protopod bearing two outer and one inner plumose setae and outer simple seta. Exopod elongate triangular, tapering, with five (right) and 10 (left) simple setae. Endopod with two distal projections, five

outer and three distal plumose sensory setae, and 12 distal simple setae.

Remarks. Our specimen lacks oostegites and an appendix masculina, but its pereon is expanded ventrally (Fig. 1), leading us to conclude that it is a female prior to spawning, rather than a manca-stage individual. The

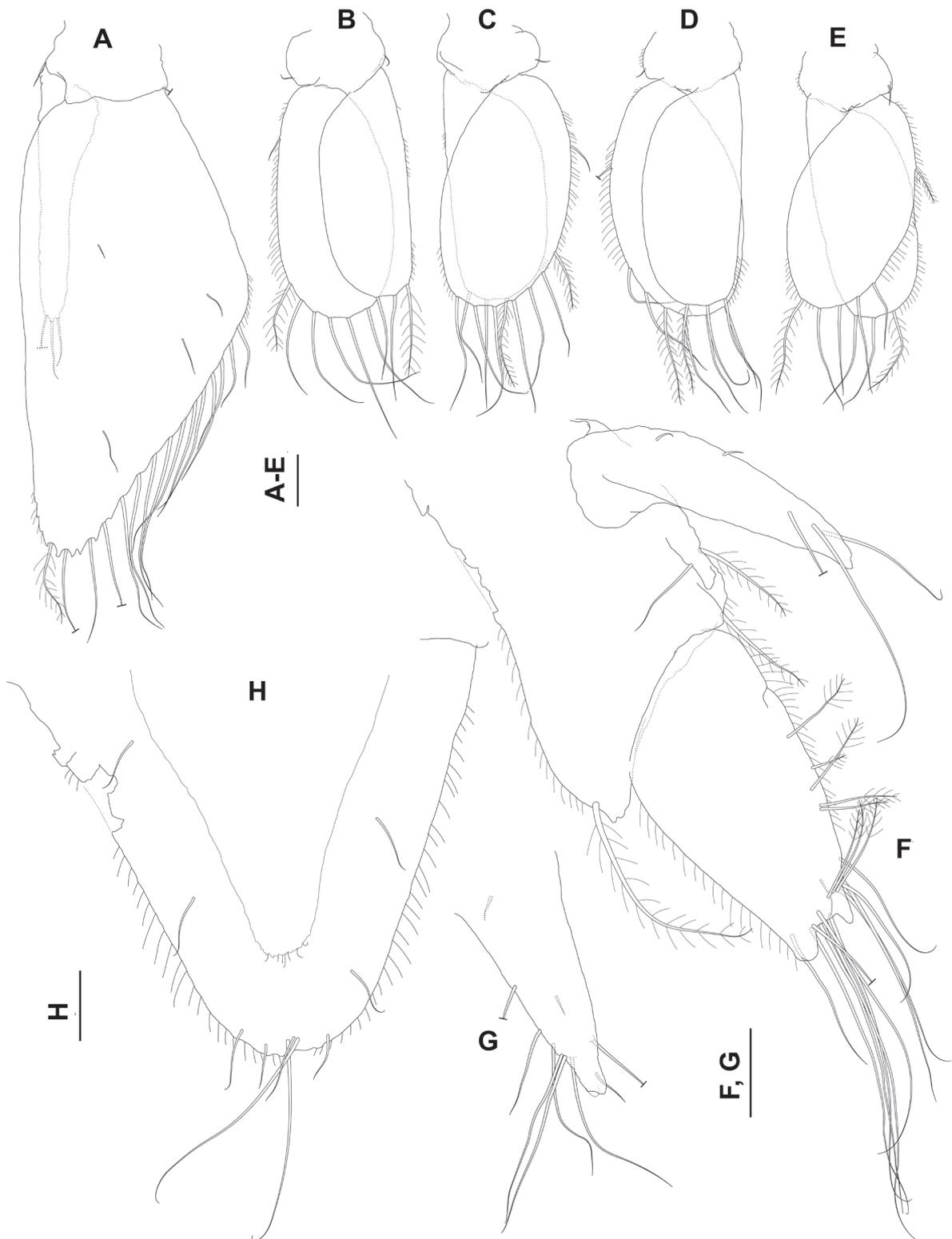


Figure 4. *Deltanthura palpus* sp. nov., holotype, female. **A–E.** Left pleopods 1–5 (most setal ornamentation omitted); **F.** Right uropod; **G.** Left uropodal exopod; **H.** Telson. Scale bars: 100 μ m.

strongly reduced pereonite 7 also supports this conclusion; this segment is not as reduced as in the mancae of non-neotenus species (cf. Frutos et al. 2011: fig. 2C, D; Wägele 1981: fig. 9M). This is the fifth neotenus genus known in Paranthuridae.

Deltanthura palpus gen. et sp. nov. differs from *Califanthura minuta*, which may belong in *Deltanthura*

(see Remarks above for the genus), in the following characters (character state of *C. minuta* in parenthesis): body length 7.03 mm (1.60 mm); carpus of pereopod 1 strongly protruding ventrodistally (not protruding); endopod of pleopod 1 short, about half length of exopod (elongate, slightly shorter than exopod); and uropodal endopod with two distal projections (no projections).

Key to the genera in Paranthuridae, modified from Poore (2001)

- 1 Pereopod 7 present in non-manca individuals 2
 – Pereopod 7 lacking in non-manca individuals 3
 2 Pleonite 6 marked off dorsally from telson; uropodal exopod linear or leaf-shaped *Paranthura*
 – Pleon and telson fused into single segment; uropodal exopod extremely reduced *Pseudanthura*
 3 Pleonite 6 entirely fused dorsally to telson; pleotelson tapering, triangular; mandible acute, with 3-articulate palp
 *Deltanthura* gen. nov.
 – Pleonite 6 marked off dorsally from telson; pleotelson oval; mandible blunt, without palp 4
 4 Pereonite 7 hidden laterally; head as long as wide 5
 – Pereonite 7 not hidden laterally; head longer than wide 6
 5 Pleonites 1–5 separated by integumental folds dorsally; pleonite 1 twice as long as pleonite 2 *Colanthura*
 – Pleonites 1–5 fused, rarely with integumental folds dorsally; pleonites 1 and 2 similar in length *Califanthura*
 6 Pleonites 2–5 fused; marine or estuarine; eyes present *Cruranthura*
 – Pleonites all free; hypogean or freshwater; eyes lacking *Cruregens*

Acknowledgements

We thank the captain and crew of RV *Tansei-maru*, Dr. Tadashi Akiyama of Okayama University, and other onboard researchers for their efforts in collecting; cruise director Dr. Tatsuo Oji of Nagoya University for organizing cruise KT08-3; and Dr. Matthew H. Dick for reviewing the manuscript and editing our English.

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Taxonomy and natural history of *Cavernocypris hokkaiensis* sp. nov., the first ostracod reported from alpine streams in Japan

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Academic editor: Kay Van Damme ♦ Received 12 January 2022 ♦ Accepted 16 March 2022 ♦ Published 5 April 2022

Abstract

We describe the cypridoidean ostracod *Cavernocypris hokkaiensis* sp. nov. from riverbed sediments in an alpine stream at an elevation of ca. 1850 m in the Taisetsu Mountains, Hokkaido, Japan. This species differs from congeners in having (1) the outer surface of the carapace smooth, with sparse, tiny setae, but without shallow pits; (2) the carapace elongate rather than triangular in lateral view; (3) the antennula consisting of seven podomeres; (4) first palpal podomere of maxillula with five dorsodistal and one ventro-subdistal setae; (5) the fifth limb lacking setae b and d; and (6) the fifth limb lacking a vibratory plate. We provided the key to the *Cavernocypris* species. We determined partial sequences for the cytochrome *c* oxidase subunit I (COI; *cox1*) and 18S rRNA (18S) genes in *C. hokkaiensis*. Our sample contained only females, and we obtained a partial 16S rRNA sequence for the endosymbiotic bacterium *Cardinium* from *C. hokkaiensis*, indicating the possibility that this ostracod species reproduces parthenogenetically. Our field survey and observations of captive individuals suggested that *C. hokkaiensis* may be endemic to the Taisetsu Mountains, with a low population density, narrow distributional range, and slow maturation to sexual maturity.

Key Words

Crustacea, cryophilic, Cypridoidea, ecology, lotic, Ostracoda

Introduction

The genus *Cavernocypris* Hartmann, 1964, one of 20 genera in the subfamily Cypridopsinae Kaufmann, 1900 (Savatenalinton 2018, 2020; Meisch et al. 2019), is distinguished from the other 19 genera by the following combination of features (cf. Smith et al. 2017): (1) carapace elongate to triangular in lateral view, (2) left valve overlapping right valve along ventral margin, (3) surface of valves smooth or with small pits, (4) swimming setae of antennae very short, (5) distal segment of maxillular palp elongate, (6) terminal segment of seventh limb not separated, and (7) uropodal ramus flagellum-like and present only in females.

To date, six *Cavernocypris* species have been described from the Palearctic and Nearctic regions

(Meisch et al. 2019; Külköylüoğlu 2020); in Japan, one species, *C. cavernosa* Smith, 2011, has been reported from Shiga and Shizuoka prefectures (Smith 2011; Tanaka et al. 2015). *Cavernocypris* species inhabit the interstitial environment of riverbed sediments, the littoral zone of mountain lakes, springs, and caves (Marmonier et al. 1989; Smith et al. 2017). Although there is little information on their ecology, Forester (1991) found *C. wardi* Marmonier, Meisch & Danielopol, 1989 only in cold-water habitats at ca. 0–14 °C and suggested that the species may be cryophilic.

Streams in the alpine vegetation zone between the treeline and the permanent snow line are cold and nutrient-poor (Niedrist and Füreder 2017). They arise from glacial melt, snowmelt, rain runoff, and groundwater springs and are highly environmentally heterogeneous (Hotaling et

al. 2017). Many organisms inhabiting alpine streams are endemics (e.g. Muhlfield et al. 2011), uniquely adapted to harsh conditions (Lencioni et al. 2009). Ostracods are often detected in ecological research in alpine streams (e.g., Suren 1993; Zbinden et al. 2008), but their taxonomy, particularly outside Europe, has not been well studied.

The Taisetsu Mountains in Daisetsuzan National Park are located in the center of Hokkaido, Japan, and consist of several gently sloping volcanic peaks in the 2000 m class. Above the treeline at ca. 1400–1500 m elevation (Amagai et al. 2018), there are several aquatic features, including alpine streams, but except for insects (e.g. Konno 2003; Konno et al. 2003), the aquatic invertebrates have not yet been investigated.

Here we describe a new species of *Cavernocypris* from an alpine stream in the Taisetsu Mountains, the first record of an ostracod from cold alpine waters in Japan. We present nucleotide sequences for this species for parts of the mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear 18S rRNA (18S) genes and provide preliminary comments on its phylogenetic position based on 18S data. We also present information on its natural history based on a field survey and preliminary rearing results. Finally, we demonstrate with molecular data (part of the mitochondrial 16S rRNA gene, 16S) the infection of this species by *Cardinium*, a group of “reproduction-manipulating” endosymbiotic bacteria (Ma and Schwander 2017).

Materials and methods

Sampling was conducted at seven sites, including four streams (Stns 1–3, 7) fed by springs, a hot spring, and/or snowmelt, two ponds (Stns 5, 6), and one waterfall basin (Stn. 4) (Table 1, Fig. 1). Bottom sediment and water were placed in a bucket and stirred, and all but the sediment was filtered through a plankton net (mesh size 63 µm). This process was repeated several times at each site. Specimens were picked from the samples using a stereomicroscope (Olympus SZX9, Japan). Geographical coordinates and the elevation were obtained from GSI Maps (Geospatial Information Authority of Japan 2022). Water temperature was measured by using an O-274 thermometer (DRETEC, Japan).

Ostracods were fixed in 80% ethanol. The methods used for dissection, preparation of slides, light microscopy, scanning electron microscopy (SEM), and drawing were as described by Munakata et al. (2021). All material studied has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, under catalog numbers ICHUM-8247 to 8252.

The following abbreviations are used in the text: Ca, carapace; LV, left valve; RV, right valve; H, height; L, length; W, width; An1, antennula; An2, antenna; Md, mandible; Mx, maxillula; L5–7, fifth, sixth, and seventh limbs, respectively; UR, uropodal ramus. The appendage chaetotaxy follows Broodbakker and Danielopol (1982) for An1, Md, and Mx; Martens (1987) for An2; Meisch (2000) for L5–7; and Meisch (2007) for UR. The following measurements were made from digital images by using ImageJ (Rasband 2022): L and H of the LV and RV (LV-L, LV-H, RV-L, and RV-H); W of the carapace (Ca-W). Measurements in the text are in millimeters, followed by the mean value and sample size in parentheses.

An attempt was made to extract total DNA from the soft parts of three individuals by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany) following the manufacturer’s protocol, but only one of the three extracts allowed successful PCR amplification. Primers used for the PCR amplification and sequencing of ostracod COI, ostracod 18S, and *Cardinium* 16S are listed in Munakata et al. (2021), except that CLO-f2 (GGTGC GTGGGCGGCTTATT) and CLO-r2 (AAAGGGTTTCGCTCGTTATAG) (Gotoh et al. 2007) were used instead of CLO-f1 and CLO-r1. PCR amplification conditions for COI with TaKaRa Ex Taq DNA polymerase (TaKaRa Bio, Japan), for 18S with KOD FX Neo (Toyobo Life Science, Japan), and for 16S with TaKaRa Ex Taq were as described by Munakata et al. (2021). All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 and a 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated using MEGA7 (Kumar et al. 2016). BLAST (Altschul et al. 1990) was used to search the International Nucleotide Sequence Database (INSD; International Nucleotide Sequence Database Collaboration 2022) for nucleotide sequences most similar to our sequences.

Table 1. List of sampling sites in Daisetsuzan National Park.

Station number and name	Environment	Coordinates	Elevation	Mean water temperature	Sampling date
1. Hokkai-sawa Stream	Stream fed by spring	43°41'08"N, 142°55'28"E	1853 m	3.5 (2.2–4.8) °C	25.viii.2020, 26.vii.2021
2. Hokkai-sawa Stream	Stream fed by spring and snowmelt	43°41'17"N, 142°54'33"E	1837 m	4.1 (2.4–5.7) °C	25.viii.2020, 26.vii.2021
3. Akaishi Stream	Stream fed by hot spring with high-H ₂ S concentration and snowmelt	43°41'23"N, 142°54'35"E	1829 m	13 °C	26.vii.2021
4. Momizi Fall	Fall basin in Akaishi Stream	43°43'72"N, 142°57'41"E	813 m	13 °C	27.vii.2021
5. Sugatami Pond	Pond	43°39'41"N, 142°49'58"E	1665 m	No data	26.viii.2020
6. Unnamed Pond	Pond	43°39'43"N, 142°49'32"E	1597 m	No data	26.viii.2020
7. Daisetsu Asahidake Spring	Stream fed by spring	43°37'59"N, 142°41'31"E	445 m	7.0 °C	26.viii.2020

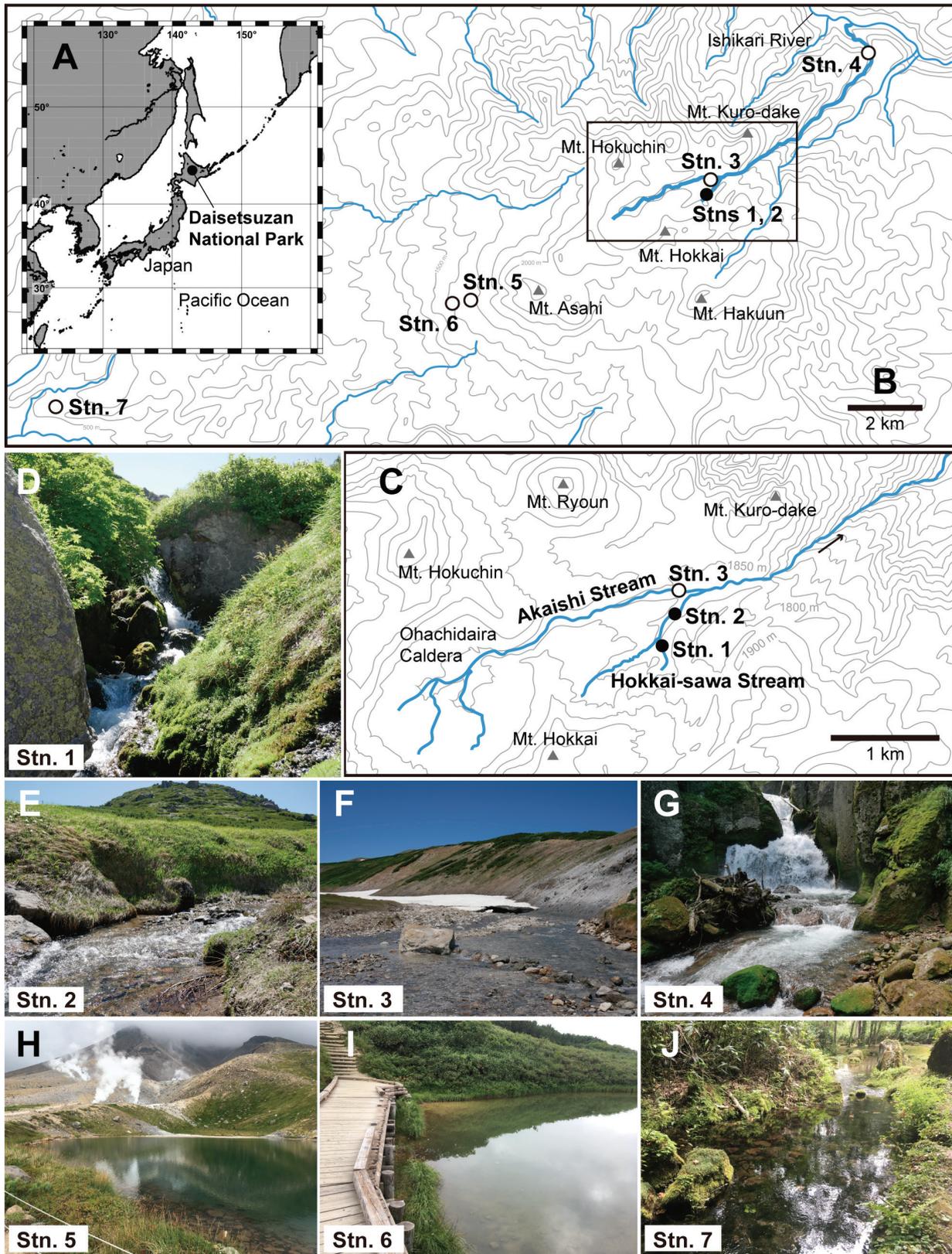


Figure 1. Sampling sites for *Cavernocypris hokkaiensis* sp. nov. **A.** Map showing location of Daisetsuzan National Park in Japan; **B.** Map showing the sampling sites (Stns 1–7) in Daisetsuzan National Park; **C.** Enlarged map of the area corresponding to the square in Fig. 1B; arrow indicates the stream flow direction; **D–J.** Photographs of the sampling sites; **D.** Hokkai-sawa Stream (Stn. 1); **E.** Hokkai-sawa Stream (Stn. 2); **F.** Akaishigawa Stream (Stn. 3); **G.** Momizi Fall (Stn. 4); **H.** Sugatami Pond (Stn. 5); **I.** Unnamed pond (Stn. 6); **J.** Daisetsu Asahidake Spring (Stn. 7). Maps were generated by using GMT6 (A Wessel et al. 2019) or were based on GSI Maps (B, C Geospatial Information Authority of Japan 2022). Filled and open circles indicate sites where *Cavernocypris* individuals were collected or not found, respectively.

To explore the phylogenetic position of this species, a maximum likelihood (ML) phylogenetic tree was constructed based on the 18S dataset comprised of 66 ostracod sequences (one our sequence, and 64 cypridoidean and one pontocypridoidean (outgroup) sequences from INSD; 1547 positions in the aligned dataset; see Suppl. material 1–3: Table S1, Alignments S1, S2). The detailed method and result are shown in Suppl. material 4: File S1.

To obtain information on the life cycle, three non-adult individuals collected on 26 July 2021 were maintained singly in wells of a tissue culture plate filled with water collected from sampling site Stn. 1 and placed in a refrigerator at a temperature of 7 °C. Detritus collected from sampling site Stn. 1 was added to each well as a food source. Observations were made twice or more per month.

Results

Field survey and observation of captive individuals

Among seven sampling sites, *Cavernocypris* ostracods were collected from only two sites in the Hokkai-sawa Stream (Stns 1 and 2) (Fig. 1B, C). Ostracod density at both sites was low, with fewer than 10 individuals per 500 ml of filtered residue. No male individuals were detected.

Three captive non-adult individuals have remained alive and active for more than five months. The body length (LV-L) of each individual was 0.47, 0.48, 0.39 mm. No molts have been observed to date (the latest observation was on 7 January 2022).

Taxonomy

Family Cyprididae Baird, 1845

Genus *Cavernocypris* Hartmann, 1964

Cavernocypris hokkaiensis sp. nov.

<http://zoobank.org/3DBCDFE2-957F-485F-A91E-036E71EC75C0>

Figs 2, 3

Etymology. The epithet *hokkaiensis* is an adjective referring to the type locality, Hokkai-sawa Stream in Japan.

New Japanese name. *Shibare-doukutsu-kaimijinko*, referring to the habitat having low water temperature. *Shibare* is derived from the Japanese verb *shibare-ru* (freeze), in a Hokkaido dialect; *Doukutsu-kaimijinko* is the Japanese name for *Cavernocypris* (Tanaka et al. 2015).

Type locality. Hokkai-sawa Stream, Daisetsuzan National Park, Hokkaido, Japan (Stn. 1: 43°41'08"N, 142°55'28"E).

Holotype: female, ICHUM-8247, one slide and one SEM stub, Stn.1, Hokkai-sawa Stream, riverbed sediment, 26 July 2021. **Paratypes** (five females): ICHUM-8248, 8249, one SEM stub and one slide for

each; ICHUM-8250, 8251, one SEM stub; ICHUM-8252, one slide, voucher specimen for LC666823 (COI) and LC666824 (18S). Collection data for ICHUM-8249, 8252 are same as holotype; ICHUM-8248, 8251 were collected from Stn. 2 (43°41'17"N, 142°54'33"E) on 25 August 2020; ICHUM-8250 was collected from Stn. 1 on 25 August 2020. All individuals were collected by Mizuho Munakata.

Description of females. Measurements (in millimeters, except for ratios) of carapace and valves: LV-L, 0.59–0.61 (0.60, N=3); LV-H, 0.30–0.31 (0.31, N= 3); LV-H/LV-L, 0.50–0.51 (0.51, N=3); RV-L, 0.58–0.61 (0.60, N=3); RV-H, 0.29–0.31 (0.30, N=3); RV-H/RV-L, 0.50–0.51 (0.51, N=3); Ca-W, 0.25–0.26 (0.26, N=2); Ca-W/LV-L, 0.41–0.42 (0.42, N=2).

Carapace (Fig. 2) translucent white, with black eye; outer surface smooth, with sparse, tiny setae but without shallow pits; widest point at about mid-length (Fig. 2A, B); LV overlapping RV at ventral margin (Fig. 2A); no dorsal hump on LV (Fig. 2B–D); outer list present in ventral margin of both valves (Fig. 2A).

LV (Figs 2C, E, G, 3A) with greatest height at about mid-length; anterior and posterior margins evenly rounded; apex of anterior margin lower than mid-height of LV and slightly higher than apex of posterior margin; in inner view, calcified inner lamella on anterior and posterior margins well developed (Figs 2E, 3A); inner list present in ventral region (Fig. 2G); mid-ventral margin with inner triangular projection (Fig. 2A, G). RV (Figs 2D, F, H, 3B) similar to LV in shape; inner list in ventral region absent (Fig. 2H). Two oblong mandibular muscle scars and four oblong adductor muscle scars on LV and RV (Figs 2E, F, 3A, B). Hinge adont.

An1 (Fig. 3C) with seven podomeres. First podomere with one dorsal and two ventrodiscal plumed setae and Wouters organ. Second podomere with dorsodistal seta reaching distal edge of third podomere and Rome organ. Third podomere with dorsodistal seta reaching distal edge of fourth podomere and ventrodiscal seta extending beyond middle of fourth podomere. Fourth podomere with two dorsodistal setae of unequal length (longer one extending to distal edge of seventh podomere) and two ventrodiscal setae reaching distal edge of sixth podomere. Fifth podomere with two dorsodistal setae of unequal length (longer one as long as podomeres 2–7) and two shorter ventrodiscal setae. Sixth podomere with four outer distal setae as long as podomeres 1–7 and shorter inner distal seta. Seventh podomere with three distal setae of unequal length and aesthetasc y_a (ca. 60% length of longest seta).

An2 (Fig. 3D) with five podomeres. First podomere (coxa) with two ventral setae. Second podomere (basis) with ventrosudistal seta reaching distal edge of third podomere. Exopodite with one long and two unequal short setae. Third (first endopodal) podomere with six inner sudistal short natatory setae extending slightly beyond distal edge of third podomere, ventrodiscal plumed seta reaching distal edge of fifth podomere, and mid-ventral

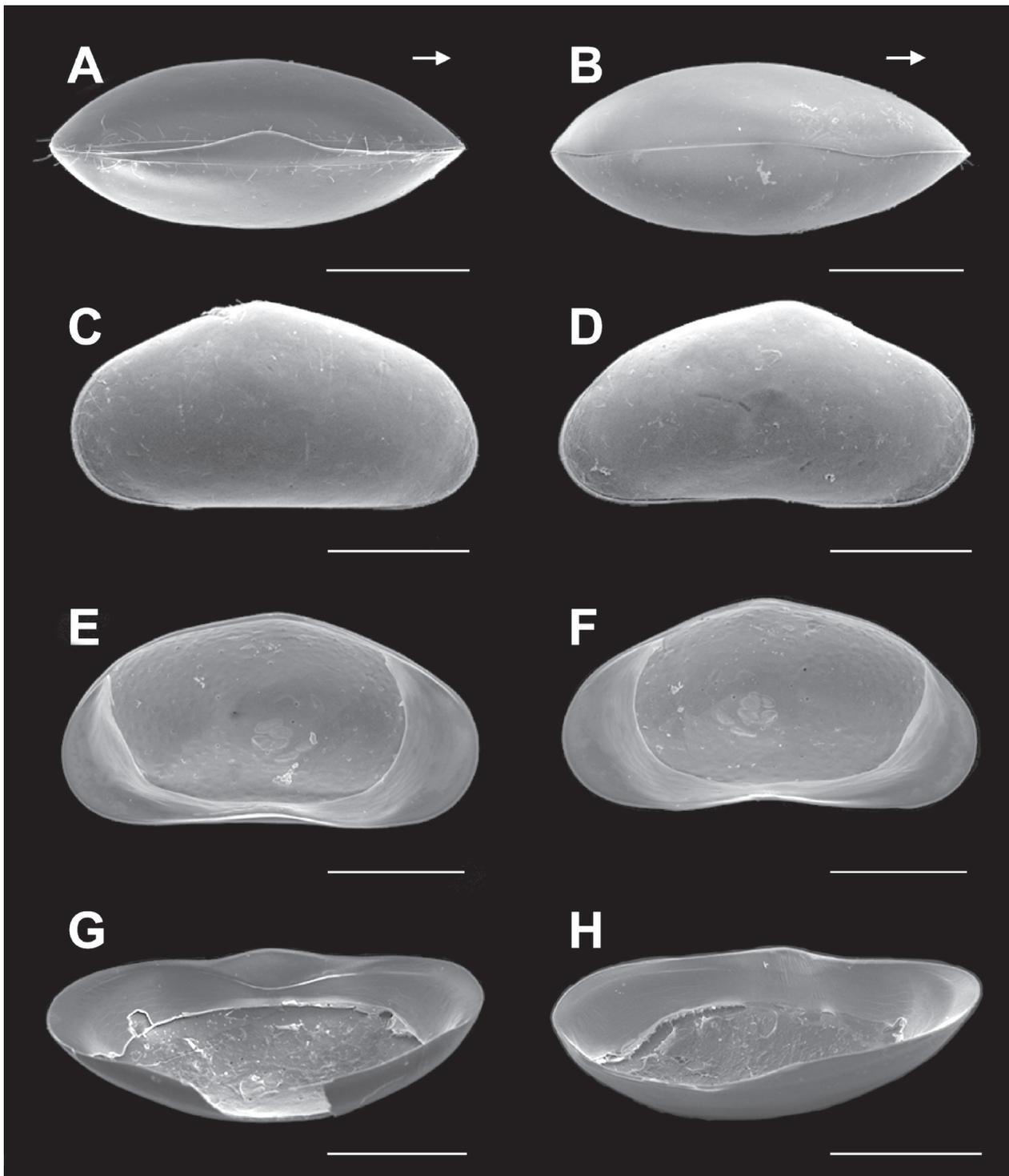


Figure 2. SEM images of carapaces and valves of female *Cavernocypris hokkaiensis* sp. nov. **A.** Paratype (ICHUM-8250); **B.** Paratype (ICHUM-8251); **C, D.** Paratype (ICHUM-8248); **E, F.** Holotype (ICHUM-8247); **G, H.** Paratype (ICHUM-8249); **A, B.** Ventral and dorsal views of whole carapace; **C, D.** Outer views of left and right valves; **E, F.** Inner views of left and right valves; **G, H.** Inner dorsal views of left and right valves; dorsal portion of left valve broken. Arrows indicate anterior direction. Scale bars: 0.2 mm.

aesthetasc Y. Fourth podomere undivided, with two mid-dorsal setae, dorsosubdistal setae z_{1-3} extending beyond middle of claws $G_{2,3}$, mid-ventral plumed setae t_{1-4} of unequal length, and distal claws G_{1-3} of nearly equal length. Fifth podomere with bifurcate aesthetasc y_3 (longer part half the length of claw G_M) and claws $G_{m,M}$; G_m ca. 70% length of G_M ; G_M reaching tip of claws G_{1-3} .

Md (Fig. 3E, F) with coxa, palp comprising four (one basal and three endopodal) podomeres, and vibratory plate. Coxa with several distal teeth and one subdistal plumed seta. First podomere (basis) with one ventrodorsal seta, ventrodorsal setae $S_{1,2}$, and ventrodorsal short seta α ; setae $S_{1,2}$ subequal in length, bearing row of long setules. Vibratory plate (exopodite) with four rays. Second (first

endopodal) podomere with three dorsodistal setae of unequal length (longest reaching tip of claws on fourth podomere), four mid-ventral long plumed setae (not extending beyond tip of claws on fourth podomere), and mid-ventral plumed short seta β (shorter than half the length of mid-ventral plumed setae). Third podomere with four dorsosubdistal setae and two ventrosubdistal setae; inner distal region with plumed seta γ and two plumed setae. Fourth podomere with two distal setae and four distal claws.

Mx (Fig. 3G) with palp comprising two podomeres, three endites, and vibratory plate (not illustrated). First palpal podomere with five dorsodistal setae of unequal length and one ventro-subdistal seta. Second palpal podomere not spatula-like, but rectangular, with two distal setae and three distal claws. First endite with two ventroproximal setae and ca. nine distal setae. Second endite with ca. eight distal setae. Third endite with two distal serrated spines and six distal setae.

L5 (Fig. 3H) with protopod and palp; vibratory plate absent. Protopod with two setae a and at least nine distal plumed setae of unequal length; setae b, c, and d absent. Palp with distal plumed setae h_{1-3} .

L6 (Fig. 3I) with six podomeres. First and second podomeres (protopod) with seta d_2 . Third (first endopodal) podomere with ventrodistal plumed seta e reaching middle of fifth podomere. Fourth podomere with ventrodistal plumed seta f reaching beyond distal edge of fifth podomere. Fifth podomere with ventrodistal plumed seta g. Sixth podomere with dorsodistal seta h_3 , ventrodistal plumed seta h_1 , and distal curved claw h_2 .

L7 (Fig. 3J) with four podomeres; third and fourth podomeres fused to form pincer organ. First podomere (protopod) with plumed setae $d_{1,2,p}$. Second (first endopodal) podomere with ventrodistal plumed seta e not reaching middle of fused podomeres 3 and 4. Fused third and fourth podomeres with mid-ventral plumed seta f not reaching tip of L7, subdistal long plumed seta h_3 , distal hook-like seta h_2 , and subdistal tiny seta h_1 .

UR (Fig. 3K) strongly reduced. Proximal part longer than wide, with one seta. Distal part flagellar in shape.

Rake organ (Fig. 3L) with stout rod and ca. eight blunt distal teeth.

Genital hooks on female copulatory organ present (not illustrated).

Genetic information and phylogenetic analysis

The partial COI sequence (658 bp, encoding 219 amino acids; LC666823), the nearly complete 18S sequence (2053 bp; LC666824), and a *Cardinium* 16S sequence (907 bp; LC666825) were determined from paratype individual ICHUM-8252.

The sequences in the INSD most similar to our sequences, determined by BLAST searches, were from the ostracod *Bennelongia scanloni* Martens et al., 2013 (KF724989.1; identity score 81.28%, query cover 99%; Martens et al. 2013) for COI, from the ostracod

Cyprretta seurati Gauthier, 1929 (AB674999.1; identity score 87.60%, query cover 89%; Hiruta et al. 2016) for 18S, and from “*Cardinium* endosymbiont of *Heterocypris spadix*” (LC589665.1; identity score 98.90%, query cover 100%; Munakata et al. 2021) for *Cardinium* 16S. In our ML tree based on 18S sequences (Suppl. material 4: File S1), *C. hokkaiensis* appears as the sister taxon to a strongly supported clade (97% ultrafast bootstrap support) comprising all other cypridoideans. Cyprididae and Cypridopsinae, in which *Cavernocypris* is classified, do not appear as monophyletic.

Discussion

Morphological comparison

Cavernocypris hokkaiensis sp. nov. resembles *C. cavernosa* and *C. danielopoli* Smith & Kamiya, 2017 in lacking setae b and d on L5, but differs from them in that (1) the outer surface of the carapace is smooth, with sparse, tiny setae, but without shallow pits (pits present in *C. cavernosa* and *C. danielopoli*); (2) the carapace is elongate rather than triangular in lateral view (triangular in *C. danielopoli*); (3) first palpal podomere of Mx has five dorsodistal and one ventro-subdistal setae (only five dorsodistal setae present in *C. danielopoli*; not described in *C. cavernosa*); and (4) L5 lacks the vibratory plate (vibratory plate present in *C. cavernosa* and *C. danielopoli*). Character states in all congeners are summarized in Table 2.

Reproductive mode

Our sample comprised only females, indicating that *C. hokkaiensis* may be parthenogenetic. The endosymbiotic bacterium *Cardinium* has previously been detected (e.g., by means of 16S sequences) in non-marine ostracods engaged in parthenogenetic or mixed reproduction, and infection with *Cardinium* might be a causative factor in the parthenogenetic reproductive mode (Schön and Martens 2019). Our study is the first to detect *Cardinium* in a species of *Cavernocypris*, implying that *C. hokkaiensis* may be parthenogenetic. It should be noted that male individuals have likewise not been reported among the congeners *C. cavernosa*, *C. danielopoli*, *C. wardi*, and some populations of *C. subterranea* (Wolf, 1920) (Marmonier et al. 1989; Külköylüoğlu and Vinyard 1998; Smith 2011; Smith et al. 2017).

Ecology, distribution, and life cycle

The results of our field survey suggest that *C. hokkaiensis* is distributed in an extremely narrow area, only in Hokkai-sawa Stream. It was not found at three sites distant from Hokkai-sawa Stream (Stns 5, 6, and 7). Its absence at two sites in Akaishi Stream (Stns 3 and 4), which Hokkai-sawa

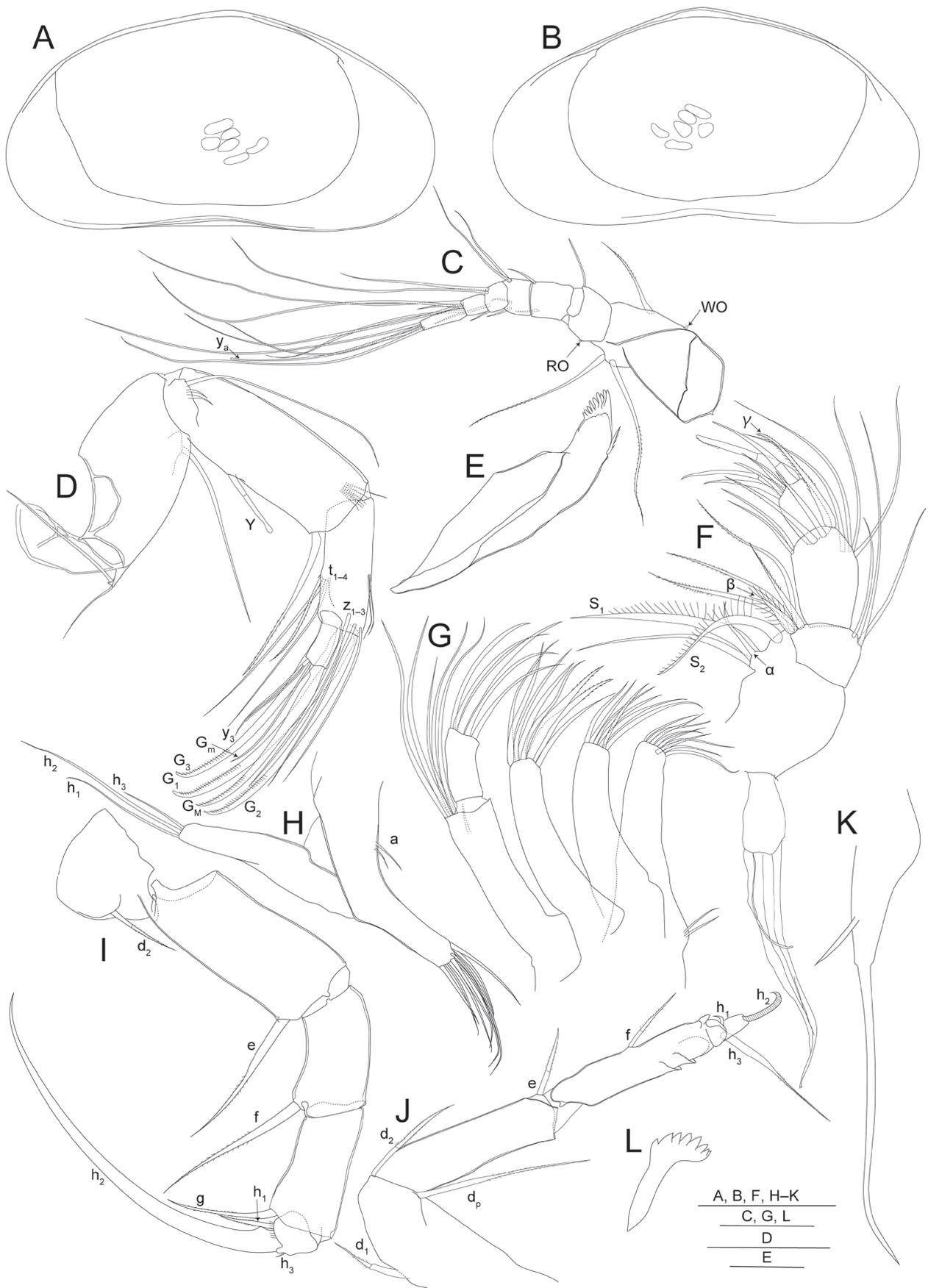


Figure 3. *Cavernocypris hokkaiensis* sp. nov., female. **A–G, I–L.** Holotype (ICHUM-8247); **H.** Paratype (ICHUM-8249); **A, B.** Inner views of left and right valves; **C.** Antennula; **D.** Antenna, outer view; **E.** Coxa of mandible; **F.** Mandible, inner view; **G.** Maxillula (vibratory plate omitted); **H–J.** Limbs 5–7; setules of distal setae on protopod and palp of limb 5 omitted; **K.** Uropodal ramus; **L.** Rake organ. Abbreviations: RO, Rome organ; WO, Wouters organ. Scale bars: 0.2 mm (**A, B**); 0.5 mm (**C–K**); 0.25 mm (**L**).

Table 2. Comparison of morphological characteristics between species of *Cavernocypris*.

	<i>C. cavernosa</i>	<i>C. coreana</i>	<i>C. danielopoli</i>	<i>C. reddelli</i>	<i>C. subterranea</i>	<i>C. wardi</i>	<i>C. hokkaiensis</i> <i>sp. nov.</i>
Shape of valves	elongate	elongate	triangular	elongate	elongate	elongate	elongate
Surface of valves	with numerous shallow pits, most distinct towards anterior and posterior margins, less distinct in central area	with very finely pitted	with elongate shallow pits in central area, and with much smaller rounded pits in posterior region	with small shallow pits	with small shallow pits in a central transverse band; pits may be reduced or even absent	smooth	smooth
Number of An1 podomeres	7	6	7	7	7	7	7
Number of setae on 1 st podomere of Mx-palp	not described	5 distal and 1 subdistal	5 distal	5 distal	5 distal and 1 subdistal	4 distal and 1 subdistal	5 distal and 1 subdistal
Seta b on L5	absent	present	absent	absent	present	present	absent
Seta d on L5	absent	absent	absent	present	present	present	absent
Number of rays comprising vibratory plate on L5	2	4	1	4	2	3	0
Reference	Smith (2011)	McKenzie (1972); Marmonier et al. (1989)	Smith et al. (2017)	Külköylüoğlu (2020)	Wolf (1920); Marmonier et al. (1989)	Marmonier et al. (1989)	this study

Stream joins, may be related to environmental differences between two streams. Hokkai-sawa Stream is fed by spring water and snowmelt, and is thus cold (1.4–6.8 °C measured in the summer season; Table 1; Konno et al. 2003) and slightly acidic (pH 6.2; Konno et al. 2003). In contrast, Akaishi Stream is fed by water from the Yudokonsen hot spring in the Ohachidaira Caldera, which has high water temperature (48 °C) and high H₂S content (245 mg/l) (Uzumasa et al. 1959), and measurements taken at Stn. 3 indicate that Akaishi Stream is warmer (13 °C) and more acidic (pH 2.8–3.3) than Hokkai-sawa Stream (Table 1; Konno et al. 2003). No pH data were available for our Stn. 4, but the water temperature was similar to that at Stn. 3 (13 °C; Table 1). Based on samples collected from sites almost identical to our Stns 1 and 3 in Hokkai-sawa and Akaishi Streams, Konno et al. (2003) found no lotic aquatic insects in common between the two streams. The warmer, more acidic condition of Akaishi Stream may be an unsuitable habitat for *C. hokkaiensis*.

Our rearing experiment, though we could prepare only three live individuals, provided preliminary data about the life history of *C. hokkaiensis*. We observed no molting by captive *C. hokkaiensis* individuals for more than five months at 7 °C. Ostracod life cycles typically comprise eight non-adult and

one adult instars, i.e., ostracods molt eight times before becoming sexually mature adults. Instars are not uniform in duration, but tend to become longer with successive instars (e.g., Heip 1976; Liberto et al. 2014). Although we could not determine the true instar for three captive individuals, their estimated instar was A-1 (0.47- and 0.48-mm individuals) or A-2 (0.39-mm individual) if this species follows Brooks's rule (Brooks 1886; Watabe and Kaesler 2004). Our result could be an artefact from the artificial conditions during culturing, but it may also be a natural phenomenon, and we could speculate that this species may require more than a year to become sexually mature.

Our field survey and observation of captive individuals may indicate that *C. hokkaiensis* is an endemic species adapted to the harsh alpine environment of the Taisetsu Mountains, with a low population density, narrow distributional range, and potentially slow maturation. If this is the case, then habitat loss and fragmentation due to anthropogenic activities, or a decrease in snowfall and snowfields due to climate change, could lead to a rapid population decline of this species. Additional ecological and biological information is necessary to confirm whether *C. hokkaiensis* is a narrow endemic, and to design an informed conservation strategy.

Key to the *Cavernocypris* species (modified after Külköylüoğlu (2020))

- 1 An1 with 7 podomeres 2
- An1 with 6 podomeres *C. coreana* (McKenzie, 1972)
- 2 6th swimming seta of An2 longer than the other 5 setae, second podomere of Md-palp with β+4 setae 3
- 6th swimming seta of An2 shorter than others, second podomere of Md-palp with β+3 setae
..... *C. reddelli* Külköylüoğlu, 2020
- 3 Seta b present on L5 4
- Seta b absent from L5 5
- 4 First palpal podomere of Mx with 5 distal and 1 subdistal setae, vibratory plate of L5 with 2 rays
..... *C. subterranea* (Wolf, 1920)
- First palpal podomere of Mx with 4 distal and 1 subdistal setae, vibratory plate of L5 with 3 rays
..... *C. wardi* Marmonier, Meisch & Danielopol, 1989

- 5 Carapace triangular in lateral view, with distinctive hump on LV *C. danielopoli* Smith & Kamiya, 2017
 – Carapace elongate in lateral view, without hump on LV 6
 6 Surface of valves covered with numerous shallow pits, vibratory plate of L5 with 2 rays *C. cavernosa* Smith, 2011
 – Surface of valves smooth, L5 without vibratory plate *C. hokkaiensis* sp. nov.

Acknowledgements

We thank Akane Saito and Sota Matsuno in the Ministry of the Environment for support in obtaining a sampling permit; Yuki Kita at Hokkaido University (HU) for supporting the field work; Akira Tsukagoshi at Shizuoka University for literature; Yuki Oya at HU for helping with molecular analyses; and Matthew H. Dick at HU for reviewing the manuscript and editing the English. Permit numbers 1910241 and 2104201 allowed field sampling of animals in Daisetsuzan National Park. This study was funded in part by a research grant from the Research Institute of Marine Invertebrates Foundation to MM.

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Supplementary material 1

Table S1

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: table (Excel format)

Explanation note: List of species included in the molecular phylogenetic analysis and respective GenBank accession numbers.

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Link: <https://doi.org/10.3897/zse.98.80442.suppl1>

Supplementary material 2

Alignment S1

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: molecular dataset (fasta format)

Explanation note: Aligned 18S sequences used for the maximum-likelihood analysis, trimmed in MEGA7 to the shortest length among the sequences.

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Link: <https://doi.org/10.3897/zse.98.80442.suppl2>

Supplementary material 3

Alignment S2

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: molecular dataset (fasta format)

Explanation note: Aligned 18S sequences used for the maximum-likelihood analysis, reduced to 1547 positions by removing alignment-ambiguous sites with Gblocks ver. 0.91b in NGPhylogeny.fr under “relaxed” parameters.

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Link: <https://doi.org/10.3897/zse.98.80442.suppl3>

Supplementary material 4

File S1

Authors: Mizuho Munakata, Hayato Tanaka, Keiichi Kakui
Data type: text with one figure (docx format)

Explanation note: Phylogenetic analysis of cypridoidean ostracods based on 18S rRNA sequences.

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Link: <https://doi.org/10.3897/zse.98.80442.suppl4>

Clarifying the type locality of *Liotyphlops wilderi* (Garman, 1883) (Serpentes, Anomalepididae), with comments on other reptiles from São Cyriaco, Minas Gerais

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<http://zoobank.org/8D91B0DE-A626-4452-8A5E-35CDD63F5B2>

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Academic editor: Pedro Taucce ♦ Received 11 January 2022 ♦ Accepted 13 April 2022 ♦ Published 5 May 2022

Abstract

The snake species *Typhlops wilderi* (today *Liotyphlops wilderi*) was described in 1883 based on specimens from São Cyriaco, in Minas Gerais, Brazil. The name of this type locality has been cited in different ways in the literature, making its geographic location confusing. Solving this question is an important issue for future taxonomy and systematic studies. After searching for information on the collector of the type series of *L. wilderi* (John Casper Branner) using the Google Scholar database, I found that São Cyriaco was a gold mining company located in the current municipality of Alvorada de Minas. Besides elucidating the type locality of *L. wilderi*, I searched for reptile specimens collected by Branner, deposited in collections registered at the VertNet Portal and SpeciesLink, and personally examined the extant material from Minas Gerais.

Key Words

Amphisbaenia, lizards, Natural History Museum, snakes, Squamata, taxonomy

Introduction

In zoological nomenclature, the type locality of a species or subspecies is the geographical (and, sometimes, stratigraphical) place of capture, collection, or observation of the name-bearing type (holotype, lectotype, neotype, or syntypes), “the objective standard of reference whereby the application of the name of a nominal taxon can be determined” (International Commission on Zoological Nomenclature 1999). It is not uncommon, however, that taxa have an uncertain or somewhat vague (e.g., “Brazil”) type locality, especially those described more than a century ago (e.g., Frost 2021; Uetz et al. 2021). A well-defined type locality is of great importance to taxonomy and systematics. If populations attributed to a single species are found to diverge sufficiently (morphologically and/or genetically, for example) to be considered two or more

subspecies or species, the population most closely related to the name-bearing type will retain the taxon name (e.g., Sturaro et al. 2018; Ascenso et al. 2019; Perez and Borges-Martins 2019). In the current “genomic era”, many historical herpetological type specimens, usually formalin-fixed, lack genetic samples, and extracting and sequencing DNA from them is still complex (Bell et al. 2020). In such cases, samples from topotypes, i.e., new specimens from (or close to) the type locality, can be a valuable contribution to systematics (Fouquet et al. 2016; Bell et al. 2020; Mângia et al. 2020). But if the type locality is uncertain or too vague, it becomes difficult to assign proper names to clades (Cacciali et al. 2018).

The snake species *Liotyphlops wilderi* (Garman, 1883) (originally *Typhlops wilderi*) is known from a few specimens from Bahia, Minas Gerais and Rio de Janeiro states, Brazil (Nogueira et al. 2019) and there is confusing

information regarding its type locality. Specimens used in the original description were said to have been collected at “São Cyriaco, Brazil” (Garman 1883). Later, the locality name was cited as “Cyriaco, near Serra Providencia, Minas Geraes, Brazil” (Hammar 1908), “São Cyprião, Minas Geraes, Brazil” (Barbour and Loveridge 1929; Marx 1958; Gans 1966; Dixon and Kofron 1983), “Cypriano, Minas Gerais State, SE Brazil, 19°45'S, 43°57'W, elevation 850 m” (a location within the city of Vespasiano) (Wallach et al. 2014), and “São Cyriaco, near the village of Santo Antônio do Rio do Peixe. Currently, Alvorada de Minas” (Nogueira et al. 2019).

Besides being the type locality of *Typhlops wilderi*, São Cyriaco is the locality of the collection of a wormlizard specimen assigned to *Amphisbaena prunicolor* (Cope 1885) (Gans 1966) a species mostly distributed in southern Brazil, Paraguay, and Argentina (Perez et al. 2012). This raises questions regarding the identity of the specimen (whether it was misidentified) and the geographic location of São Cyriaco (if it could somehow be in southern, instead of southeastern Brazil).

The wormlizard specimen and the type series of *Liotyphlops wilderi* were collected by the geologist John Casper Branner (1850–1922). Born in New Market, U.S.A., from a distinguished family, Branner entered Cornell University in 1870, where he met the geologist Charles Hartt, who invited him for a trip to Brazil in 1874 (Penrose 1925). This expedition set the stage for the Comissão Geológica do Império do Brasil (Geological Commission of the Brazilian Empire), with Hartt as its director and Branner as his assistant from 1875 to 1877 (Penrose 1925). Following the termination of the commission, in 1879 and 1880 Branner became assistant of the mining engineer James E. Mills, who was superintendent of a U.S. mining company in the state of Minas Gerais (Branner 1902). He returned to his home country in 1880, but a few months later came back to Brazil, employed by the inventor Thomas A. Edison to search for a vegetable fiber to strengthen incandescent lights (Penrose 1925). In 1882, working for the U.S. Department of Agriculture, Branner traveled to study the insects affecting cotton and sugar cane cultures in Brazil (Penrose 1925; Oliveira 2014).

In 1899, as a professor at Stanford University, Branner returned to Brazil with collaborators for the ‘Branner-Agassiz Expedition’, funded by Alexander Agassiz (son of Louis Agassiz), to study the geology of ocean reefs, atolls, and volcanic islands, besides zoology (particularly ichthyology) traveling along the coast of Rio Grande do Norte and Bahia states (northeastern Brazil) and visiting islands such as the Fernando de Noronha archipelago (Penrose 1925; Oliveira 2014). Another visit occurred in 1907 to study the black diamonds districts of Bahia and the geology of Alagoas and Sergipe states, also in northeastern Brazil (Penrose 1925). In 1911, Branner led the ‘Stanford Expedition to Brazil’ (or ‘Hopkins-Branner Expedition’ (Schmidt and Inger 1951)), intended to explore the Brazilian coast from the northeast to the mouth of the Amazon river, in the north (Oliveira 2014).

The original objectives were not accomplished, but the team made geological and zoological collections in the states of Rio Grande do Norte, Ceará, Pará, Amazonas, and Rondônia, as well as Bolivia (Oliveira 2014, 2018).

Despite being a geologist, Branner was trained as a naturalist, making observations, and collecting material other than of geological interest, even before his interdisciplinary expeditions as a professor at Stanford. This led to the publication, for example, of an account of the identification of the supposedly deadly peanut-headed lantern fly (*Fulgora lanternaria*) (Branner 1885) and of notes on the fauna of Fernando de Noronha (Branner 1888). He also collected specimens that would later become the types of new species, like the treefrog *Dendropsophus branneri* (Cochran, 1948) and the aforementioned snake *Liotyphlops wilderi*. To find the correct name and current location of São Cyriaco, type locality of *L. wilderi*, I decided to investigate in more detail the life and work of J. C. Branner. Additionally, I aimed to examine the specimen identified as *Amphisbaena prunicolor* collected by Branner at São Cyriaco, as well as other reptile specimens from the same locality housed in natural history collections.

Methods

With the intention of finding published records that could shed light on the geographic location and the current name of the type locality of *Liotyphlops wilderi*, in early 2019 I used the Google Scholar database to search for the following keywords: “São Cyriaco” AND Branner; “São Cyprião” AND Branner; “Cyprião” AND Branner; “Cypriano” AND Branner. I also searched at the VertNet Portal (<http://portal.vertnet.org>) and SpeciesLink (<https://specieslink.net>) for reptile specimens collected by J. C. Branner, deposited in collections registered in those databases, and in April 2019 I personally examined the extant specimens, all deposited in U.S. museums. To ensure a proper identification of these specimens, I compared their external morphology (mainly scale counts, but also the color pattern and measurements when necessary) with the original descriptions or updated taxonomic studies (Gans 1966; Roze 1967; Peters and Donoso-Barros 1970; Peters and Orejas-Miranda 1970; Dixon and Kofron 1983; Dixon 1989; Etheridge and Williams 1991; Silva and Sites 1999; Vanzolini 2002; Campbell and Lamar 2004; Rodrigues et al. 2006; Centeno et al. 2010; Perez et al. 2012; Costa et al. 2015, 2019; Breitman et al. 2018; Santos and Reis 2018). I took measurements; snout-vent length (SVL) and tail length (TL), with a ruler to the nearest millimeter and photographs in dorsal view of all but one of the specimens (one syntype of *L. wilderi*), for the purpose of illustration.

Results and discussion

The search for the correct name and location of the type locality of *Liotyphlops wilderi* was successful. As stated

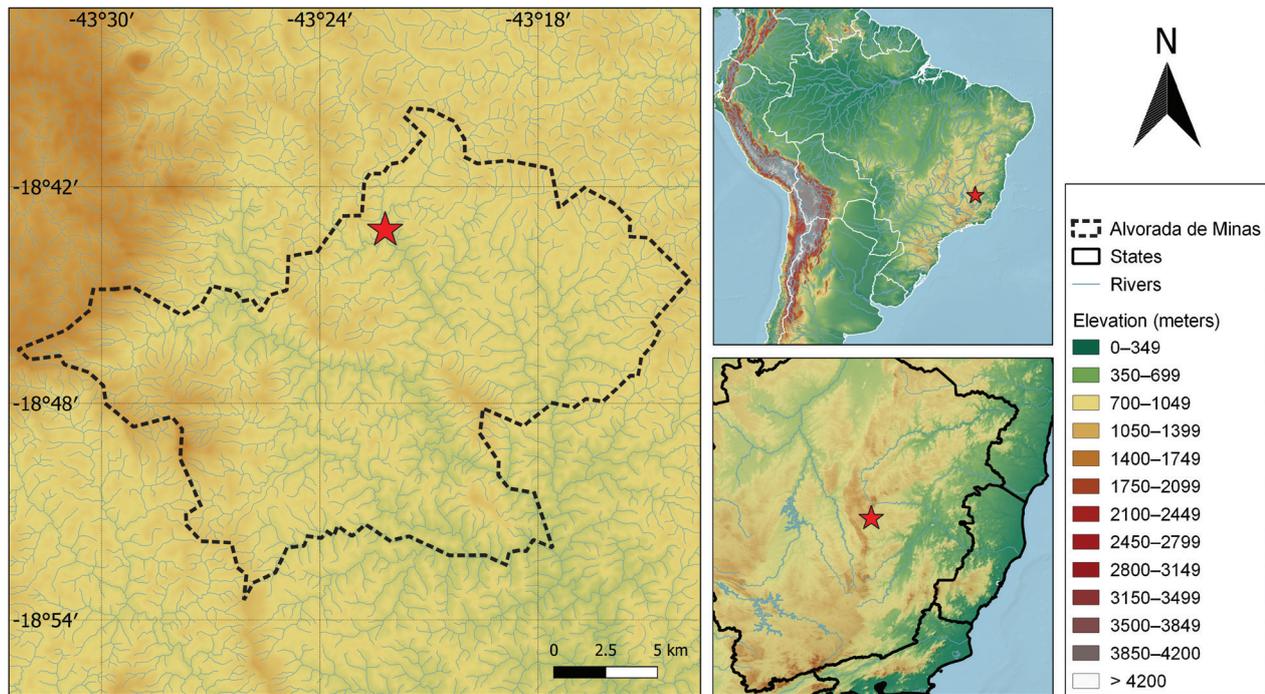


Figure 1. Map of the municipality of Alvorada de Minas, state of Minas Gerais, Brazil, type locality of *Liotyphlops wilderi*, where the São Cyriaco Gold Mining Company was established in the 19th century. The star is placed over the city of Alvorada de Minas.

in the original description (Garman 1883) São Cyriaco is the correct name. The São Cyriaco Gold Mining Company of Boston received authorization from the Brazilian Empire for operation at Minas Gerais in 1878 (Senado Federal 1878). The mining was established close to the village of Santo Antônio do Rio do Peixe, an area said to contain fertile soil, many fields, hills, and forests (Mills 1878). John Casper Branner worked in São Cyriaco in 1879 and 1880 as an interpreter and assistant engineer to superintendent James E. Mills (Branner 1902). The village of Santo Antônio do Rio do Peixe later became a district of the municipality of Serro, and in 1962 became a separate municipality named Alvorada de Minas (Carvalho 2018; Neves and Simões 2021) (18.72°S, 43.37°W) (Fig. 1). This information is briefly cited, without details, by Nogueira et al. (2019). Hammar (1908) cites “Cyriaco, near Serra Providencia”. The mines of São Cyriaco were located in the Espinhaço Mountain range (Gontijo 2008), but I did not find any reference to a ‘serra’ (mountain) named Providência in the region. It may be possible that its name has changed.

Only 18 squamate reptiles collected by John C. Branner in Minas Gerais are registered at VertNet and six are registered at SpeciesLink (all of the latter are at VertNet). Those specimens were deposited at the collections of the Field Museum (FMNH) (one specimen), the Museum of Comparative Zoology (MCZ) (six specimens), and the Cornell University Museum of Vertebrates (CUMV) (11 specimens). The specimens were collected by Branner before he became a professor at Stanford University, when he was working at São Cyriaco Gold Mining Company. He sent the specimens to his alma mater, Cornell University, whence some were later exchanged with the

MCZ. One syntype of *L. wilderi* was sent from MCZ to the FMNH, according to the collections’ catalogues. Unfortunately, one specimen from MCZ and all but three specimens from CUMV are missing (Charles M. Dardia, in litt., 2019). Therefore, to the best of my knowledge, only nine specimens of reptiles collected by J. C. Branner in Minas Gerais remain in museum collections. Below, I provide information on those specimens, all personally examined, and discuss reidentifications when needed.

ANOMALEPIDIDAE

Liotyphlops wilderi (Garman, 1883). Two specimens. MCZ R-5126 (syntype) (Fig. 2A). Minas Gerais, São Cyriaco. Sex unknown; SVL 163 mm; TL 5 mm. FMNH 73387 (syntype). Minas Gerais, São Cyriaco. Sex unknown; SVL 156 mm; TL 4.6 mm.

ELAPIDAE

Micrurus corallinus (Merrem, 1820). Two specimens. CUMV 1925 (Fig. 2B). Minas Gerais, São Cyriaco. Male. SVL 415 mm; TL 66 mm. MCZ R-5568 (Fig. 2C). Minas Gerais, São Cyriaco. Male; SVL 350 mm; TL 61 mm.

Micrurus frontalis (Duméril, Bibron & Duméril, 1854). One specimen. CUMV 1928 (Fig. 2D). Minas Gerais, São Cyriaco. Sex unknown; SVL ~300 mm; TL 21 mm. The specimen is desiccated, making the SVL measurement less precise. It was originally identified as *M. lemniscatus* Linnaeus, 1758. Despite its poor condition, a pattern of black triads is visible at some parts of the



Figure 2. Reptiles collected by John Casper Branner in Minas Gerais between 1878 and 1879. **A.** *Liotyphlops wilderi* (MCZ R-5126, syntype); **B.** *Micrurus corallinus* (CUMV 1925); **C.** *Micrurus corallinus* (MCZ R-5568); **D.** *Micrurus frontalis* (CUMV 1928); **E.** *Erythrolamprus maryellenae* (CUMV 1930); **F.** *Amphisbaena metallurga* (MCZ R-5124); **G.** *Enyalius bilineatus* (MCZ R-5567); **H.** *Urostrophus vauitieri* (MCZ R-5566). Scale bars: 10 mm.

body. Among the *Micrurus* species known for the region, only *M. frontalis* and *M. carvalhoi* Roze, 1967 (formerly *M. lemniscatus carvalhoi*) show a triad pattern (Campbell and Lamar 2004; Nogueira et al. 2019), and can be distinguished from each other mainly by the presence of a light band on the snout of *M. carvalhoi*, absent in *M. frontalis*.

DIPSADIDAE

Erythrolamprus maryellenae (Dixon, 1985). One specimen. CUMV 1930 (Fig. 2E). Minas Gerais, São Cyriaco. Sex unknown; SVL 280 mm; TL 90 mm. The specimen was identified as *Liophis merremii* (= *Erythrolamprus miliaris merremii* (Wied, 1821)). I reidentified it as *E. maryellenae* by its having 19-19-17 dorsal scale rows without apical pits, 152 ventrals, a divided cloacal shield, 66 paired subcaudals, eight supralabials (fourth and fifth contacting the eye), and 10 infralabials (Dixon 1989).

AMPHISBAENIDAE

Amphisbaena metallurga Costa, Resende, Teixeira Jr., Dal Vechio & Clemente, 2015. One specimen. MCZ R-5124 (Fig. 2F). Minas Gerais, São Cyriaco. Sex unknown; SVL 123 mm; TL 14 mm. Garman (1883) cited three specimens (with no collection catalogue information) of *Amphisbaena darwinii* Duméril & Bibron, 1839, one of which may be MCZ R-5124 (the other two may be lost and I did not find any additional record of them). Gans (1966) examined MCZ R-5124 and identified it as *A. prunicolor* (Cope, 1885). Specimens of *Amphisbaena prunicolor* have a dark purplish-brown color that fades to pale brown in preservative. The color pattern is still clearly visible in the holotype (Academy of Natural Sciences, Philadelphia; ANSP 12969), collected in late 19th century (Perez et al. 2012). In contrast, MCZ R-5124 presents a uniform cream color pattern. Furthermore, *A. prunicolor* exhibits a row of postmalar scales (Gans 1966; Perez et al. 2012), absent in MCZ R-5124. Gans (1966) noted this, as can be seen in the document with the character data for each specimen he examined (Document Number 8998 in the Library of Congress, Washington, D.C., U.S.A.). There, Gans noted “2+4[8]” for the “chin segments” of MCZ R-5124. The brackets indicate a row of scales posterior to the mouth commissure, which should not be counted as a postmalar row but rather as the first body annulus, as can be seen in his annotations of *A. albocingulata* Boettger, 1885. The color pattern and the morphology of MCZ R-5124 (205 body annuli, three lateral annuli, 19 caudal annuli, autotomic site at 8th caudal annulus, lateral sulcus present, dorsal and ventral sulci absent, 12 dorsal and 14 ventral segments at a midbody annulus, four pre-cloacal pores sequentially arranged, three supralabials, three infralabials, two scales on first postgenial row, four scales on second postgenial row, and no postmalars) best

fit the diagnosis of *Amphisbaena metallurga*, whose type locality is Conceição do Mato Dentro, a neighboring municipality south of Alvorada de Minas (Costa et al. 2015, 2019). MCZ R-5124 increases the range of body annuli of *A. metallurga* from 185–199 to 185–205 and the range of caudal annuli from 23–25 to 19–25. Therefore, the record of *Amphisbaena prunicolor* for São Cyriaco, Minas Gerais, is here invalidated.

LEIOSAURIDAE

Enyalius bilineatus Duméril & Bibron, 1837. One specimen. MCZ R-5567 (Fig. 2G). Minas Gerais, unknown locality. Sex unknown; SVL 77 mm; TL 168 mm (tip broken).

Urostrophus vautieri Duméril & Bibron, 1837. One specimen. MCZ R-5566 (Fig. 2H). Minas Gerais, unknown locality. Sex unknown; SVL 76 mm; TL 95 mm.

According to the collection catalogues, the nine missing specimens were: 1) CUMV 1926, Minas Gerais, São Cyriaco – recorded as *Erythrolamprus aesculapii monozona* (Jan, 1863) (Serpentes: Dipsadidae); the taxonomy of *E. aesculapii* needs clarification (Curcio et al. 2015), but the name *E. a. venustissimus* (Wied, 1821) has been used for non-Amazonian populations, including for morphs with fused black rings (Costa and Bérnils 2018). 2) CUMV 1924, Minas Gerais, São Cyriaco – recorded as *Elapomorphus quinquelineatus* (Raddi, 1820) (Serpentes: Dipsadidae). 3–4) CUMV 1927 and 1939, Minas Gerais, São Cyriaco – recorded as *Oxyrhopus trigeminus* Duméril, Bibron & Duméril, 1854 (Serpentes: Dipsadidae), possibly correct, but we cannot dismiss *Oxyrhopus guibei* Hoge & Romano, 1978, described almost a century after the specimen’s collection, quite similar to *O. trigeminus* (Zaher and Caramaschi 1992) and also expected to occur in the region (Nogueira et al. 2019). 5) CUMV 1931, Minas Gerais, São Cyriaco – recorded as *Liophis reginae* (Linnaeus, 1758) (= *Erythrolamprus reginae*; Serpentes: Dipsadidae), most probably *Erythrolamprus macrosoma* (Amaral, 1935), formerly a subspecies of *E. reginae* that recently received full species status and is expected to occur in the region, contrary to *E. reginae* sensu stricto (Ascenso et al. 2019). 6–7) CUMV 1933 and 1935, Minas Gerais, São Cyriaco – recorded as *Liophis cobella* (Linnaeus, 1758), (= *Erythrolamprus cobella*; Serpentes: Dipsadidae) today restricted to northern South America (Nogueira et al. 2019); no species of the *E. cobella* group is expected to occur in the region (Fernandes et al. 2002) and possibly the referred specimens are *E. poecilogyrus poecilogyrus* (Wied, 1824), a common species in southeastern Brazil (Nogueira et al. 2019). 8) CUMV 1936, Minas Gerais, São Cyriaco – one syntype of *Typhlops wilderi* Garman, 1883 (Serpentes: Anomalepididae). 9) MCZ R-144556, Minas Gerais, unknown locality – *Enyalius bilineatus* (Iguania: Leiosauridae).

Minas Gerais has one of the richest reptile fauna among Brazilian states (Costa et al. 2022), probably

related to its large area and presence of varied ecoregions (Drummond et al. 2005). During the 19th century, Minas Gerais was visited by naturalists who traveled mainly along the mining districts of the Espinhaço mountain range (Papavero 1971). John C. Branner was a geologist but collected reptile specimens while working for the São Cyriaco Gold Mining Company, in the Espinhaço range. Unfortunately, half of the 18 specimens collected by him in Minas Gerais that had been catalogued in museums are lost. But the remaining material includes valuable specimens such as two syntypes of *Liotyphlops wilderi* and a wormlizard reidentified here as *Amphisbaena metallurga*, a recently named taxon. São Cyriaco (now Alvorada de Minas) is the fifth locality from which *A. metallurga* is known to occur (Costa et al. 2015, 2019; Dal Vechio et al. 2018), and all but one (Morro do Pilar) previous records of this species are in areas impacted by mining activities.

In the so called ‘century of extinctions’, when biodiversity is facing a crisis by human activities (Ceballos et al. 2015), taxonomy is crucial for conservation biology (Dubois 2003; Hortal et al. 2015). In this regard, reviewing and updating information on doubtful type localities is important to overcome some problems that hamper advances in taxonomy and systematics (Bell et al. 2020). *Liotyphlops wilderi* has a relatively broad geographic range (Nogueira et al. 2019), although being known from few specimens (Centeno et al. 2010; Santos and Reis 2018). The two remaining syntypes are discolored, dissected, and lack genetic samples. Knowing the exact type locality will allow the future collection of topotypes. Such new specimens would be useful for a morphological reanalysis of the species and its inclusion in molecular phylogenies. If future research finds that specimens assigned to *L. wilderi* belong to more than one species, specimens from its type locality will be very important.

Acknowledgments

I am grateful to Alan Resetar and Joshua Mata (FMNH), Charles M. Dardia (CUMV), James Hanken and Jose Rosado (MCZ) for allowing access to specimens under their care; to Eva Lynn Gans and the Gans Collections and Charitable Fund, for making available to me the document of Carl Gans deposited in the Library of Congress. To Pedro Taucce (subject editor), Angele Martins, Rodrigo C. Gonzalez, and Thais B. Guedes for valuable comments on a previous version of this article that greatly improved the final text. To Ross D. MacCulloch for his English review. This work was funded by a visiting scholarship from the Field Museum (2019), and a postdoctoral fellowship by Programa Nacional de Pós-Doutorado, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PNPD/CAPES) at Universidade Federal de Viçosa (2018–2019).

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A new genus of Australian orb-weaving spider with extreme sexual size dimorphism (Araneae, Araneidae)

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Academic editor: Danilo Harms ♦ Received 23 February 2022 ♦ Accepted 17 April 2022 ♦ Published 5 May 2022

Abstract

The new Australian orb-weaving spider genus *Mangrovia* in the family Araneidae Clerck, 1757 is described. It is characterised by extreme sexual size-dimorphism (eSSD) with females (total length 8–10 mm) ca. 3 to 5 times larger than males (2.5–3 mm). Whilst *Mangrovia* shares with the informal Australian ‘backobourkiine’ clade a single seta on the male pedipalp patella, the genus is probably more closely related to the ‘zealaraneines’ or associated genera. In addition to eSSD and the single patellar spine, the genus is characterised by a distinct subterminal embolus branch in males. The new genus includes two species: the type species *Mangrovia albida* (L. Koch, 1871) **comb. nov.** (= *Epeira fastidiosa* Keyserling, 1887, **new syn.**) from Queensland and *Mangrovia occidentalis* **sp. nov.** from Western Australia. Both species are apparently coastal and occur in mangroves, but also in riparian woodland. Spiders were found resting in rolled-up leaves adjacent to their orb-web.

Key Words

Backobourkiines, new combination, new species, systematics, taxonomy, zealaraneines

Introduction

Extreme sexual size dimorphism (eSSD), a phenomenon where one sex – generally the female – is at least twice as big as the other, is not a common phenomenon in spiders and mostly expressed in orb-weaving taxa (family Araneidae Clerck, 1757, incl. Nephilinae Simon, 1894 and Phonognathinae Simon, 1894) and comb-footed spiders (family Theridiidae) (Hormiga et al. 2000; Kuntner and Coddington 2020; Yu et al. 2022). There are several hypotheses explaining eSSD, for example an increase of female size due to fecundity advantage (Head 1995; Coddington et al. 1998), male dwarfism due to selection for early maturation (Gunnarson and Johnsson 1990; Vollrath and Parker 1992), or gravity advantages (Moya-Laraño et al. 2002). However, a recent review of eSSD in spiders suggests that the phenomenon is most

likely the expression of a complex of evolutionary forces, originating both in natural and sexual selection (Kuntner and Coddington 2020).

The incidence of eSSD is low within the traditional Araneinae Clerck, 1757 (sensu Scharff and Coddington 1997); at the genus level, only *Kaira* O. Pickard-Cambridge, 1889 has significantly smaller males than females (Levi 1993b; Hormiga et al. 2000), although eSSD is known to occur in other traditional ‘araneine’ genera at the species level (e.g. Piel 1996; Framenau et al. 2010). A recent multi-gene molecular phylogenetic analysis of the Araneidae did not confirm a monophyletic ‘Araneinae’; however, this study also found multiple origins (and reversals) of eSSD at the genus level in the Araneidae (Scharff et al. 2020) (see also Yu et al. (2022) for a re-analysis of the dataset with focus on *Polytus* Simon, 1881 and *Cephalonotus* Simon, 1895).

Scharff et al.'s (2020) comprehensive analysis of world-wide Araneidae has transformed our understanding of araneid relationships, in particular with respect to the traditional subfamily Araneinae. The Australian species formed several well supported clades at the subfamily level, although Scharff et al. (2020) did not establish formal subfamilies for them due to their poor taxonomic knowledge and morphological circumscription. Of these groups, the 'backobourkiines' are now best known due to some recent taxonomic studies (e.g. Framenau et al. 2010, 2021a, b, 2022; Framenau 2011; Joseph and Framenau 2012). A uniting character appears to be the presence of a single macroseta on the male pedipalp patella and the median apophysis of the male pedipalp forming an arch over the radix (Scharff et al. 2020). The backobourkiines include Australian genera, such as *Acroaspis* Karsch, 1878, *Backobourkia* Framenau, Dupérré, Blackledge & Vink, 2010, *Carepalxis* L. Koch, 1872 (also known from the Nearctic), *Hortophora* Framenau & Castanheira, 2021, *Novakiella* Court & Forster, 1993, *Plebs* Joseph & Framenau, 2012 (also known in the Oriental region) and *Socca* Framenau, Castanheira & Vink, 2022. There are two currently known cases of eSSD in the backobourkiines (Scharff et al. 2020). In *Backobourkia* eSSD is expressed at the species level, as only one of three species in the genus, *B. collina* (Keyserling, 1886), is highly dimorphic (Framenau et al. 2010). In contrast, in a species-group represented by *Parawixia dehaani* (Doleschall, 1859) in Scharff et al.'s (2020) analysis – referred to as 'Araneus dehaani-group' in Yin et al. (1997) – eSSD appears to be expressed at the genus-level as a second species in this group, *Araneus albomaculatus* Yin, Wang, Xie & Peng, 1990, is also extremely size-dimorphic (Yin et al. 1997). The male of the third species in the group, *A. shunguangensis* Yin, Wang, Xie & Peng, 1990, is currently unknown. The *dehaani*-group apparently represents a new genus in the backobourkiines with *P. dehaani* clearly not belonging to *Parawixia* based on molecular data (Scharff et al. 2020).

A second well-supported group including Australian orb-weavers are the 'zealaraneines', which largely include New Zealand genera such as *Colaranea* Court & Forster, 1988, *Cryptaranea* Court & Forster, 1988 and *Zealaranea* Court & Forster, 1988, but also the Australian species *Araneus albotriangulus* (Keyserling, 1887) and *A. talipedatus* (Keyserling, 1887), both misplaced at the genus level and representing new genera (Scharff et al. 2020). Most closely related to the zealaraneines appear to be true *Araneus* Clerck, 1757, but also species in the large genus *Neoscona* Simon, 1895 (Scharff et al. 2020). Zealaraneines have no known synapomorphies, but in contrast to backobourkiines, males have two pedipalp patellar spines and the median apophysis does not form an arch over the radix (see Court and Forster 1988; figs 354–358, 360–362). There is no known case of eSSD in the zealaraneines where females are generally only slightly larger than males (Court and Forster 1988).

Our current revision of Australian orb-weaving spiders recovered a novel case of eSSD. Females of *Araneus albidus* (L. Koch, 1871), a species originally described based on a female only, are approximately three to five times larger than males. As in the *dehaani*-group, eSSD appears to act at the genus level, as a second undescribed species very similar to *A. albidus* is also extremely size-dimorphic. The male pedipalps of *A. albidus* (and the undescribed species) have only one patellar spine suggesting close affinities with the backobourkiines. However, other genitalic characters, for example the shape of the median apophysis of the male pedipalp, do not match any of the genera currently recognised in this group and these species may therefore be a zealaraneine, or not belong to any of these two well-supported indigenous Australo-Oriental groups.

The aim of this study is to describe these two species in a new genus as a working hypothesis for future phylogenetic analyses of Australian Araneidae, specifically the species of the backobourkiines and zealaraneines *sensu* Scharff et al. (2020).

Materials and methods

Descriptions and terminology follow recent publications on Australian orb-weaving spiders (e.g. Framenau et al. 2010, 2021a, b, 2022). Fresh and well-preserved specimens were used instead of historic and often poorly preserved type specimens for redescrptions. Colour patterns were described based on specimens preserved in ca. 75% ethanol. The descriptions of the male pedipalp relate to their position as a limb, i.e. a full view of the bulb with the cymbium in the background of an image is a retrolateral view as in Araneidae the cymbium is situated mesally. Our standard views of the pedipalp therefore generally show the ventral view – to illustrate the generally diagnostic median apophysis – or the dorsal view – with the tegulum in full view – as the cymbium is situated to the side in the images.

Male pedipalps were expanded by alternately submerging them for ca. 20 min in warm 10%KOH and distilled water until maximum expansion was reached. Female genitalia were dissected and then cleared in warm 10%KOH for 20 mins and transferred into lactic acid on a microscopic slide under a coverglass to further clear internal features for imaging. Measurements are given in millimetres taken at an accuracy of one tenth of a millimetre, except for eye and labium sizes measured at a hundredth of a millimetre.

Images of preserved specimens were taken in different focal planes with a Nikon D300 digital SLR camera attached to a Leica M16A stereomicroscope and combined with Auto Montage (vers. 5.02) software from Syncroscopy to increase depth of field. We used 2 Nikon RIC1 wireless speedlights instead of fibre optics to illuminate the exposures. The latter were used as guide-light for focusing. Microscopic images of cleared epigynes and

expanded pedipalps were taken in different focal planes (ca. 20–30 images) on a Leica DMC4500 digital camera mounted to a Leica M205C stereomicroscope and combined using the Leica Application Suite X, v. 3.6.0.20104. All photos were edited and mounted in the software Photoshop CC 2020.

Maps were compiled in the software package QGIS 3.22.3 ‘Białowieża’ (<https://qgis.org/en/site/>; accessed 20 February 2022). Geographic coordinates were extracted directly from original labels or the registration data as provided by the museums. When no detailed geographic information was available, localities were estimated based on Google Earth v. 7.3.4.8248 (64-bit) (<https://earth.google.com/web/>; accessed 21 February 2022).

Abbreviations

Collections

AM	Australian Museum, Sydney, Australia
NHMUK	Natural History Museum, London, United Kingdom
QM	Queensland Museum, Brisbane, Australia
WAM	Western Australian Museum, Perth, Australia
ZMH	Zoologisches Museum Hamburg, Hamburg, Germany

Morphology

AME, ALE	anterior median (lateral) eyes
PME, PLE	posterior median (lateral) eyes

Results

Taxonomy

Class Arachnida Cuvier, 1812

Order Araneae Clerck, 1757

Family Araneidae Clerck, 1757

Mangrovia gen. nov.

<http://zoobank.org/AFC772CC-2A82-4533-BF66-0317BAACE2AD>

Type-species. *Epeira albida* L. Koch, 1871, designated here.

Etymology. The genus-group name is derived from the general habitat preferences of the two species, which are often found in coastal mangroves and woodlands. The gender is feminine.

Diagnosis. Within an Australian context, *Mangrovia* gen. nov. males have only a single patellar spine on the pedipalp, a character considered a putative synapomorphy of the backobourkiines. However, *Mangrovia* gen. nov. differ considerably from members of the backobourkiines by somatic and genital morphology. Both species in the genus display eSSD with females about 3–5 times larger than males, but eSSD is absent in the backobourkiines

(with the exception of *B. collina* and species in the *dehaani*-group (sensu Yin et al. 1997)). The median apophysis in backobourkiines is elongate transverse with the base forming an arch over the radix, but it is short with an apical, spine-like projection in *Mangrovia* gen. nov. The female epigyne in *Mangrovia* gen. nov. has a terminal pocket, absent in all backobourkiines.

Mangrovia gen. nov. differs from the members of the zealaraneines as currently known (see Introduction section) by its eSSD, absent in any known zealaraneines, and the presence of only one patellar spine on the male pedipalp, whereas there are always two in zealaraneines (Court and Forster 1988). The median apophysis of zealaraneines is elongate transverse, with a variable number of spine-like protrusions, but much shorter and only with a single spine-like protrusion in *Mangrovia* gen. nov.

Outside the backobourkiines and zealaraneines, *Mangrovia* gen. nov. appears most similar to species of *Neoscona* (Berman and Levi 1971; Grasshoff 1986; Levi 1993a), in particular with respect to the male genitalia. However, eSSD is absent in any known *Neoscona* species and males of *Neoscona* have distinctly enlarged tibiae on the second leg with numerous and strong spines, often in diagnostic arrangement. *Mangrovia* gen. nov. males do not have enlarged tibiae II. In contrast to *Mangrovia* gen. nov., males in *Neoscona* have two patellar spines on the pedipalp, not one, and the embolus of *Mangrovia* gen. nov. has a subterminal side branch (e.g. Fig. 2B, C), not present in *Neoscona*. An embolus lamella is absent in *Mangrovia* gen. nov. males, but present in *Neoscona* (e.g. Levi 1993a; fig. 6).

Description. Small to medium-sized orb-weaving spiders with eSSD (TL males ca. 2.5–3 mm, females ca. 8–10 mm). Carapace (Figs 1A, 3A, 6A, 8A) rounded pear-shaped, longer than wide; colouration variable from light brown to reddish-brown with dusky edges on males. Eyes: AME largest, row of PE slightly recurved, lateral eyes almost touching, PLE separated from PME by approximately their diameter in males and by more than their diameter in females. Chelicerae paturon and fangs yellowish- to reddish-brown. Females with four promarginal teeth and three retromarginal teeth, males with three promarginal teeth and two retromarginal teeth. Labium wider than long, subtriangular, with front end bulging and white. Endites elongate-rounded, beige to light brown with antero-mesal corner shiny and white. Sternum heart-shaped, slightly longer than wide, with dark edges (Figs 1B, 3B, 6B, 8B). Legs: leg formula I > II > IV > III (males) or I > IV > II > III (females). Abdomen about as long as wide, dorsum with dark folium pattern in males, varying from uniformly grey to beige with anterior black area on females; venter black with a pair of white guanine spots. Genitalia: male pedipalp patella with a single strong spine (e.g. Fig. 1C, D); paracymbium poorly developed (Figs 1D, 2C); radix elongate (Figs 2B, 7A); no obvious stipes, possibly fused with radix (see Discussion); median apophysis oval, bearing a heavily sclerotized and acute apical

process (Figs 1C, 2A, 6C); conductor conspicuous, robust, basally sclerotised but apically fleshy (Figs 1C, 2C, 6A); terminal apophysis well-developed, somewhat spoon-shaped, not sclerotised (Figs 2A, B, 7A, B); subterminal apophysis elongate and thin (Figs 1D, 2A, C, 6D); embolus heavily sclerotized, basally inflated, then straight, thin and elongated, with subterminal short branch (Figs 2B, C, 4C, D, 7A). Epigyne base plate wider than long; scape elongate and reaching posteriorly beyond the base plate and bearing a terminal pocket; atrium rounded and very conspicuous, located almost on the border of genital area (Figs 3C–E, 8C–E); spermathecae ovoid, separated by less than their diameter; fertilisation duct basally convoluted and attaching posteriorly to spermathecae (Fig. 4A, B).

Composition. *Mangrovia albida* (L. Koch, 1871) comb. nov. and *Mangrovia occidentalis* sp. nov.

Distribution. Australia (Queensland and Western Australia) (Figs 5, 9).

***Mangrovia albida* (L. Koch, 1871) comb. nov.**

Figs 1A–D, 2A–D, 3A–E, 4A, 5

Epeira albida L. Koch 1871: 83–84, plate 7, figs 2, 2a.

Araneus albidus (L. Koch).- Rainbow 1911.- 181.

Epeira fastidiosa Keyserling 1887: 183–184, plate 16, figs 1, 1a.

New synonymy.

Araneus fastidiosus (Keyserling).- Rainbow 1916: 101, plate 21, figs 16, 17.

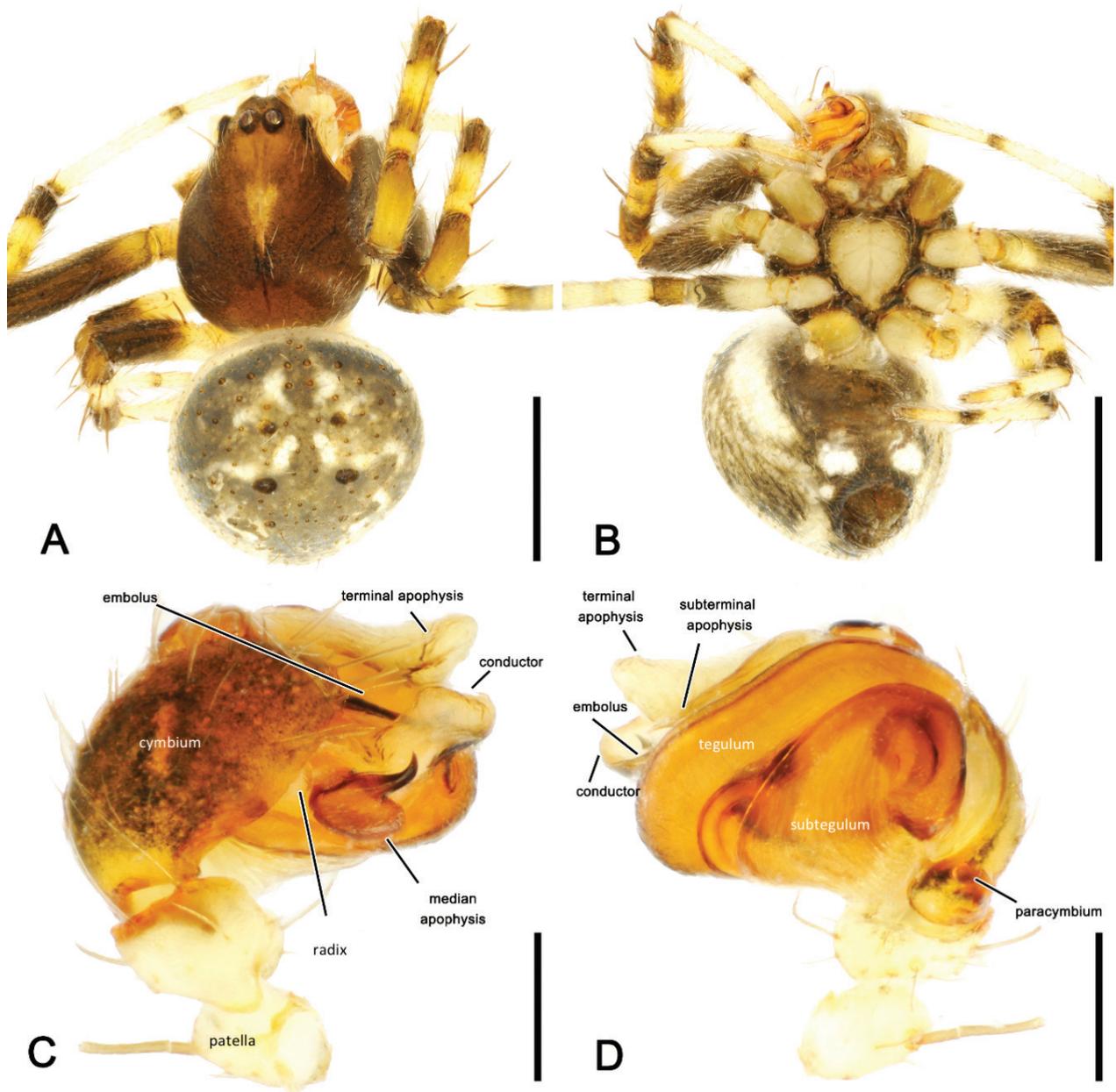


Figure 1. *Mangrovia albida* comb. nov., male (QM S67277). **A.** Habitus, dorsal view; **B.** Habitus, ventral view; **C.** Left pedipalp, ventral view; **D.** Left pedipalp, dorsal view. Scale bars: 1 mm (A, B); 0.1 mm (C, D).

Type material. *Holotype* of *Epeira albida* L. Koch 1871: female, Rockhampton, (23°23'S, 150°30'E, Queensland, Australia) (NHMUK 1915.3.5.65), examined.

Holotype of *Epeira fastidiosa* Keyserling, 1887: male Rockingham, (23°23'S, 150°30'E, Queensland, Australia) (ZMH (Rack 1961)-catalog 237), examined.

Other material examined. AUSTRALIA: Queensland: 1 female, Brisbane, 27°28'S, 153°01'E (AM KS.32873); 1 male, Buhot Creek, Burbank, 27°35'S, 153°10'E (QM S67277); 1 female, Bundaberg Forest, 24°52'S, 152°21'E (QM); 1 female, Baldwins Swamp (QM S25331); 1 female, Cabbage Tree Creek, 25°27'S, 150°01'E (QM); 2 female, 4 juv., Cabbage Tree Point, 25°27'S, 150°01'E

(QM); 1 female, Cabbage Tree Point, Beenleigh, 25°27'S, 150°01'E (QM); 1 female, 1 juv., Camira, 27°38'S, 152°55'E (QM); 1 male, D'Aguiar National Park, Lepidozamia Road, 27°17'S, 152°45'E (AM KS.128413); 7 females, Glasshouse Mountains, 26°53'55.3"S, 152°56'56.0"E (QM); 1 female, Karawatha Forest, 27°37'S, 153°05'E, (QM S65833); 1 male, Koah Road, 16°49'S, 145°31'E (QM S83480); 1 female, Mareeba, 17°S, 145°26'E (AM KS.32640); 1 female, Molangdool, 24°45'S, 151°33'E (AM KS.98754); 1 female, Mt Chalmers, near Rockhampton, 23°20'S, 150°40'E (QM S15531); 1 male, Rockhampton, 23°23'S, 150°30'E (NHMUK 1890.7.1.4150); 1 female, same locality

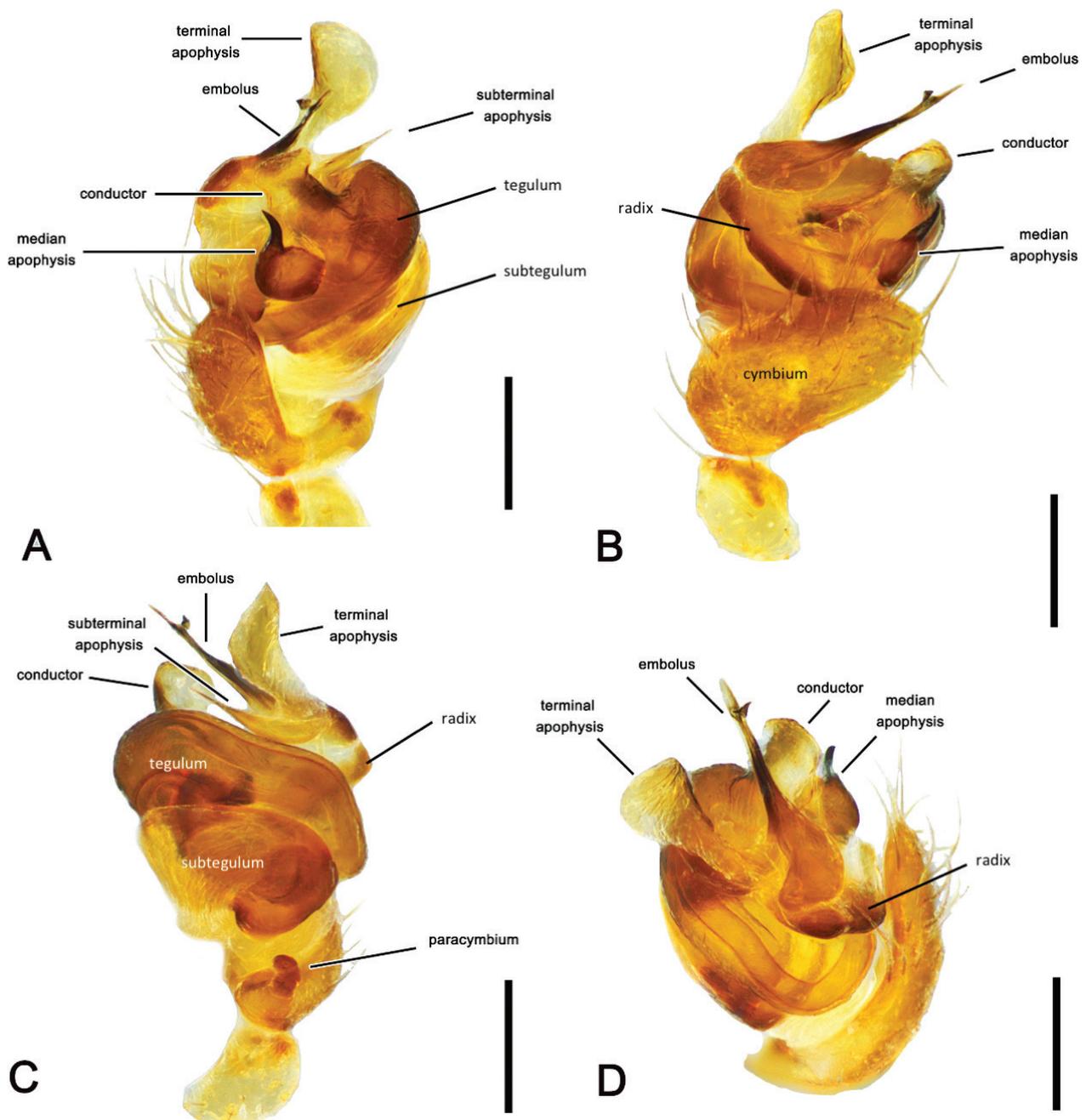


Figure 2. *Mangrovia albida* comb. nov., expanded male left pedipalp (QM S67277). **A.** Apico-ventral view; **B.** Mesal view; **C.** Retrolateral view; **D.** Dorsal view. Scale bars: 0.1 mm (A–D).

(NHMUK 1890.7.1.4171); 1 female, same locality (NHMUK 77.37); 1 immature female, same locality (ZMH Rack (1961)-catalog 218).

Diagnosis. Males *M. albida* comb. nov. can be separated from *M. occidentalis* sp. nov. by subtle differences in key pedipalp sclerites, specifically the conductor is less elongate (Figs 2B, 7B), and the terminal apophysis is less bent apically (Figs 1C, 6C). In addition, the subterminal branch of the embolus appears larger in *M. albida* comb. nov. than in *M. occidentalis* sp. nov. (Figs 2B, 7A). Females of *M. albida* comb. nov. are distinguished from those of *M. occidentalis* sp. nov. by the shorter scape and its wider tip (scape longer and thinner with thinner tip in *M. occidentalis* sp. nov.) (Figs 3C, 8C).

Description. *Male* (based on QM S67277): Total length: 2.8. Carapace (Fig. 1A) 1.4 long, 1.2 wide; dark brown, lighter in cephalic area. Eyes diameter AME 0.14, ALE 0.07, PME 0.10, PLE 0.07; row of eyes: AME 0.38, PME 0.29, PLE 0.65. Chelicerae small with paturon dark grey basally and beige apically; two promarginal teeth, the apical larger, three retromarginal teeth of similar size. Legs (Fig. 1A, B) yellow-brown with distinct dark brown annulations. Leg formula I > II > IV > III; length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – 1.6 + 0.6 + 1.0 + 1.0 + 0.6 = 4.8, II – 1.3 + 0.5 + 0.9 + 0.9 + 0.5 = 4.1, III – 0.8 + 0.3 + 0.3 + 0.4 + 0.4 = 2.3, IV – 1.2 + 0.4 + 0.7 + 0.7 + 0.5 = 3.5. Labium and endites beige. Sternum 0.5 long, 0.4 wide, beige with dark brown contour (Fig. 1B). Abdomen (Fig. 1A, B) 1.6 long,

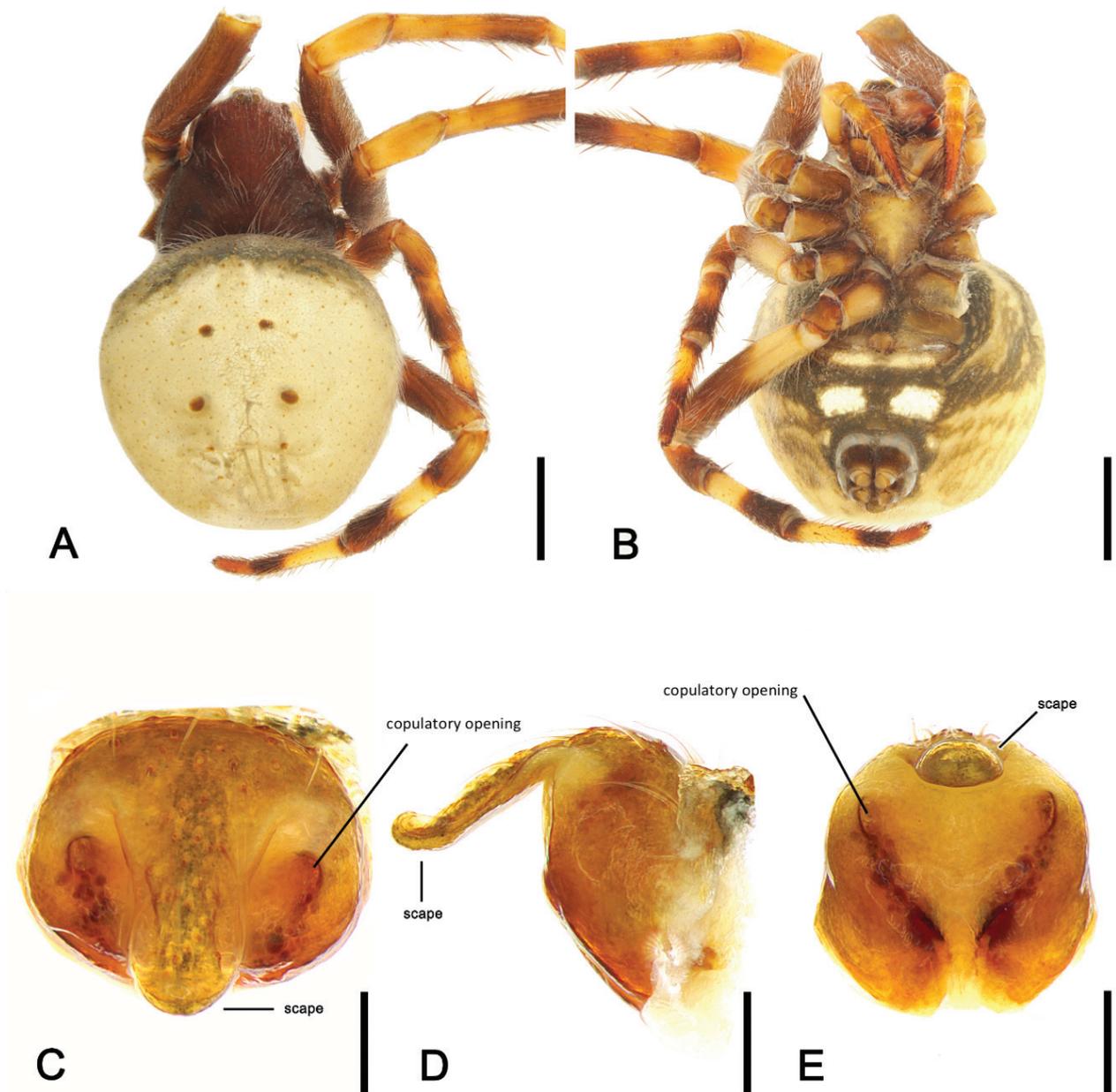


Figure 3. *Mangrovia albida* comb. nov., female (AM KS.98754). **A.** Habitus, dorsal view; **B.** Habitus, ventral view; **C.** Epigyne, ventral view; **D.** Epigyne, lateral view; **E.** Epigyne, posterior view. Scale bars: 2 mm (A, B); 0.2 mm (C–E).

1.3 wide; rounded; dorsum with instinct dark folium pattern bordered by irregular, wavy light band; two small triangular white spots anteriorly sparsely covered with long brown setae; laterally beige with greyish bands; venter dark brown with two large, rounded white spots near spinnerets. Pedipalp (Figs 1C, D, 2A–D) length of segments (femur + patella + tibia + cymbium = total length): $0.3 + 0.1 + 0.2 + 0.5 = 1.1$; cymbium broad, tegulum and subtegulum well-developed; conductor broad, basally slightly sclerotised, otherwise fleshy; median apophysis oval with an apical, slightly curved spine-like prong; radix elongate; terminal apophysis well-developed, with rounded distal portion; subterminal apophysis originating near the basis of terminal apophysis, thin and sclerotized; embolus basally inflated, otherwise thin, straight and with distinct subterminal branch.

Female (based on AM KS.98754): Total length 8.6. Carapace (Fig. 3A) 3.9 long, 3.5 wide; pear-shaped, uniformly dark reddish-brown, weak cover of long and short white setae. Eyes diameter AME 0.27, ALE 0.18, PME 0.20, PLE 0.18; row of eyes: AME 0.70, PME 0.54, PLE 1.98. Chelicerae paturon reddish brown; four promarginal teeth, apical and third largest; three retromarginal teeth of similar size. Legs colouration similar to male (Fig. 3A, B). Pedipalp length (femur + patella + tibia + tarsus = total length): $1.2 + 0.5 + 0.8 + 1.3 = 3.8$. Leg formula $I > IV > II > III$; length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – $4.2 + 1.8 + 3.0 + 3.1 + 1.1 = 13.2$, II – $3.8 + 1.7 + 2.7 + 0.9 + 1 = 10.1$, III – $2.4 + 1.1 + 1.4 + 1.5 + 0.9 = 7.3$, IV – $3.6 + 1.6 + 2.2 + 2.3 + 0.9 = 10.6$. Labium and endites light brown. Sternum almost heart-shaped, yellowish-brown with dusky contour (Fig. 3B). Abdomen (Fig. 3A, B) 5.5 long, 4.7 wide; dorsum beige,

with black band on anterior margin; four pairs of dark brown sigillae; venter as in male, but with thin white band behind epigastric furrow area. Epigyne (Fig. 3C, D, 4A) oval, around 1.5 times wider than long; copulatory openings lateral to scape; scape broad lip with terminal pocket; spermathecae sub-spherical, almost touching; fertilisation ducts basally convoluted and attaching posteriorly to spermathecae (Fig. 4A).

Variation. Total length males 2.7–3.0 ($n = 3$), females 8.3–9.7 ($n = 5$). There is very little colour variation in both males and females, although the folium pattern in males can be very distinct.

Remarks. Rack (1961) listed the holotype of *Epeira albida* L. Koch, 1871 as her catalog number 218 in the collection of the ZMH; however, this cannot be the holotype as this specimen is an immature female and the original description and illustrations by L. Koch (1871) clearly show a mature female with fully developed epigyne. We here consider a female in the collection of the NHMUK London the holotype of *E. albida*. This specimen has the typical label handwritten by L. Koch and matches well the description in L. Koch (1871).

Keyserling (1887) described *Epeira fastidiosa* Keyserling, 1887 based on a mature male. The holotype of this species matches in all diagnostic characters the males that are here recognised as conspecific with the female *M. albida* comb. nov. We therefore consider *E. fastidiosa* a junior synonym of *M. albida* comb. nov. Similarly to *M. albida* comb. nov., the designation of the holotype of *E. fastidiosa* remains ambiguous. Rack (1961) considered a male in the ZMH (Rack (1961)-catalog no. 237) as holotype of the species, but there is also a male in the NHMUK (1890.7.1.4150) from the type locality Rockingham that could be the holotype, as part

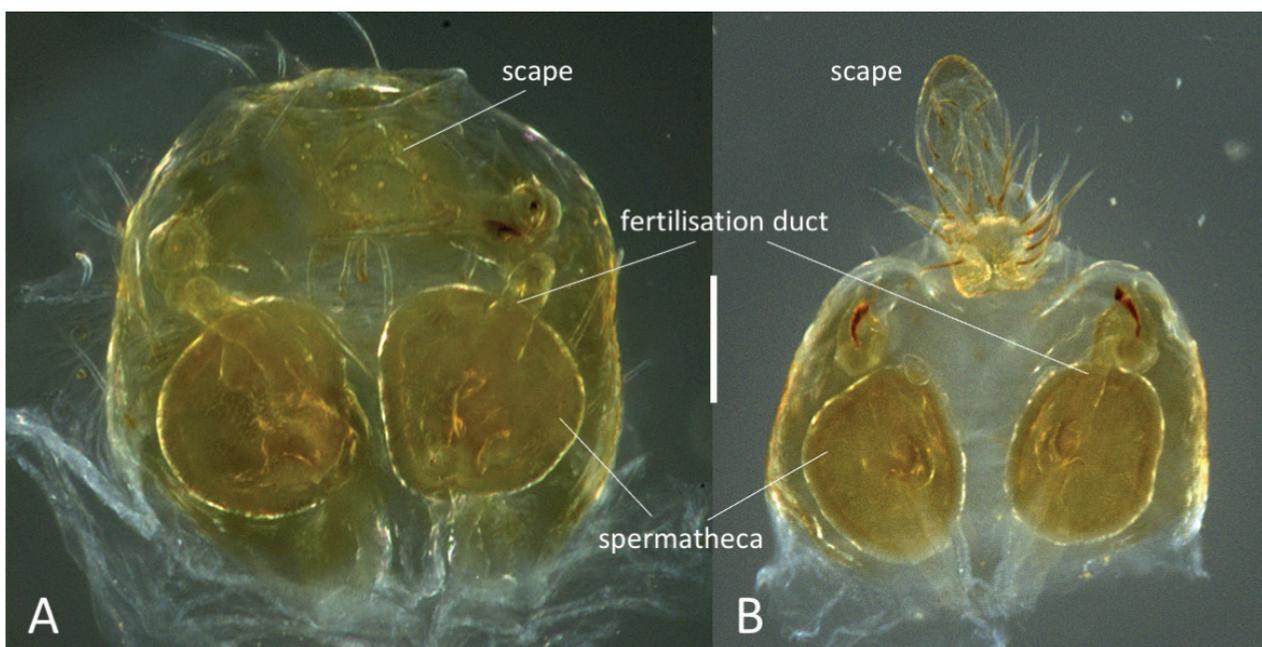


Figure 4. *Mangrovia albida* comb. nov. (AM KS.98754) and *M. occidentalis* sp. nov. (WAM T75326), cleared female epigynes, posterior view. **A.** *Mangrovia albida* comb. nov.; **B.** *M. occidentalis* sp. nov. Scale bar: 0.2 mm (A, B).

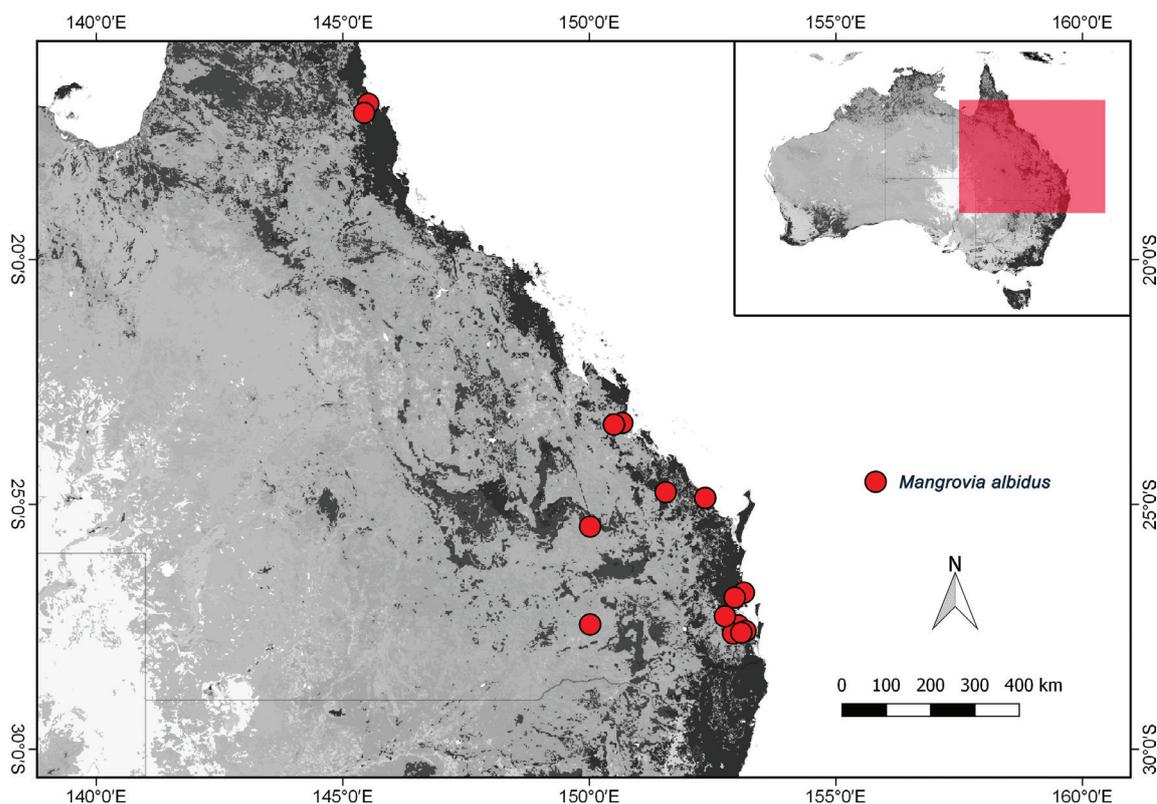


Figure 5. Distribution records of *Mangrovia albida* comb. nov.

of the Keyserling-collection was sold to the NHMUK (J. Beccaloni, pers. comm.). Both specimens are very similar and match Keyserling's (1887) original description. We here follow Rack's (1961) initial designation of the holotype of *E. fastidiosa* to maintain consistency with previous literature.

Habitat preferences and life history. Habitat descriptions found on labels with museum specimens include 'mangroves' and 'riparian', where spiders were found in rolled leaves near the orb-web. Mature spiders were found between December and April.

Distribution. This species is only known from coastal Queensland (Fig. 5).

Mangrovia occidentalis sp. nov.

<http://zoobank.org/7985F3FE-1B1E-479D-82EC-5E4BAC183D7F>

Figs 4B, 6A–D, 7A, B, 8A–E, 9

Type material. *Holotype* male, Cape Range National Park, Yardie Creek (22°20'S, 113°48'E, Western Australia, Australia), 7 July 1987, B. Y. Main (WAM T77397).

Etymology. The specific epithet is a Latin adjective in apposition – *occidentalis* – meaning western, and it refers to its distribution that is limited to coastal Western Australia.

Other material examined. AUSTRALIA: Western Australia: 1 female, Bay of Rest, 22°18'S, 114°08'E (WAM T75793); 1 female, same locality (WAM T75827); 1 female, 1 juv., Cape Range, 22°10'S, 114°E (AM KS.62723); 3 juv., Cape Range National Park, Yardie Creek, 22°20'S,

113°48'E (WAM T157108); 1 female, same locality (WAM T75322); 1 female, same locality (WAM T75326); 1 female with eggsac, same locality (WAM T115115); 1 female, same locality (WAM T75327); 1 female, Cooke Point, Port Headland, 20°19'S, 118°36'E (WAM T75325); 2 females, Faure Island, North, 25°48'S, 113°53'E (WAM T67857); 3 females, Faure Island, West, 25°52'S, 113°53'E (WAM T67854); 1 female, same locality (WAM T67855); 1 female, same locality (WAM T67856); 1 female, Karratha, NW airport, 20°44'S, 116°51'E (WAM T75840); 1 female, King Sound, Derby Jetty, 16°49'S, 123°28'E (WAM T75777).

Diagnosis. See above for *Mangrovia albida* comb. nov.

Description. *Male* (based on holotype, WAM T77397): Total length 2.2. Carapace (Fig. 6A) 1.0 long, 0.9 wide; light brown with darker lateral flanks; few white setae. Eyes diameter AME 0.09, ALE 0.05, PME 0.08, PLE 0.05; row of eyes: AME 0.29, PME 0.22, PLE 0.50. Chelicerae paturon dark grey; two promarginal teeth, the apical slightly larger, three retromarginal teeth of similar size. Legs (Fig. 6A, B) beige with distal parts of femora, tibiae and patellae brown. Leg formula I > II > IV > III; length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – 1.0 + 0.4 + 0.8 + 0.7 + 0.4 = 3.3, II – 0.9 + 0.3 + 0.6 + 0.9 + 0.4 = 3.1, III – 0.6 + 0.2 + 0.3 + 0.3 + 0.3 = 1.7, IV – 0.8 + 0.3 + 0.5 + 0.5 + 0.4 = 2.5. Labium and endites beige. Sternum (Fig. 6B) heart-shaped, yellowish brown, with darker contour. Abdomen (Fig. 6A, B) 1.1 long, 1.2 wide; olive-grey with indistinct darker folium pattern; laterally yellowish-brown; ventral olive-brown, four irregular white guanine spots. Pedipalp (Figs 6C, D, 7A, B) length of segments (femur

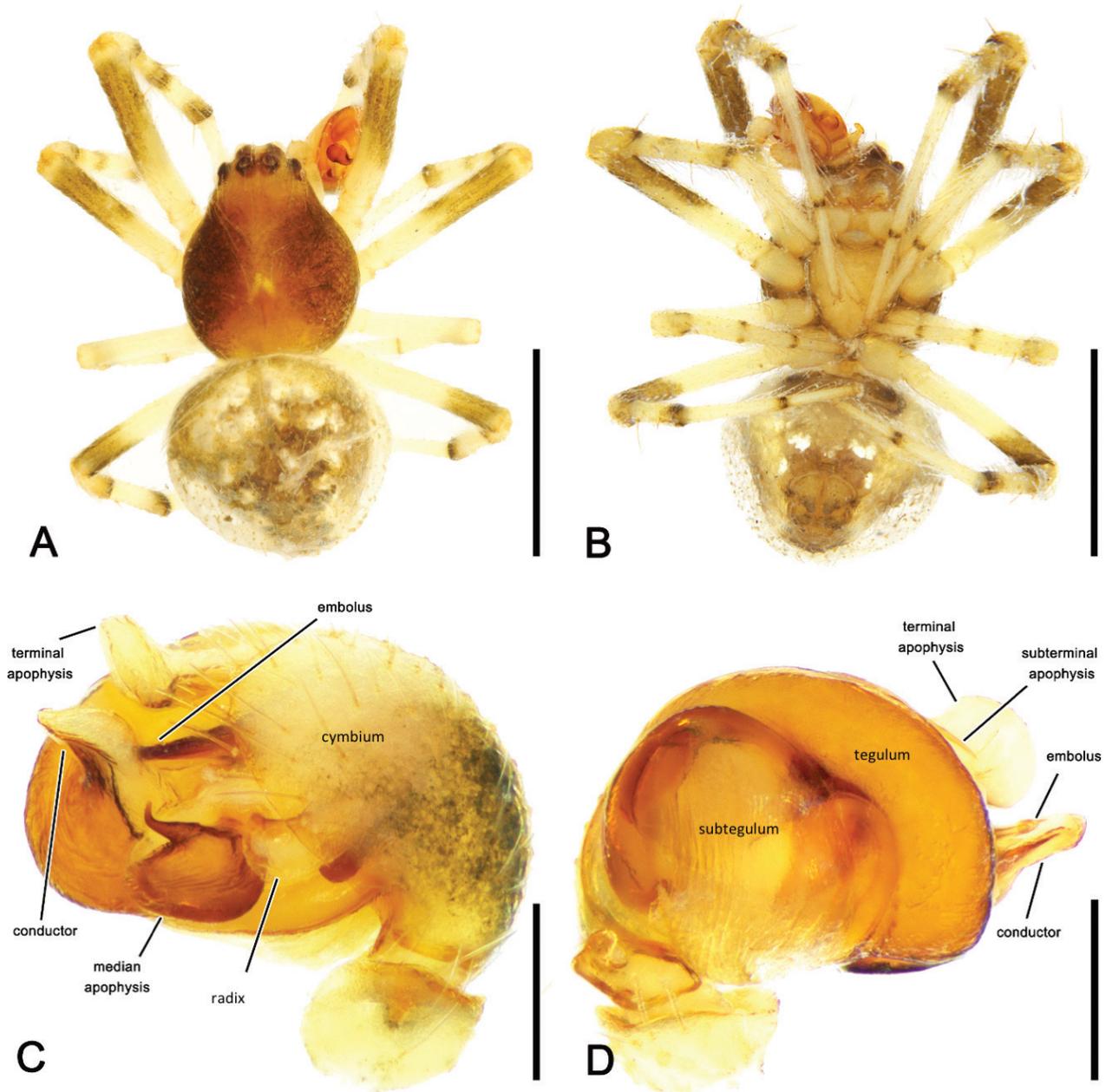


Figure 6. *Mangrovia occidentalis* sp. nov., male (WAM T77397). **A.** Habitus, dorsal view; **B.** Habitus, ventral view; **C.** Right pedipalp, ventral view; **D.** Right pedipalp, dorsal view. Scale bars: 1 mm (A, B); 0.1 mm (C, D).

+ patella + tibia + cymbium = total length): 0.2 + 0.1 + 0.2 + 0.4 = 0.9; cymbium broad, tegulum and subtegulum well-developed; conductor broadly elongate, basally slightly sclerotised, otherwise fleshy; median apophysis oval with an apical, slightly curved spine-like prong; radix elongate; terminal apophysis well-developed, with rounded distal portion, bent apically; subterminal apophysis originating near the basis of terminal apophysis, thin and sclerotized; embolus basally inflated, otherwise thin, straight and with small subterminal branch.

Female (based on WAM T75326): Total length 10.8. Carapace (Fig. 8A) 3.8 long, 3.6 wide; pear-shaped, uniformly reddish-brown; few white setae. Eyes diameter AME 0.25, ALE 0.16, PME 0.18, PLE 0.16; row of

eyes: AME 0.65, PME 0.47, PLE 1.64. Chelicerae reddish-brown; four promarginal teeth, second basal largest; three retromarginal teeth of similar size. Legs (Fig. 8A, B) reddish-brown. Pedipalp length (femur + patella + tibia + tarsus = total length): 1.2 + 0.6 + 0.9 + 1.5 = 4.2. Leg formula I > IV > II > III; length of segments (femur + patella + tibia + metatarsus + tarsus = total length): I – 4.6 + 2.1 + 3.5 + 3.4 + 1.1 = 14.7, II – 4.0 + 1.8 + 3.1 + 0.9 + 1.0 = 10.8, III – 2.5 + 1.2 + 1.5 + 1.6 + 0.8 = 7.6, IV – 3.8 + 1.6 + 2.3 + 2.9 + 0.9 = 11.5. Labium and endites reddish-brown. Sternum (Fig. 8B) heart-shaped, reddish-brown with two small lighter patches along the median line. Abdomen (Fig. 8A, B) 6.5 long, 6.3 wide; dorsally beige, with a black band along anterior margin; four pairs of dark brown

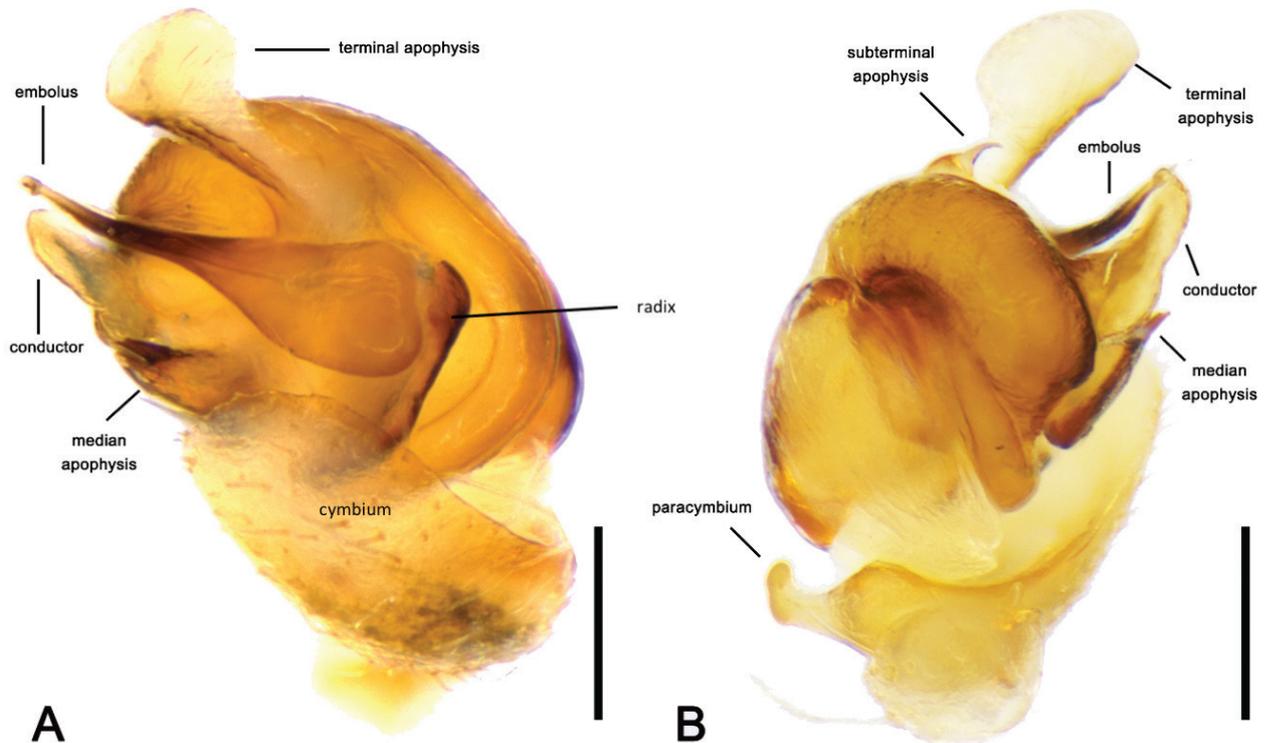


Figure 7. *Mangrovia occidentalis* sp. nov., expanded right left pedipalp (WAM T77397). **A.** Apico-dorsal view; **B.** Mesal view. Scale bars: 0.1 mm (**A, B**).

sigillae; venter olive-brown, with transverse guanine band posterior of epigastric furrow, somewhat lighter anterior of spinnerets and two small white spot antero-lateral of spinnerets. Epigyne (Figs 4B, 8C–E) almost twice as wide as long, copulatory openings laterally of scape; scape almost twice as long as epigyne plate, narrow, basally and apically wider, and with terminal pocket. Spermathecae ovoid; fertilisation ducts basally convoluted and attaching posteriorly to spermathecae (Fig. 4B).

Variation. Male only known from holotype; the spine on the median apophysis of the left pedipalp was broken off, therefore the right pedipalp is illustrated here. Female total length 8.7–10.6 (n = 8); there was little colour variation in females although the abdomen venter showed distinct white guanine spots in most specimens.

Habitat preferences and life history. Collection data on labels with museum specimens of *M. occidentalis* sp. nov. exclusively lists ‘mangroves’ as habitat, where, similar to *M. albida* comb. nov., spiders were collected mainly from rolled leaves near the orb-web. Mature spiders were mainly collected in May, July and September with a single record in February.

Distribution. This species is only known from coastal Western Australia (Fig. 9).

Discussion

Mangrovia gen. nov. males have a single patellar spine on the male pedipalp, a character that was noted by Scharff et al. (2020) to occur in all backbourkiines, an

Australasian clade in the analysis. This character was earlier recognised as taxonomically informative when employed in a study of Chinese araneids to key out two groups combined, species previously listed in *Zilla* C.L. Koch, 1834 (now all recognised as belonging to *Plebs* Joseph and Framenau, 2012) and the *dehaani*-group (Yin et al. 1997). Both *Plebs* and the *dehaani*-group have been confirmed as backbourkiines by molecular data (Scharff et al. 2020) showing that the single patellar spines can possibly serve as good diagnostic character beyond the Australian continent.

An association of *Mangrovia* gen. nov. with the backbourkiines is, however, poorly supported otherwise, as genital morphology of its two species is very unlike other representatives of this clade. The median apophysis of the male pedipalp does not form an arch over the radix as in all other backbourkiines and a basal extension of the conductor, referred to as paramedian apophysis or conductor lobe is absent (e.g. Framenau et al. 2010, 2021b; Framenau 2011). In addition, the epigyne scape has a terminal pocket, not found in any other backbourkiines, but otherwise common, for example, in members of the zealaraneines, a largely New Zealand clade (Court and Forster 1988; Scharff et al. 2020). An association of *Mangrovia* gen. nov. with zealaraneines is also more likely due to the similarities of its genitalia to *Neoscona*, a genus that was, albeit without statistical support, part of a sister group to zealaraneines and true *Araneus* combined (Scharff et al. 2020). It is therefore possible that a potential loss of a patellar spine in comparison to other zealaraneines, *Neoscona* and *Araneus* is an artefact of males becoming

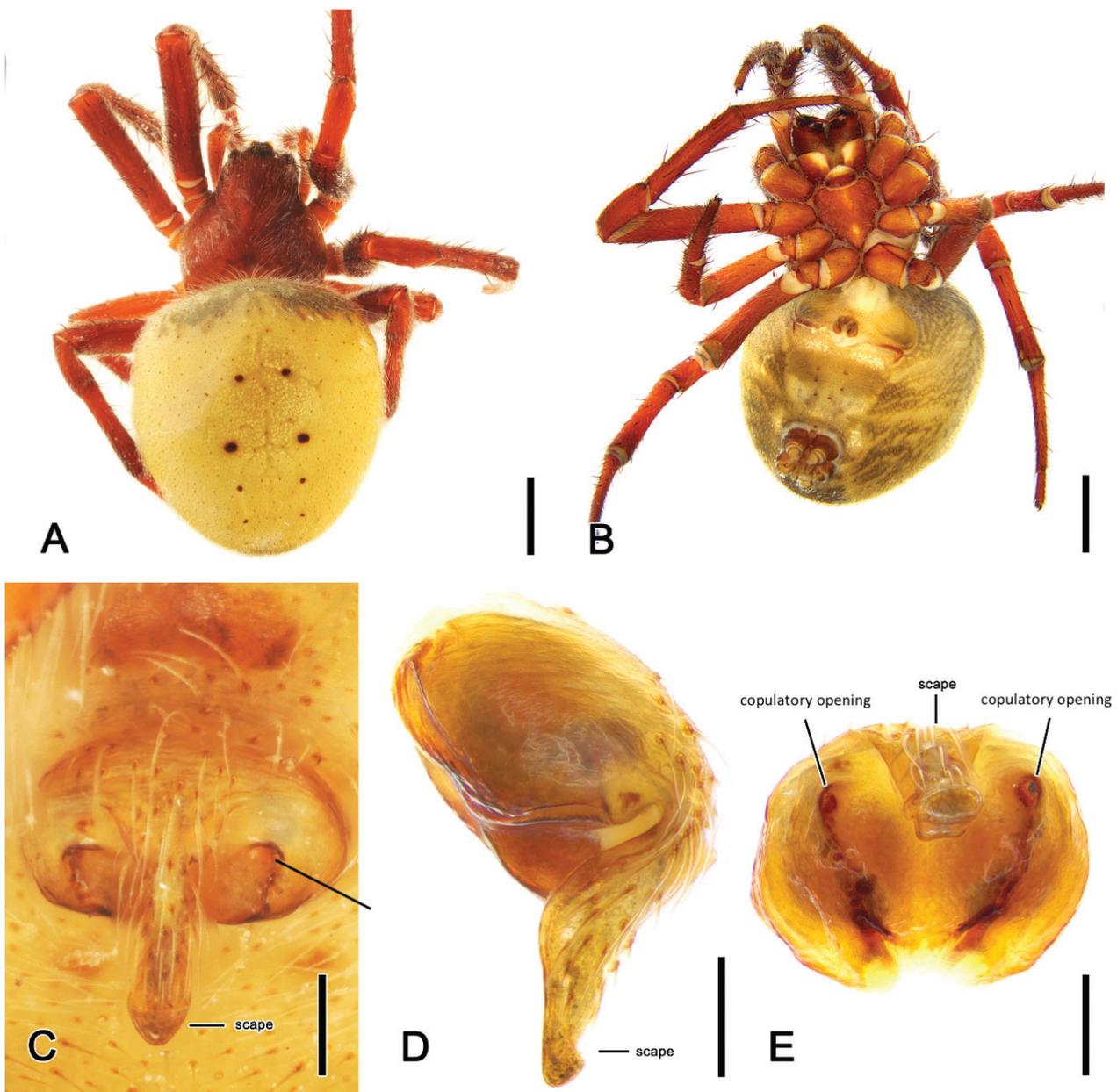


Figure 8. *Mangrovia occidentalis* sp. nov., female (WAM T75326). **A.** Habitus, dorsal view; **B.** Habitus, ventral view; **C.** Epigyne, ventral view; **D.** Epigyne, lateral view; **E.** Epigyne, posterior view. Scale bars: 2 mm (**A, B**); 0.2 mm (**C–E**).

smaller. Confidently placing *Mangrovia* gen. nov. in a phylogenetic context within the Araneidae will require, at a minimum, its inclusion into a phylogeny based on data from the molecular study by Scharff et al. (2020), although it has to be recognised that that study did not provide a fully resolved phylogenetic hypothesis of this spider family.

The pedipalp morphology of *Mangrovia* gen. nov. is very similar to that of *Neoscona*, although there are some differences, in particular in the apical section of the pedipalp, including terminal and subterminal apophyses and embolus. The *Neoscona* pedipalp also has three terminal sclerites, i.e. terminal apophysis, embolus lamella (Levi 1993a; “Terminal-Lamelle” in Grasshoff 1986) and the embolus. It is likely that the sclerite we termed subtermi-

nal apophysis is homologous to Levi’s (1993a) embolus lamella, but its shape is generally very different – and very variable – in *Neoscona* compared to the thin, needle-like structure in *Mangrovia* gen. nov. (Figs 2A, 7B). The embolus has a unique subterminal side-branch in *Mangrovia* gen. nov., the functional significance of which is unknown. ‘Capped’ emboli are not uncommon in the Araneidae (see Scharff and Coddington 1997) and are generally associated with the embolus breaking during copulation, possibly acting as mating plug in the epigyne to prevent the female from mating again. The function of the side branch of the *Mangrovia* gen. nov. embolus remains unknown.

As in *Neoscona*, the radix and stipes are either fused in *Mangrovia* gen. nov. or a stipes is absent. There

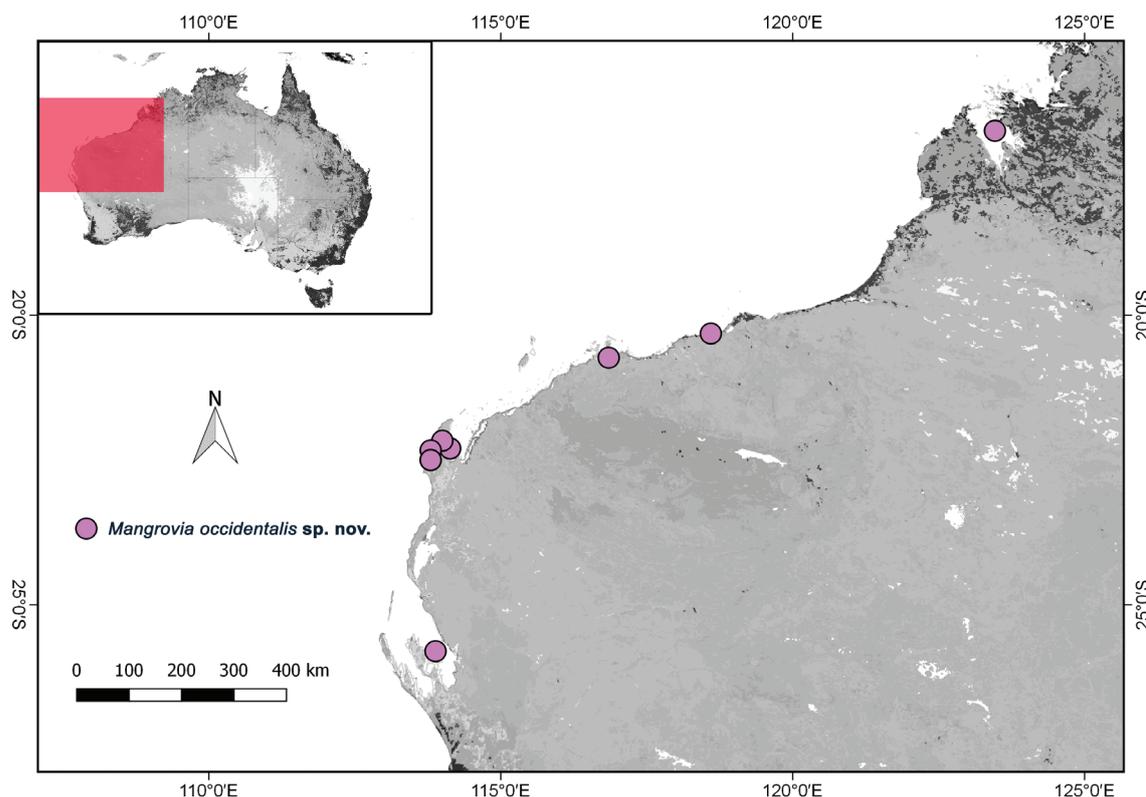


Figure 9. Distribution records of *Mangrovia occidentalis* sp. nov.

seem to be a less sclerotised short section in the radix at least in *M. albida* comb. nov. (Fig. 2C, D), which may indicate a fusion point of radix and stipes, but a similar section is not necessarily visible in *M. occidentalis* sp. nov. (Fig. 7A). Other basal pedipalp sclerites are very similar to *Neoscona*, in particular the shape of the median apophysis. Similar to many *Neoscona* (Berman and Levi 1971; Grasshoff 1986; Levi 1993a), this sclerite cannot be used to differentiate between the two *Mangrovia* gen. nov. species due to its similarity in both species. Its apical spine has likely an important function during copulation interlocking with the terminal pocket of the epigyne. Few studies have investigated genitalia during copulation in the Araneidae to explore the functional roles of the different pedipalp sclerites. But the median apophysis interlocks with the terminal pocket of the scape in *Larinia jeskovi* Marusik, 1987 (Mouginot et al. 2015).

Both *Mangrovia* gen. nov. species appear to be specialists of coastal habitats, particularly inhabiting subtropical mangroves. Here, the apparently nocturnal spiders hide in a self-constructed rolled-leaf retreat adjacent to the web during the day. In Australia, constructing a retreat from a rolled leaf has been observed in other orb-weaving spiders. Apparently similar to *Mangrovia* gen. nov., *Araneus praesignis* (L. Koch, 1872) roll a leaf of the plant that harbours the spider's web (Whyte and Anderson 2017). In contrast, *Araneus dimidiatus* (L. Koch, 1871) incorporates a dead rolled leaf into the periphery of its web where the spider hides (Framenau et al. 2014). Both species are not true *Araneus* with phylogenetic relationships poorly resolved (Scharff et al. 2020). Australian species in the

araneid subfamily Zygiellinae Wunderlich, 2004, i.e. in the genera *Artifex* Kallal & Hormiga, 2018, *Deliochus* Simon, 1894 and *Phonognatha* Simon, 1894, also suspend a dead rolled leaf in the web, but in contrast to *A. dimidiatus* it is suspended near the hub (Kallal and Hormiga 2018). It is clear that leaf-curling behaviour has evolved through different evolutionary pathways a number of times in the phylogenetic history of the Araneidae in Australia and resolving their origin requires testing with empirical data, specifically the compilation of a fully resolved phylogenetic hypothesis including all groups displaying this behaviour.

Acknowledgements

We acknowledge the support of all museum curators and scientists who facilitated loans of specimens or visits to their respective institutions: Graham Milledge (retired) and Helen Smith (AM), Janet Beccaloni (NHMUK), Robert Raven and Owen Seeman (QM), Mark Harvey and Julianne Waldo (WAM), and Hieronymus Dasty (retired), Danilo Harms and Nadine Dupérré (ZMH). We thank Renner Baptista and Cor Vink for constructive comments as a reviewer of the manuscript. Funding for a revision of the Australian Araneidae was provided by the Australian Biological Resources Study (ABRS) (grant no. 205-24 [2005–2008] to VWF and N. Scharff and grant number 4-EHPVRMK [2021–2023] to VWF, P. Castanheira, N. Scharff, D. Dimitrov, A. Chopra and R. Baptista). PdeSC received funding through a Synthesis project grant (GB-TAF-TA3-008) to visit the NHMUK in London.

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Phylogenetic relationships of a new catfish of the genus *Trichomycterus* (Siluriformes, Trichomycteridae) from the Brazilian Cerrado, and the role of Cenozoic events in the diversification of mountain catfishes

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Academic editor: Nicolas Hubert ♦ Received 4 March 2022 ♦ Accepted 5 May 2022 ♦ Published 23 May 2022

Abstract

The Brazilian Cerrado highlands shelter the headwaters of the three largest South American hydrographic basins, where a great species diversity is concentrated, but some biological groups are still insufficiently known. The focal taxa of this study are trichomycterid catfishes of the subgenus *Cryptocambeva*, genus *Trichomycterus*, endemic to mountain areas of south-eastern Brazil. The primary objective of this study is to test through a molecular phylogeny if a new species collected in streams of the upper Rio Paraná basin draining the Serra da Canastra is sister to *T. macrotrichopterus*, endemic to the upper Rio São Francisco at another facet of the Serra da Canastra, as suggested by morphological data. The analysis corroborated sister group relationships between these two species, besides supporting four main clades in *Cryptocambeva*, each of them endemic to distinct mountain regions. A time-calibrated analysis supported the divergence timing between the new species and *T. macrotrichopterus* at the Pliocene, which is chronologically compatible with the final period of intense fluvial configuration re-arrangement, when São Francisco headwater streams were captured by the Paraná basin. The new species herein described is similar to *T. macrotrichopterus* and distinguished from all other species of *Cryptocambeva* by having a long pectoral-fin filament. These two species are distinguished from each other by characteristics of the latero-sensory system, colour pattern and bone morphology.

Key Words

molecular systematics, mountain biodiversity, osteology, paleo-drainages, Rio Paraná basin

Introduction

Studies on the Cerrado biota have quickly increased since the 1980s (Oliveira and Marquis 2002). However, some groups are still insufficiently known, including mountain catfishes of the Trichomycterinae (hereafter trichomycterines), the largest subfamily of the Neotropical siluriform family Trichomycteridae (Katz et al. 2018; Costa and Katz 2021). Trichomycterines occur in all areas of the Cerrado, but they are particularly diverse in mountain

ranges of south-eastern Brazil (Costa 1992; Triques and Vono 2004; Alencar and Costa 2006; Barbosa and Costa 2010; Costa and Katz 2021; Costa et al. 2021a, b).

The great trichomycterine species diversity concentrated in mountain ranges of south-eastern Brazil is probably a consequence of the past Cenozoic scenario, characterised by intense re-arrangements of the hydrographic systems due to generalised uplift during the Neogene (Riccomini et al. 2004; Valadão 2009). Events of drainage capture by neighbouring basins were frequent

until the Pliocene (Rezende et al. 2018), probably shaping the distribution pattern of fish species (Costa and Katz 2021; Costa et al. 2022a, b). Substantial evidence supports the upper and middle sections of the Rio Grande drainage, presently a main tributary of the upper Rio Paraná basin, as being formerly connected to the Rio São Francisco basin, a configuration that was changed after the capture of the Rio Grande drainage by the Rio Paraná basin during the Middle Miocene (Rezende et al. 2018).

The main focus of this study is an undescribed species of *Trichomycterus*, subgenus *Cryptocambeva* Costa, 2021, from the upper Rio Araguari drainage, upper Rio Paraná basin. *Cryptocambeva* comprises 16 species and is diagnosable by a unique morphology of the latero-posterior portion of the neurocranium and adjacent posterior region, including a relatively small posttemporo-supracleithrum separated by large interspaces from adjacent bones, and a narrow and long lateral extremity of the pterotic, with its tip extending beyond the lateral margin of the neurocranium (Costa 2021). The new taxon here described exhibits a long pectoral-fin filament, suggesting it is closely related to *T. macrotrichopterus* Barbosa & Costa, 2010, the only other species of *Cryptocambeva* having a similar long filament (Barbosa and Costa 2010). More interestingly, these two species were only found in rivers drainages separated by the Serra da Canastra, a mountain range of about 3,000 km² that is part of a series of mountain ranges situated between the upper Rio Paraná and upper Rio São Francisco basins. The northeastern facet of the Serra da Canastra is a major watershed divide between the headwaters of the Rio Araguari drainage, of the Rio Paranaíba drainage, upper Rio Paraná basin, where the new taxon was found, and the headwaters of the main course of the Rio São Francisco basin, where can be found the type locality of *T. macrotrichopterus* (Costa & Barbosa, 2010).

The primary objectives of this study are to perform a multigene phylogenetic analysis to test the phylogenetic positioning of the new taxon and to provide a formal description to it. The secondary objective is to conduct a dating analysis in order to establish if the estimated divergence timing for *Cryptocambeva* lineages from south-eastern Brazil is compatible with the available model for the temporal drainage network evolution.

Materials and methods

Specimens

Material used in this study included specimens previously deposited in ichthyological collections: Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJ), and Museu de Zoologia, Universidade de São Paulo (MZUSP); and specimens collected in recent field studies using small dip nets (40 X 30 cm) and sieves (diameter 40–60 cm), and deposited in Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão (CICCAA). Collecting

permits were given by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit numbers: 76588-1 and 38553-11) and IEF (Instituto Estadual de Florestas; permit number: 040/2020). Photographs of live specimens here presented were taken between about one-and-a-half hours and three hours after fish capture. Euthanasia followed methods approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 065/18), using a buffered solution of tricaine methane sulphonate (MS-222) at a concentration of 250 mg/L, following AVMA (American Veterinary Medical Association) Guidelines (Leary et al. 2013) and the European Commission DGXI consensus for fish euthanasia (Close et al. 1996, 1997). Specimens were fixed in formalin for two weeks, and subsequently preserved in 70% ethanol, except specimens used in molecular analysis that were fixed in absolute ethanol. Comparative material is mainly deposited in UFRJ, but also includes specimens deposited in MZUSP, as well as in Museu de Ciências e Tecnologia, Pontifícia Universidade Católica, Porto Alegre (MCP), Muséum national d'Histoire Natural, Paris (MNHN), and Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ): *Trichomycterus brasiliensis* Lütken, 1874: MNHN 9575, 1 syntype (photograph); MNHN 18890303, 1 syntype (radiograph); UFRJ 4833, 5 ex.; UFRJ 4834, 3 ex. (C&S); UFRJ 4923, 2 ex.; UFRJ 4223, 2 ex.; Rio das Velhas basin, southeastern Brazil. *Trichomycterus bruno* Barbosa & Costa, 2010: UFRJ 6030, holotype; UFRJ 5649, 11 paratypes; UFRJ 5658, 5 paratypes (C&S); UFRJ 5660, 2 paratypes; Rio Itabapoana basin, southeastern Brazil. *Trichomycterus candidus* (Miranda-Ribeiro, 1949): MNRJ 5209, holotype; MNRJ 11762, 14 paratypes; MNRJ 5356, 21 ex.; UFRJ 4926, 31 ex.; UFRJ 4928, 5 ex. (C&S); Rio Grande basin, southeastern Brazil. *Trichomycterus claudiae* Barbosa & Costa, 2010: UFRJ 6027, holotype; UFRJ 5684, 9 paratypes; UFRJ 5685, 3 paratypes (C&S); Rio Paraíba do Sul basin, southeastern Brazil. *Trichomycterus fuliginosus* Barbosa & Costa, 2010: UFRJ 6029, holotype; UFRJ 718, 5 paratypes; UFRJ 5207, 2 paratypes (C&S); UFRJ 3248, 1 paratype; MNRJ 18177, 7 (4 C&S) paratypes; Rio Paraíba do Sul basin, southeastern Brazil. *Trichomycterus giarettai* Barbosa & Katz, 2016: UFRJ 10109, holotype; UFRJ 9676, 8 paratypes; UFRJ 9739, 3 paratypes (C&S); Rio Paranaíba basin, central Brazil. *Trichomycterus macrotrichopterus* Barbosa & Costa, 2010: UFRJ 6031, holotype; UFRJ 5775, 3 paratypes; UFRJ 5776, 2 (C&S); UFRJ 8354, 1 ex.; upper Rio São Francisco basin, southeastern Brazil. *Trichomycterus mariamole* Barbosa & Costa, 2010: UFRJ 6026, holotype; UFRJ 5666, 17 paratypes; UFRJ 5142, 15 paratypes; UFRJ 5400, 3 paratypes (C&S); UFRJ 5401, 3 paratypes (C&S); UFRJ 5247, 15 paratypes; UFRJ 5688, 6 paratypes; UFRJ 7609, 4 ex.; UFRJ 7604, 6 ex.; UFRJ 1147, 2 ex.; Rio Paraíba do Sul basin, southeastern Brazil. *Trichomycterus mimonha* Costa, 1992: MZUSP 43343, holotype; MZUSP 43344, 7 paratypes; UFRJ 641, 7 paratypes; UFRJ 5209, 1 ex. (C&S); UFRJ 4731, 22 ex.; UFRJ 5665, 2 ex.; Rio Paraíba do Sul basin, southeastern Brazil. *Trichomycterus mirissumba* Costa, 1992: UFRJ

642,3 paratypes; UFRJ 4729,12 ex.; UFRJ 4730, 5 ex. (C&S); UFRJ 1300, 5 ex.; UFRJ 3391, 2 ex.; UFRJ 4729, 12 ex.; UFRJ 10486, 3 ex.; UFRJ 11843, 1 ex.; UFRJ 11656, 1 ex.; UFRJ 3864, 1 ex.; UFRJ 11677, 4; UFRJ 1638, 1 ex.; UFRJ 3366, 7 ex.; UFRJ 4100, 2 ex.; Rio Paraíba do Sul basin, southeastern Brazil. *Trichomycterus potschi* Barbosa & Costa, 2003: MCP 29061, holotype; UFRJ 4727, 11 paratypes; MCP 29062, 2 paratypes; UFRJ 4728, 5 paratypes (C&S); UFRJ 1636, 2 ex.; UFRJ 11002, 17 ex.; UFRJ 719, 10 ex.; Rio de Janeiro coastal river basins, southeastern Brazil. *Trichomycterus novalimensis* Barbosa & Costa, 2010: MZUSP 104536, holotype; MZUSP 37145, 15 paratypes; Rio das Velhas basin, southeastern Brazil. *Trichomycterus rubiginosus* Barbosa & Costa, 2010: MZUSP 104537, holotype; MZUSP 37168, 20 paratypes; Rio Paraopeba basin, southeastern Brazil. *Trichomycterus vermiculatus* (Eigenmann, 1917): FMNH 58077, holotype (x-rays); UFRJ 11787, 21 ex.; UFRJ 6095, 3 ex.; UFRJ 5462, 8 ex.; UFRJ 5465, 3 ex. (C&S); UFRJ 5463, 3 ex. (C&S); UFRJ 12564, 1 ex.; UFRJ 12563, 1 ex.; UFRJ 5464, 4 ex.; UFRJ 582, 12 ex.; UFRJ 7241, 2 ex.; UFRJ 3592, 16 ex.; UFRJ 571, 1 ex.; UFRJ 1143, 4 ex.; UFRJ 1131, 5 ex.; UFRJ 720, 3 ex.; Rio Paraíba do Sul basin, southeastern Brazil.

Morphological data

Measurements were made using landmarks proposed by Costa (1992) as modified by Costa et al. (2020a) and presented as percent of standard length (SL), or head length in measurements of head parts. Fin-ray counts and formulae were according to Bockmann and Sazima (2004), modified by Costa et al. (2020a), in which lower case Roman numerals indicate procurrent unsegmented unbranched rays of unpaired fins, upper case Roman numerals indicate segmented unbranched rays of any fin, and Arabic numerals indicate segmented branched rays of any fin. Vertebra counts included all free vertebrae, considering the compound caudal centrum as a single element. Taylor and Van Dyke's (1985) methodology was used to clear and stain specimens for osteological examination. Osteological terminology followed Costa (2021), and latero-sensory system terminology followed Arratia and Huaquin's (1995) pore nomenclature, modified by Bockmann and Sazima (2004). Illustrations of bones were made in a stereomicroscope Zeiss Stemi SV 6 with camera lucida. Bone landmark measurements were according to Costa and Katz (2021). Comparative material is listed in Costa (2021).

DNA extraction, amplification and sequencing

DNA was extracted from muscle tissues of the caudal peduncle, using DNeasy Blood & Tissue Kit (Qiagen), according to manufacturer's protocol. Amplification of DNA fragments was made using polymerase chain reaction

(PCR) method, with primers RAG2 TRICHO F and RAG2 TRICHO R (Costa et al. 2020b), and RAG2 MCF and RAG2 MCR (Cramer et al. 2011) for nuclear gene recombination activating 2 (RAG2); Cytb Siluri F and Cytb Siluri R (Villa-Verde et al. 2012) for the mitochondrial gene cytochrome b (CYTB); and ND4 H3 L11935 and H12857 (Palumbi et al. 1991) for the mitochondrial gene NADH dehydrogenase subunit 4 (ND4). Double-stranded PCR amplifications were made 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: initial denaturation for 2–5 min at 94–95 °C; 35 cycles of denaturation for 0.5–1 min at 94–95 °C, annealing for 1–1.5 min at 45–52 °C and extension for 1–1.5 min at 72 °C; and terminal extension for 4–8 min at 72 °C. In all PCR reactions, negative controls without DNA were used to check contaminations. The 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 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 as follows: (1) 35 cycles of 10 s at 96 °C, 5 s at 54 °C and 4 min at 60 °C.

Phylogenetic analyses

The new species and twelve species representing all lineages of *Cryptocambeva* were included as terminal taxa in the analyses, besides four trichomycterine out-groups: *Trichomycterus albinotatus* Costa, 1992, a member of the subgenus *Humboldtglanis* Costa, 2021 that is sister to *Cryptocambeva* (Costa 2021); *Trichomycterus nigricans* Valenciennes, 1832, a distantly related species (Costa 2021); *Cambeva cf. cubataonis* (Bizerril, 1994), a member of the clade sister to *Trichomycterus s.s.* (Katz et al. 2018); and '*Trichomycterus areolatus* Valenciennes, 1846, a trichomycterine that is distantly related to *Trichomycterus s.s.* (Katz et al. 2018). GenBank accession numbers are provided in the Suppl. material 1: Tables S1, S2.

Alignment was conducted in Clustal W (Chenna et al. 2003) algorithm implemented in MEGAX (Kumar et al. 2018). No gap was found in alignments. The concatenated dataset was 2545 pb (1033 for CYTB, 693 for ND4, 819 for RAG2). This dataset was first analysed using a Maximum Likelihood (ML) approach in IQTREE 1.6.12 (Nguyen et al. 2015; Trifinopoulos et al. 2016), with partitions including each codon position for each gene, for which the best-fitting models as of molecular evolution, as described by Chernomor et al. (2016), were calculated using the Bayesian information criterion (BIC) of ModelFinder (Kalyaanamoorthy et al. 2017),

implemented in IQ-TREE. The list of partitions and their respective models of nucleotide substitution appear in the Suppl. material 1: Tables S1, S2. Three methods for assessing the reliability of internal branches were used in the ML analysis: the Shimodaira-Hasegawa-like procedure support (SH-aLRT; Guindon et al. 2010), the Bayesian-like transformation of SH-aLRT support (aBayes; Anisimova et al. 2011) and the ultrafast bootstrap support (UFBoot; Minh et al. 2013; Hoang et al. 2018), using 1000 replicates and default parameters as implemented in IQ-TREE.

The concatenated dataset was additionally analysed using Bayesian Inference (BI) with MrBayes 3.2.5 (Ronquist et al. 2012), using the best partition scheme and best-fit models of substitution (Suppl. material 1: Tables S1, S2) identified according to the Bayesian information criterion (BIC; Schwarz 1978) of PartitionFinder 2.1.1 (Lanfear et al. 2016), with the following parameters: two independent Markov chain Monte Carlo (MCMC) runs of two chains each for 30 million generations, with a tree sampling frequency of every 1000 generations. The convergence of the MCMC chains and the burn-in value

were assessed by evaluating the stationary phase of the chains using Tracer 1.7.1 (Rambaut et al. 2018). The BI final consensus tree and the Bayesian posterior probabilities were generated with the remaining tree samples after removing the first 25% samples as burn-in.

Divergence-time estimation

Terminal taxa were the same as above, but including additional outgroups: the trichomycterines *Scleronema minutum* (Boulenger, 1891) and *Ituglanis boitata* Ferrer, Donin & Malabarba, 2015; *Microcambeva ribeirae* Costa, Lima & Bizerril, 2004, a member of the Microcambevinae that is sister to the Trichomycterinae (Costa et al. 2020b); *Trichogenes longipinnis*, a basal trichomycterid (Katz et al. 2018); *Callichthys callichthys* (Linnaeus, 1758), a member of the loricarioid family Callichthyidae; *Nematogenys inermis* (Guichenot, 1848), the sister group to all other loricarioids (Betancur-R et al. 2015); and *Diplomystes nahuelbutaensis* Arratia, 1987, a basal member of the clade sister to the Loricarioidei (Sullivan et al. 2006; Betancur-R et al. 2015),

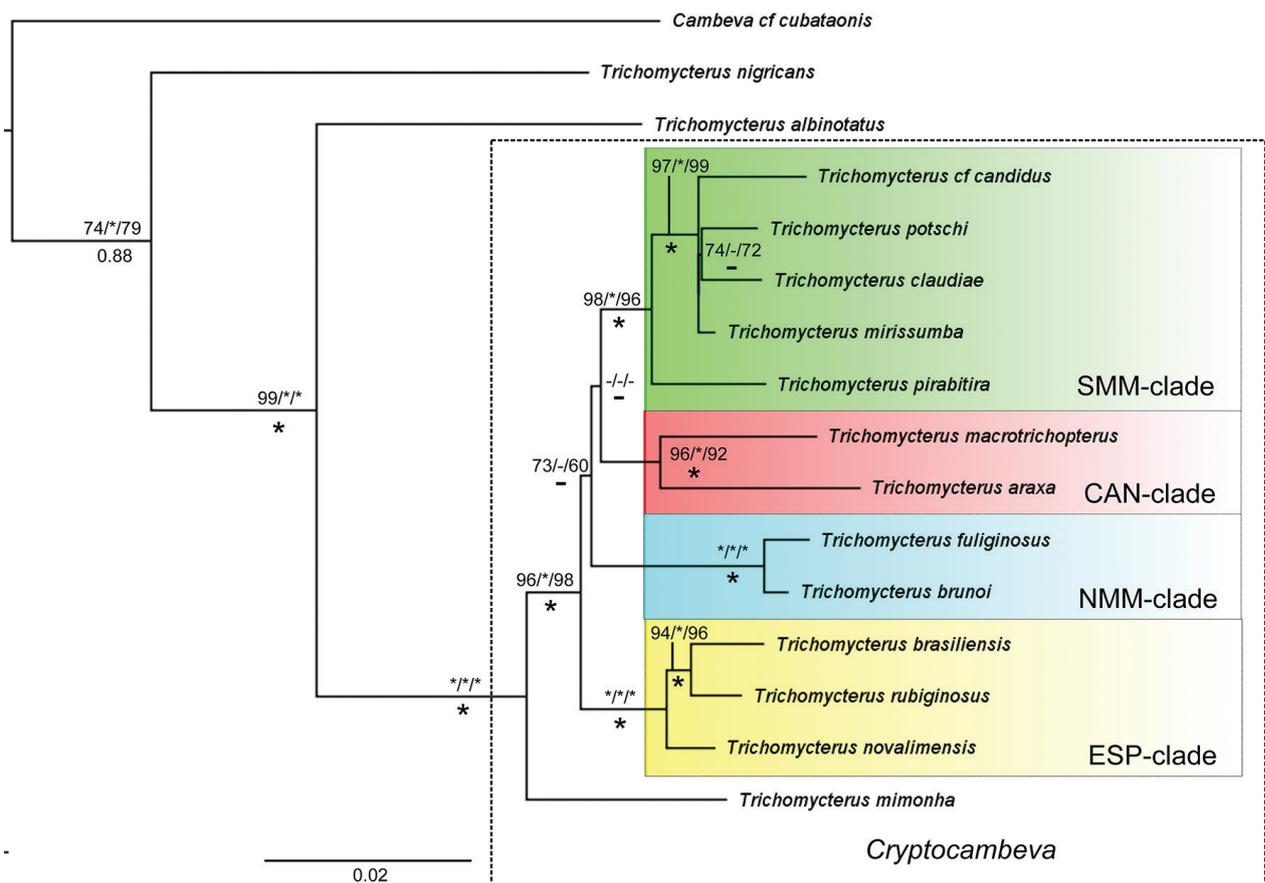


Figure 1. Phylogenetic tree generated by Maximum Likelihood analysis for 13 species of *Cryptocambeva* and four trichomycterine outgroups (most external out-group, '*Trichomycterus areolatus*', not depicted in the figure), using a multigene data set (CYTB, ND4 and RAG2, total of 2545 bp); numbers above branches are: SH-aLRT support (%) / aBayes support / ultrafast bootstrap support (%); numbers below branches are posterior probabilities from the Bayesian Inference; asterisks indicate maximum support values; minus sign indicate support values below 0.8 for aBayes support and posterior probability, and below 50% for SH-aLRT support and ultrafast bootstrap support. CAN-clade means Canastra clade; ESP-clade, Espinhaço clade; NMM-clade, northern Mantiqueira-Mar clade, and SMM-clade, southern Mantiqueira-Mar clade.

in which the analysis was rooted. DNA markers were the same as above; in addition, the best partition scheme and best-fit models were also calculated as above. The analysis was performed in BEAST v.1.10.4 (Suchard et al. 2018) using a relaxed molecular clock approach (uncorrelated relaxed molecular clock), and a Birth-Death process for the tree prior (Gernhard 2008). A single secondary calibration point was placed at the stem of the clade Trichomycteridae (normal prior distribution with mean age of 103.2 Mya, minimum age of 100.3 Mya, and standard deviation 1.5), an age estimated by Betancur-R et al. (2015). Two independent runs of Markov Chain Monte Carlo (MCMC), each runs with 50×10^6 generations were performed with sample frequency of 1000. The value of parameters of the analyses, convergence of the MCMC chains, effective sample size and the stationary distribution were evaluated using Tracer v. 1.7.1. Generated trees were combined in LogCombiner v.1.10.4 (Suchard et al. 2018) after applying a burn-in of the first 25% in each run. TreeAnnotator v.1.10.4 (Suchard et al. 2018) was used to obtain the maximum credibility tree and posterior probabilities.

Taxonomic accounts

Osteological structures included in the description are those that have informative variability to diagnose species of the eastern South American trichomycterine clade (e.g. Costa et

al. 2021c), comprising the mesethmoid and adjacent bones, jaw suspensorium and adjacent opercular bones, and the parurohyal. In the list of specimens, C&S means specimens cleared and stained for osteological examination. Geographical names are not tentatively translated to English, but follow Portuguese regional terms, making easier field identification and avoiding common errors when translating them.

Results

Phylogenetic analyses and divergence dating

The phylogenetic analyses generated identical trees, which corroborated the new taxon as sister to *T. macrotrichopterus* with high support values (Fig. 1). Like in previous analyses (e.g. Costa 2021), *Trichomycterus mimonha* Costa, 1992 appeared as sister to a clade containing all other species belonging to *Cryptocambeva*. This inclusive clade comprises four major well-supported clades that are geographically disjunct (Fig. 2): 1) the southern Mantiqueira-Mar clade (hereafter SMM-clade), comprising species endemic to river basins draining the southern Serra da Mantiqueira and the adjacent portion of the Serra do Mar; 2) the Canastra clade (hereafter CAN-clade), sister to the SMM-clade and comprising species endemic to the river basins draining the Serra da Canastra; 3) the northern

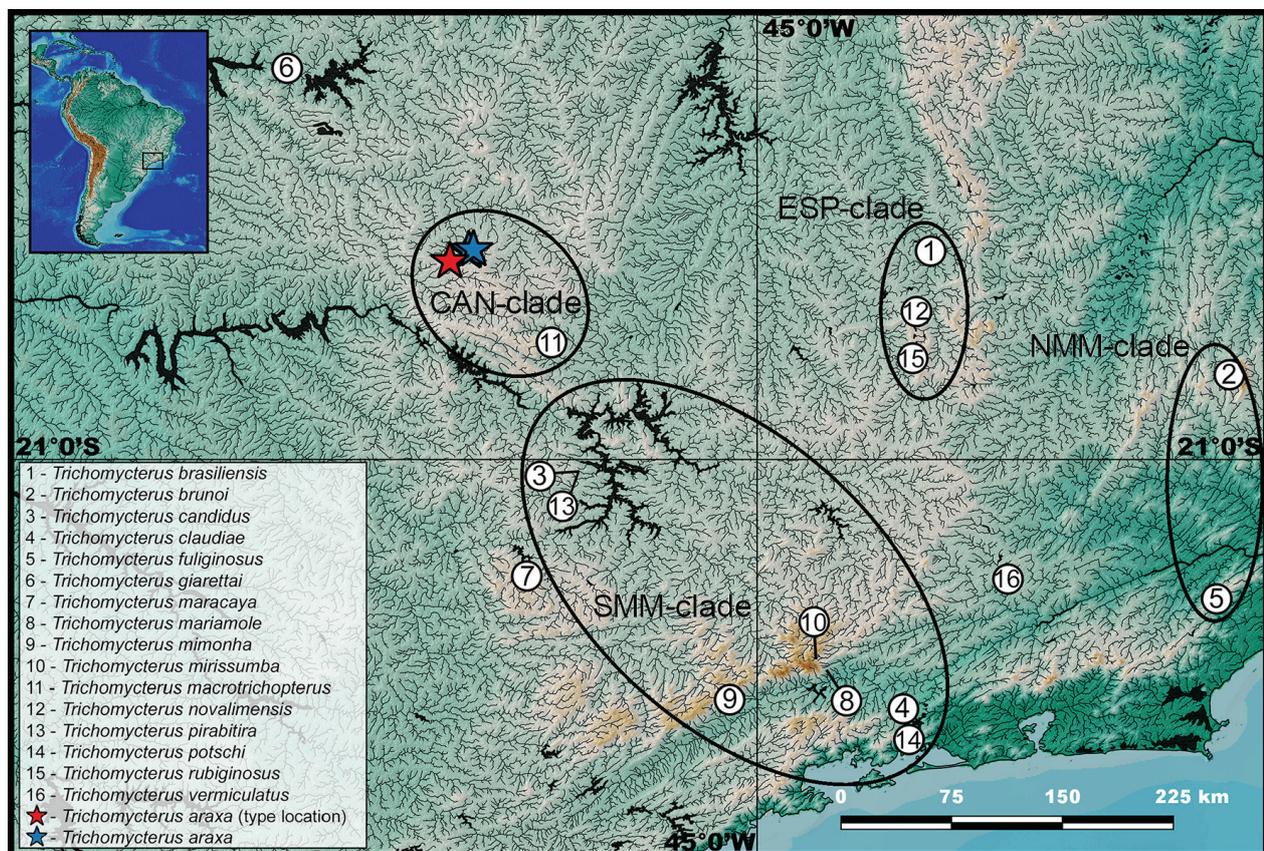


Figure 2. Geographical distribution of *Cryptocambeva*. CAN-clade means Canastra clade; ESP-clade, Espinhaço clade; NMM-clade, northern Mantiqueira-Mar clade, and SMM-clade, southern Mantiqueira-Mar clade.

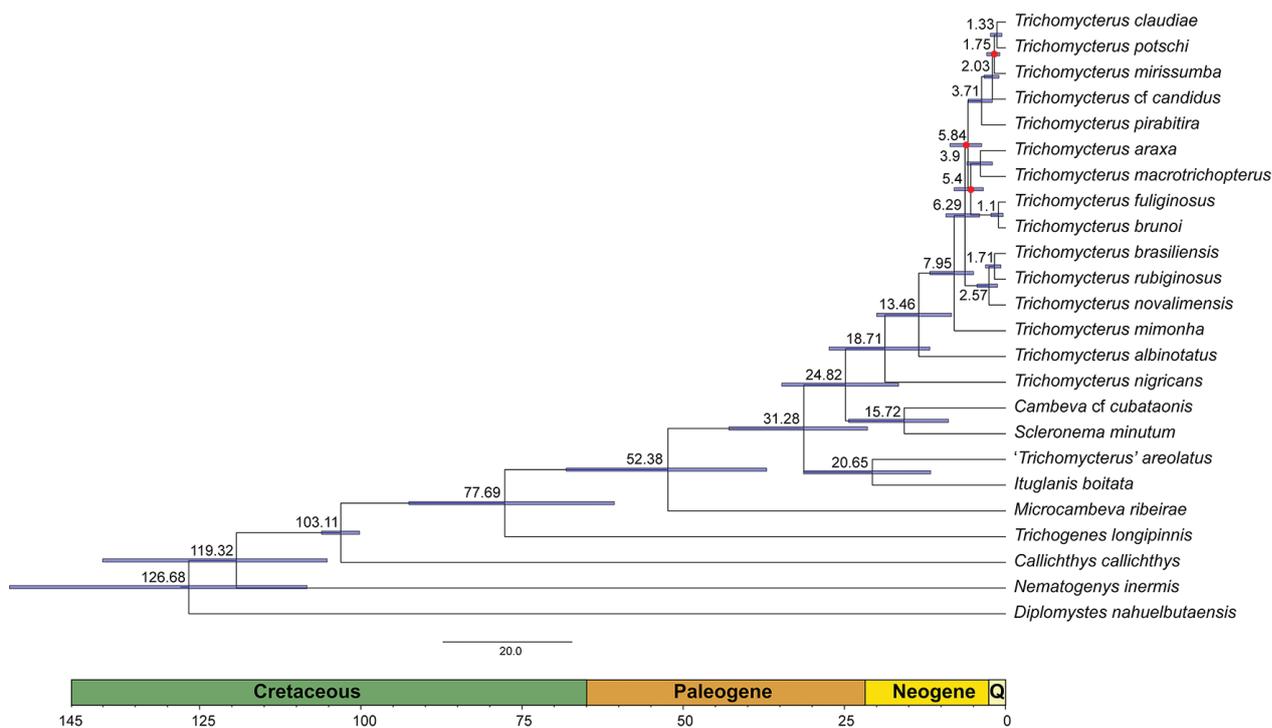


Figure 3. Time-scaled maximum credibility tree obtained from the Bayesian analysis in Beast for 13 species of *Cryptocambeva* and 11 outgroups, using a multigene data set (CYTB, ND4 and RAG2, total of 2545 bp). Numbers above the branches indicates the average age of the nodes and bars represent the 95% highest posterior densities intervals for the ages. Red dots indicate the nodes with posterior probabilities values inferior to 80. The time scale is express in millions of years.

Mantiqueira-Mar clade (hereafter NMM-clade), sister to the SMM-clade plus CAN-clade, and comprising species endemic to the river basins draining the northern Serra da Mantiqueira and the adjacent portion of the Serra do Mar; 4) and the Espinhaço clade (hereafter ESP-clade), sister to the SMM-clade plus CAN-clade plus NMM-clade, and comprising species endemic to the river basins draining the Serra do Espinhaço (Fig. 1). The time-calibrated analysis (Fig. 3) indicated an initial diversification of *Cryptocambeva*, with the splitting of *T. mimonha* and the clade containing the remaining species of the genus, during the Late Miocene, at about 8 Ma. According to the analysis, the divergence between the new species and *T. macrotrichopterus* occurred during the Pliocene, at about 4 Ma.

Taxonomical accounts

Trichomycterus araxa sp. nov.

<http://zoobank.org/367D112A-6969-4316-8456-D7AA9DE987AE>
Figs 4–7, 8A–C, Table 1

Holotype. UFRJ 7029, 53.6 mm SL; Brazil: Minas Gerais State: Araxá Municipality: stream tributary to Rio Capivara, a tributary of Rio Quebra Anzol, a tributary of the Rio Araguari subdrainage, Rio Paranaíba drainage, Rio Paraná basin, at Reserva Particular do Patrimônio Natural São Sebastião, 19°40'26"S, 47°02'24"W, about 970 m asl; A.M. Katz et al., 3 November 2021.

Paratypes. All from Brazil: Minas Gerais State: Araxá Municipality: Rio Araguari subdrainage, Rio Paranaíba drainage, Rio Paraná basin. UFRJ 7030, 18 ex., 28.1–60.7 mm SL; CICCAA 05603, 10 ex., 28.5–56.3 mm SL; all collected with holotype. – UFRJ 7031, 3 ex. (C&S), 36.7–57.7 mm SL; about 1.7 km below the type locality, 19°40'02"S, 47°01'34"W, about 940 m asl; W.M.S. Sampaio et al., 5 August 2021. – MZUSP 114937, 9 ex., 19.6–64.0 mm SL; stream tributary to Rio Quebra Anzol, 19°36'27"S, 46°54'57"W, about 983 m asl; O.T. Oyakawa et al., 26 June 2013. – MZUSP 114925, 15 ex., 14.4–55.2 mm SL; stream tributary to Rio Quebra Anzol at Araxá City, 19°35'37"S, 46°52'57"W, about 963 m asl; O.T. Oyakawa et al., 25 June 2013. – MZUSP 114918, 13 ex., 16.4–52.4 mm SL; stream tributary to Rio Quebra Anzol near Santuário Nossa Senhora de Fátima, 19°35'56"S, 46°54'51"W, about 1020 m asl; O.T. Oyakawa et al., 26 June 2013.

Additional specimens (non-types). MZUSP 109239, 2 ex.; Domo de Salitre; L.F. Salvador & M.J. Pozza, no date.

Diagnosis. *Trichomycterus araxa* is distinguished from all other species of the subgenus *Cryptocambeva* by the presence of a black median longitudinal stripe on the caudal fin in juvenile specimens below about 40 mm SL (Fig. 6; vs. black median longitudinal stripe always absent). *Trichomycterus araxa* differs from all other species of *Cryptocambeva*, except *T. macrotrichopterus*, by having a long pectoral-fin filament, its length about 40–60% of pectoral-fin length in specimens about 50 mm SL or larger (vs. short, about



Figure 4. *Trichomycterus araxa* sp. nov., holotype, UFRJ 7029, 53.6 mm SL **A.** Left lateral view; **B.** Dorsal view; **C.** Ventral view.

5–20%). *Trichomycterus araxa* is also distinguished from *T. macrotrichopterus* by the presence of the anterior infraorbital canal, supported by an elliptical antorbital (Fig. 8A; vs. anterior infraorbital canal absent, antorbital circular, Fig. 8D), a moderately deep opercular odontode patch (Fig. 8B; vs. slender, Fig. 8E), posterior margin of the metapterygoid, anterior margin of the hyomandibula anterior outgrowth and dorso-posterior margin of the quadrate slightly curved (Fig. 8B; vs. strongly waved, Fig. 8E), and absence of a prominent projection on the lateral margin of the lateral ethmoid, ventrally overlapping the sesamoid supraorbital (Fig. 8A; vs. presence, Fig. 8C).

Description. General morphology (Figs 4–7). Morphometric data appear in Table 1. Body moderately slender, subcylindrical, slightly depressed anteriorly, compressed posteriorly. Greatest body depth at vertical immediately anterior to pelvic-fin base. Dorsal and ventral profiles of head and trunk slightly convex, about straight on caudal peduncle. Anus and urogenital papilla at vertical just anterior to middle of dorsal-fin base. Head sub-trapezoidal in dorsal view. Anterior profile of snout slightly convex in dorsal view. Eye small, dorsally positioned in head, about equidistant from mouth and posterior border of opercle. Posterior nostril nearer anterior nostril than orbit. Tip of nasal barbel posteriorly reaching opercle or area slightly



Figure 5. Live holotype of *Trichomycterus araxa* sp. nov., UFRJ 7029, 53.6 mm SL, left lateral view.

Table 1. Morphometric data of *Trichomycterus araxa* sp. nov.

	Holotype	Paratypes (n = 10)	Mean
Standard length (mm)	53.6	43.3–64.0	53.5
<i>Percent of standard length</i>			
Body depth	19.3	15.8–20.9	18.5
Caudal peduncle depth	14.7	13.4–15.8	14.7
Body width	11.7	10.4–13.2	12.3
Caudal peduncle width	4.1	3.8–5.6	4.6
Pre-dorsal length	64.2	63.2–67.5	65.3
Pre-pelvic length	59.6	58.0–62.3	60.0
Dorsal-fin base length	11.6	11.4–14.2	12.4
Anal-fin base length	8.7	9.0–11.6	9.8
Caudal-fin length	19.0	17.3–20.2	18.9
Pectoral-fin length	15.0	12.3–14.6	13.3
Pelvic-fin length	10.2	9.7–11.3	10.5
Head length	20.2	19.4–21.6	20.1
<i>Percent of head length</i>			
Head depth	54.9	51.0–67.9	56.2
Head width	83.4	82.8–95.7	87.7
Snout length	47.2	42.4–48.7	45.9
Interorbital length	30.2	25.4–33.5	29.4
Preorbital length	11.6	9.4–13.6	11.5
Eye diameter	11.1	9.2–11.1	10.2

posterior to it; tip of maxillary barbel posteriorly reaching pectoral-fin base; rictal barbel posteriorly reaching area between interopercular patch of odontodes and pectoral-fin base. Mouth subterminal. Jaw teeth irregularly arranged, pointed, 35–49 in premaxilla, 32–45 in dentary. Head and trunk skin with minute skin papillae.

Dorsal and anal fins subtriangular, distal margin slightly convex; total dorsal-fin rays 10 or 11 (i–ii + II + 7), total anal-fin rays 9 (ii + II + 5); anal-fin origin at vertical through base of 5th branched dorsal-fin ray. Pectoral fin subtriangular in dorsal view, posterior margin slightly convex, first pectoral-fin ray terminating in long filament, reaching about 40–60% of pectoral-fin length in specimens above about 50 mm SL; total pectoral-fin rays 7 (I + 6). Pelvic fin subtruncate, its posterior extremity at vertical through middle of dorsal-fin base and posterior to urogenital aperture; pelvic-fin bases medially in contact; total pelvic-fin rays 5 (I + 4). Caudal

fin subtruncate, posterior corners rounded; total principal caudal-fin rays 13 (I + 11 + I), total dorsal procurent rays 20–23 (xix–xxii + I), total ventral procurent rays 16 or 17 (xv–xvi + I).

Laterosensory system. Supraorbital sensory canal continuous, posteriorly connected to posterior section of infraorbital canal. Supraorbital pores 3, all paired: s1, adjacent to medial margin of anterior nostril; s3, adjacent and just posterior to medial margin of posterior nostril; s6, at transverse line through posterior half of orbit. Pores s6 nearer its symmetrical homologous pore than orbit. Infraorbital sensory canal arranged in 2 segments; anterior section isolated, with two pores: i1, at transverse line through anterior nostril, i3, at transverse line just anterior to posterior nostril; posterior segment posteriorly connected to postorbital canal, with 2 pores: i10, adjacent to ventral margin of orbit, i11, posterior to orbit. Postorbital canal with 2 pores: po1, at vertical line above posterior portion of interopercular patch of odontodes, po2, at vertical line above posterior portion of opercular patch of odontodes. Lateral line of body short, with 1 pore just posterior to head.

Osteology (Fig. 8A–C). Mesethmoid T-shaped in dorsal view, cornu extremity rounded. Antorbital elliptical, short, dorso-posteriorly carrying thin latero-sensory canal. Sesamoid supraorbital slender, without processes. Premaxilla sub-rectangular in dorsal view, slightly narrowing laterally. Maxilla boomerang-shaped, shorter than premaxilla. Autopalatine sub-rectangular in dorsal view when excluding its postero-lateral process, its width about half its length including anterior cartilage; medial margin slightly sinuous, lateral margin about straight; posterolateral process well-developed, subtriangular, its length slightly shorter than autopalatine length excluding anterior cartilage. Lateral ethmoid with minute lateral projection. Metapterygoid sub-triangular, slightly longer than deep. Quadrate L-shaped, vertical branch wider, dorsoposterior margin in close proximity to hyomandibula outgrowth.

Hyomandibula long, with well-developed anterior outgrowth; middle portion of dorsal margin of hyomandibula slightly concave. Opercle slender, with moderately



Figure 6. *Trichomycterus araxa* sp. nov., paratype, UFRJ 7030, 26.4 mm SL: **A.** Left lateral view; **B.** Dorsal view; **C.** Ventral view.

deep odontode patch with 12–16 odontodes transversely arranged. Opercular odontodes pointed, anterior odontodes narrow and straight, posterior odontodes slightly broader, slightly curved. Dorsal process of opercle short. Opercular articular facet for hyomandibula with prominent rounded extension, articular facet for preopercle well developed, rounded. Interopercular patch of odontodes long, about four fifths of hyomandibula length, with 26–31 odontodes. Interopercular odontodes pointed, arranged in irregular longitudinal rows. Preopercle slender, narrowing anteriorly.

Parurohyal robust, lateral process subtriangular, latero-posteriorly directed, tip pointed; parurohyal head well-developed, with prominent anterolateral paired process; middle foramen oval circular; posterior process long, slightly shorter or equal to distance between anterior margin of parurohyal and anterior insertion of posterior process. Branchiostegal rays 8. Vertebrae 35. Ribs 12 or 13. Dorsal-fin origin at vertical through centrum of 19th vertebra; anal-fin origin at vertical through centrum of 22nd or 23rd vertebra. Two dorsal hypural plates, corresponding to hypurals 4 + 5 and 3, respectively; single

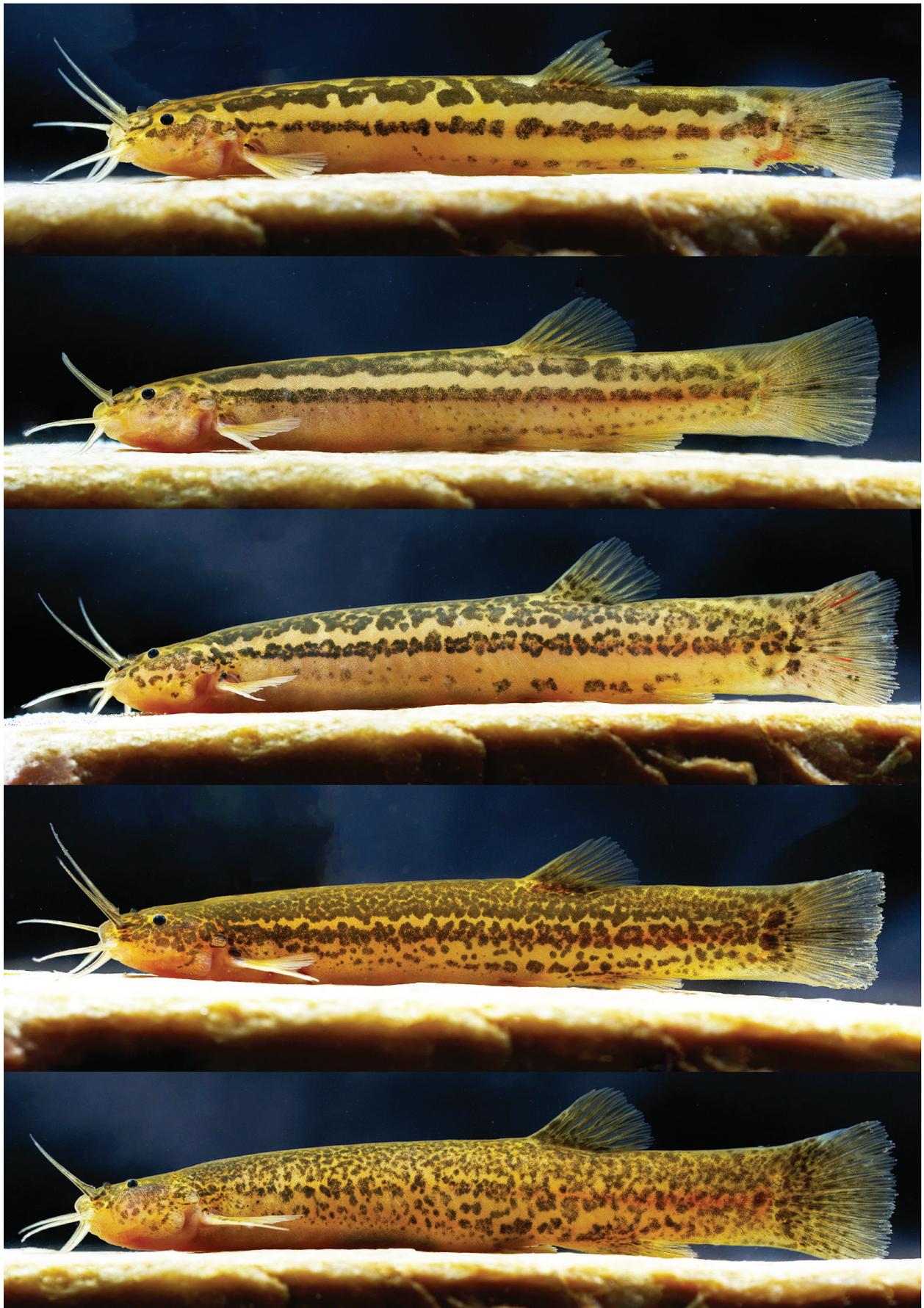


Figure 7. Live paratypes of *Trichomycterus araxa* sp. nov., UFRJ 7030, left lateral view: **A.** 40.6 mm SL; **B.** 39.3 mm SL; **C.** 53.7 mm SL; **D.** 45.8 mm SL; **E.** 45.8 mm SL.

ventral hypural plate corresponding to hypurals 1 and 2, and parhypural.

Colouration (Figs 4–7). Colouration of preserved specimens in alcohol similar to colouration in live specimens, except for yellow colouration being paler after preservation. Flank, dorsum and head side pale yellow, with variably shaped dark brown to black marks in adults. Smaller specimens (14.4–21.8 mm SL) with unspotted colouration and black narrow stripe along flank longitudinal midline. In specimens above about 25 mm SL, dark marks variably arranged and shaped, gradually changing in larger specimens, becoming more spotted and longitudinal midline stripe becoming fragmented or disappearing. Some specimens with dark marks arranged in three longitudinal zones (Fig. 7A–C), often forming large, coalesced blotches along dorsal region. In other specimens, longitudinal zones little or not distinct (Figs 4, 5, 7D, E), with spots being smaller and more scattered on flank in some specimens (Fig. 7E). Venter always white to yellowish white, paired fins hyaline. In smaller juveniles, about 14–40 mm SL (Fig. 6), fins hyaline, caudal fin with broad median black stripe anteriorly joined to flank midline stripe; in larger specimens,

unpaired fins gradually becoming faintly spotted on their bases, and caudal black stripe becoming inconspicuous in larger specimens (Figs 4, 5, 7).

Etymology. The name *araxa* is a reference to the occurrence of the new species in the region of Araxá, a historical Brazilian city founded in the 18th century, during the colonial period. The word *araxa* is possibly derived from the Tupi-Guarani to designate some native people formerly inhabiting the region.

Distribution and habitat notes. *Trichomycterus araxa* occurs in the Rio Quebra Anzol drainage, which is part of the Rio Paranaíba drainage, a main tributary of the upper Rio Paraná basin, in altitudes about 940–1020 m asl (Fig. 2). In the type locality area, the new species was collected in shallow, moderate fast-flowing streams, about 40–50 cm deep, and about 3–5 m wide, with a well-preserved marginal vegetation. The stream bottom comprised gravel, sand and pebbles. The new species was collected buried in the stream bank, but smaller specimens were sporadically seen swimming above gravel substratum. See Costa et al. (2022a) for a list of species occurring in this area, where this species is identified as *Trichomycterus* sp.

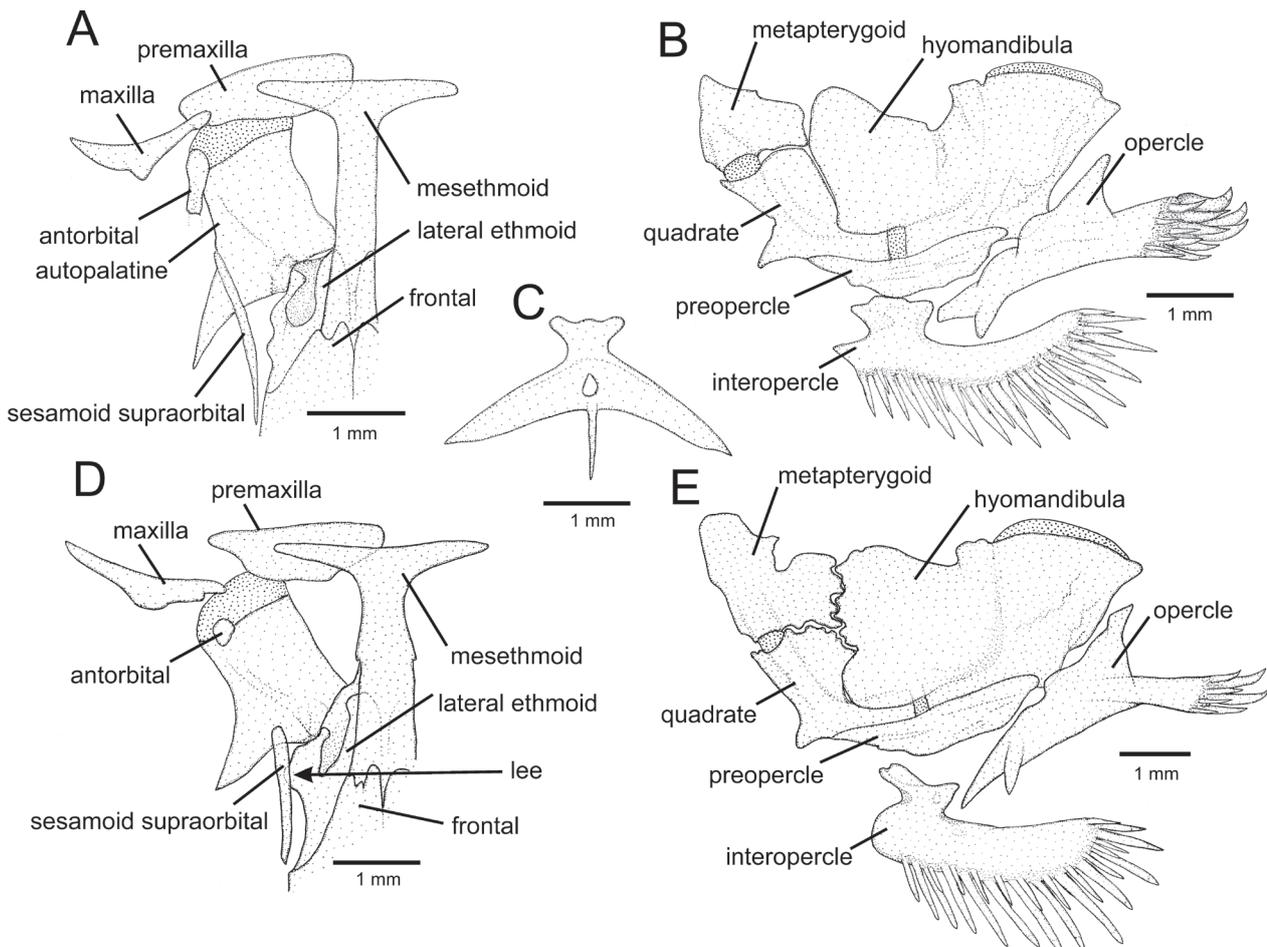


Figure 8. Osteological features in *Trichomycterus araxa* sp. nov. (A–C) and *T. macrotrichopterus* (D, E). A, D. Mesethmoidal region, middle and left portions, dorsal view; B, E. Left jaw suspensorium and opercular apparatus, lateral view; C. Parurohyal, ventral view. lee, lateral ethmoid expansion. Larger stippling represents cartilages.

Discussion

Phylogenetic relationships

Cryptocambeva occurs in a broad area of eastern South America, comprising different river basins and mountain ranges of south-eastern Brazil (Fig. 2; Costa 2021). *Trichomycterus araxa* is the second species of *Cryptocambeva* described from the Rio Paranaíba drainage. The first one was *Trichomycterus giarettai* Barbosa & Katz, 2016, occurring in another area of the drainage, at lower elevation, about 590 m asl (Barbosa and Katz 2016). Material of this species was not available for the present molecular phylogenetic analysis, but morphological data suggest that *T. giarettai* is closely related to *Trichomycterus candidus* (Miranda Ribeiro, 1949), not to *T. araxa*. *Trichomycterus giarettai* shares with *T. candidus* a thin premaxilla with few teeth (23–30), a condition that does not occur in *T. araxa* (i.e. 51–58 teeth) and in other species of *Cryptocambeva* and closely related subgenera, besides *T. giarettai* and *T. candidus* sharing a similar colour pattern, consisting of minute dark brown to black dots over a pale brownish yellow ground (Barbosa and Costa 2003: fig. 1; Barbosa and Katz 2016: fig. 1).

The phylogenetic analyses highly supported *T. araxa* as sister to *T. macrotrichopterus* (Fig. 1). However, the only morphological character state found to corroborate this sister group relationship was the presence of a long pectoral-fin filament, a condition not occurring in other species of *Cryptocambeva*, but sporadically occurring in other subgenera of *Trichomycterus* from eastern South America (Costa 1992). On the other hand, *T. araxa* does not exhibit any of the unique osteological features that are present in *T. macrotrichopterus*, including a prominent projection on the lateral margin of the lateral ethmoid (Fig. 8C), a strongly waved margin of the posterior margin of metapterygoid, anterior margin of hyomandibula anterior outgrowth and dorso-posterior margin of quadrate (Fig. 8D), and a distinctively slender opercular odontode patch (Fig. 8D), three conditions that do not occur in any other congener of the subgenus *Cryptocambeva*. In addition, it is remarkable that these species were found in different habitats, with *T. araxa* occurring in moderate fast-flowing streams with predominantly gravel bottom, and *T. macrotrichopterus* found in a fast-flowing river with large rocks on the bottom, just below waterfalls.

Timing of species diversification and biogeographical implications

Recent studies have shown a high concentration of closely related trichomycterine species in different South American regions, often exhibiting geographical distribution patterns restricted to some neighbouring river drainages around mountain and high plateau areas (e.g. Hayes et al. 2020; Costa et al. 2021c). However, the factors responsible for such distribution patterns and the high concentration of species in these areas have not been investigated. Herein we

tentatively associate the great species diversity of mountain trichomycterine catfishes concentrated between the headwaters of the Paraná and São Francisco basins with Cenozoic paleogeographic episodes that resulted in the present hydrographic configuration. The estimated age for the start of species diversification in *Cryptocambeva*, at the Middle Miocene, is chronologically compatible with the generalised uplift relief of south-eastern Brazil at this period as proposed by recent geological studies (Valadão 2009; Rezende et al. 2018). After an initial divergence during the Late Miocene, at about 8 Ma, separating *T. mimonha* lineage from another lineage comprising the most recent common ancestor of a clade containing all other species of *Cryptocambeva*, four main clades had their origins almost synchronically at about 6 Ma, still during the Late Miocene (Fig. 3). These clades are geographically disjunct, each one involving different mountain ranges and river basins (Figs 1, 2).

The divergence timing between *T. araxa* from the upper Rio Araguari and its sister group, *T. macrotrichopterus*, from the upper section of the main canal of the Rio São Francisco, at the Pliocene (Fig. 3), is also compatible with the period of intense fluvial re-arrangement reported for the region (Rezende et al. 2018). The upper Paranaíba uplift separating the Paraná and São Francisco river basins occurred in an older period, still in the Cretaceous (Campos and Dardenne 1997), but events of river segment captures changing river basin configuration in the upper Rio Paraná basin were frequent between the Middle Miocene and Pliocene (Rezende et al. 2018). Therefore, the divergence between *T. araxa* and *T. macrotrichopterus* estimated to have occurred at the Pliocene, is possibly derived from a drainage capture of the upper São Francisco by the upper Paranaíba drainage in the northeastern facet of the Serra da Canastra. This was a common paleogeographic event occurring in the region, well documented for the Rio Grande drainage, which captured a river drainage from the upper São Francisco basin (Rezende et al. 2018). However, this hypothesis should be still tested through the phylogenetic study of other fish groups inhabiting the same areas.

Acknowledgements

We are grateful to Valter M. Azevedo-Santos, for discussion about fish distribution patterns in the upper Paraná basin and help during type material collection. The manuscript benefitted from reviews provided by Francisco Langeani and Paulo Brito. Thanks are also due to the Meio Ambiente team of the Companhia Brasileira de Metalurgia e Mineração, for allowing collections and use of specimens from the farms comprising the RPPN São Sebastião and Monte Alto, and to Aléssio Datovo and Michel Gianeti by the loan of specimens. This work was partly supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant 304755/2020-6 to WJEMC), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; grant E-26/201.213/2021 to WJEMC, E-26/202.005/2020 to AMK, and E-26/202.327/2018 to JLM). This study was also supported by CAPES

(Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Finance Code 001) through Programa de Pós-Graduação em: Biodiversidade e Biologia Evolutiva/UFRJ; Genética/UFRJ; and Zoologia, Museu Nacional/UFRJ.

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Supplementary material 1

Tables S1, S2

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Data type: pdf file

Explanation note: **Table S1**. Terminal taxa for molecular phylogeny and respective GenBank accession numbers. **Table S2**. Best-fitting models.

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Link: <https://doi.org/10.3897/zse.98.83109.suppl1>

A new *Leptobrachella* species (Anura, Megophryidae) from South China, with comments on the taxonomic status of *L. chishuiensis* and *L. purpurus*

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Academic editor: Rafe Brown ♦ Received 18 August 2021 ♦ Accepted 16 May 2022 ♦ Published 3 June 2022

Abstract

A new species of Leaf Litter Toad, *Leptobrachella shimentaina* sp. nov., is described from the Shimentai and Luokeng nature reserves of northern Guangdong Province, southern China. The new taxon can be distinguished from all recognized congeners by a combination of discrete morphological character state differences relating to its small body size (SVL 26.4–28.9 mm in six adult males, 30.1 and 30.7 mm in two adult females); a number of apparently fixed color pattern character differences (including eye coloration and color pattern features from dorsal, ventral, and dorsolateral surfaces of its head, body, limbs, and ventrum); the morphological and discrete characteristics of the external phenotype (the skin texture of dorsum and ventrum, the presence of supra-axillary and ventrolateral glands, the wide dermal fringes and rudimentary webbing on toes, and the uninterrupted longitudinal ridges under toes). Two samples of this new species previously were proposed as representing a new, unnamed species. We now substantiate this claim by providing diagnostic comparisons of discrete character differences. In addition, we also discuss taxonomic uncertainty surrounding the identity of two congeners, *L. chishuiensis* and *L. purpurus*, which we interpret as indicative of taxonomic inflation in the species-rich subfamily Megophryidae.

Key Words

Leptobrachella shimentaina sp. nov., *L. chishuiensis*, *L. purpurus*, morphology, taxonomy

Introduction

The genus *Leptobrachella* Smith, 1925 recently was found to be paraphyletic with *Leptolalax* Dubois, 1983 based on a comprehensive molecular analysis combining fragments of mitochondrial and nuclear DNA markers by Chen et al. (2018). They suggested to synonymize two genera on account of their results clearly nested *Leptobrachella* within *Leptolalax*, though the type species *Leptobrachella mjobergi* Smith, 1925 was not included.

With 92 species, the forest-dependent genus *Leptobrachella* is widely distributed in southern China, Myanmar, northeastern India, Indochina region, Borneo and Natuna Island (Frost 2022). Species diversity in *Leptobrachella* may be underestimated, as suggested by 15 undescribed species proposed by Chen et al. (2018).

One of the most widely-distributed species, *Leptobrachella liui* (Fei & Ye, 1990) has been reported from Fujian, Guangdong, Guizhou, Hunan, and Zhejiang, provinces, Guangxi Zhuang Autonomous Region, and Hong Kong

SAR, China (Fei and Ye 1992; Fei et al. 2009; Fei et al. 2012). Later, Li et al. (2011) recognized *Leptobrachella* populations from Hong Kong and Guangdong (Xinyi City, Fengkai County, Shenzhen City) as the species *L. pelodytoides* (Boulenger, 1893). However, more recently, populations in Shenzhen of Guangdong and Hong Kong have been proposed as another species, *L. laui* (Sung, Yang & Wang, 2014), and those in Xinyi have been described as *L. yunkaiensis* Wang, Li, Lyu & Wang, 2018. Although the taxonomic status of the population from Fengkai, western Guangdong remained unresolved due to the lack of molecular data, another noteworthy lineage from Shimentai Nature Reserve, northern Guangdong was indicated by Chen et al. (2018), as a putatively undescribed species ("*Leptobrachella* sp. 6").

In this paper we evaluate discrete character state differences and phylogenetic relationships of seven additional specimens from Shimentai Nature Reserve and a single specimen from the adjacent Luokeng Nature Reserve (northern Guangdong, southern China Fig. 1), which substantiate the recognition of "*Leptobrachella* sp. 6" (Chen et al. 2018) as a distinctive new species.

Material and methods

Phylogenetic analyses

Eighteen new individuals were sequenced for phylogenetic analyses, and 71 sequences were obtained from

GenBank (Suppl. material 1: Table S1). Our sampling includes individuals from most recognized congeners from China and neighboring countries. The 16S ribosomal RNA mitochondrial gene (16S rRNA) fragment was sequenced for new samples; DNA extraction, PCR, and sequencing follow Wang et al. (2020).

Sequences were aligned with Clustal X 2.0 (Thompson et al. 1997) with default parameters. For GenBank sequences missing intervening sequence segments, we filled blank nucleotide positions "N" to indicate missing data. The aligned data was trimmed using default parameters and allowing no gap positions in Gblocks version 0.91b (Castresana 2000). We ran Jmodeltest v2.1.2 (Darriba et al. 2012; with Akaike and Bayesian information criteria) on our alignment and obtained the best-fitting nucleotide substitution model of GTR + I + G (General Time Reversible model, with variable sites modelled according to the Gamma distribution, and a portion of the sites invariant). Phylogenetic analysis was conducted using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012) and maximum likelihood (ML) in maximum likelihood (ML) in Randomized Axelerated Maximum Likelihood (RAxML, Stamatakis 2006) with RAxML GUI 1.3 (Silvestro and Michalak 2012). For the ML analysis, an optimal tree was obtained and branch supports were evaluated with 1000 rapid bootstrapping replicates. For the BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1000 iterations. The first 25% of the samples were discarded as burn-in, leaving 7500 samples

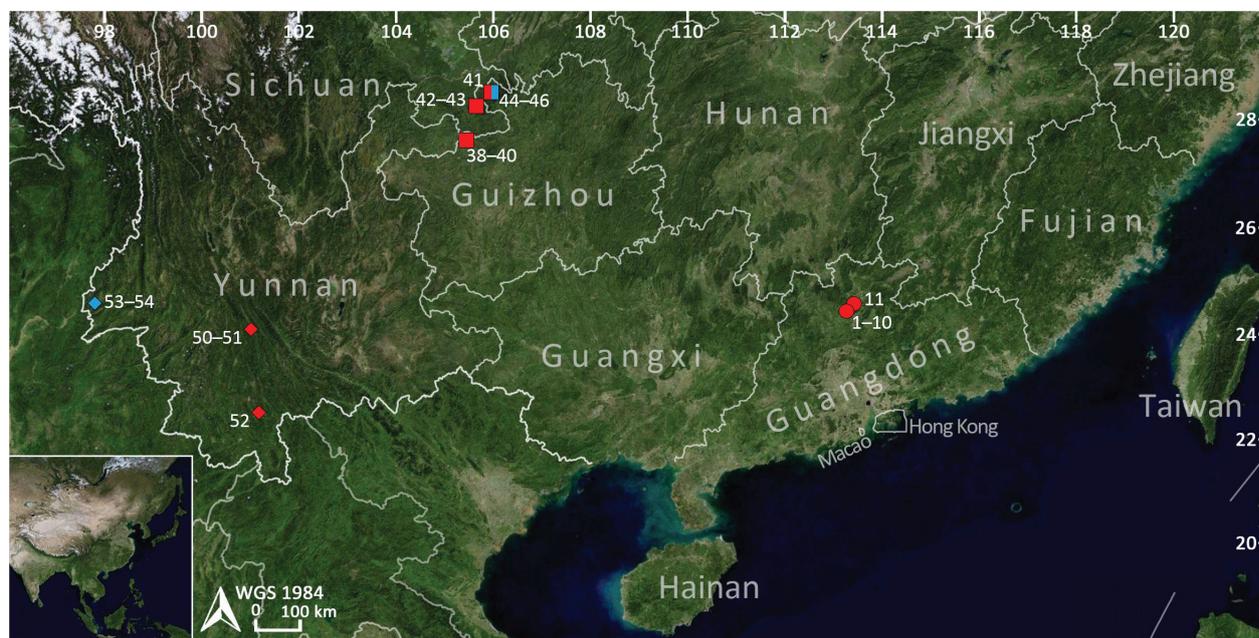


Figure 1. Localities of *Leptobrachella shimentaina* sp. nov. (samples ID 1–10, Shimentai Nature Reserve, Guangdong, China; sample ID 11, Luokeng Nature Reserve, Guangdong, China); *Leptobrachella bijie* (samples ID 38–40, Zhaozishan Nature Reserve, Guizhou, China; sample ID 41, Chishui, Guizhou, China; samples ID 42–43, Huagaoxi Nature Reserve, Guizhou, China); *Leptobrachella chishuiensis* (samples ID 44–46, Chishui, Guizhou, China); *Leptobrachella alpina* (samples ID 50–51, Mt Huangcaoling, Yunnan, China; sample ID 52, Pu'er, Yunnan, China); and *Leptobrachella purpurus* (samples ID 53–54, Yingjiang, Yunnan, China). Numbers correspond to the ID numbers in Suppl. material 1: Table S1.

in the final summary. Convergence of the Markov Chain Monte Carlo simulations was assessed by PSRF < 0.01 and ESS (effective sample size) value > 200 using Tracer 1.4 (<http://tree.bio.ed.ac.uk/software/tracer/>). Nodes having ML bootstrap values (BS) ≥ 70 and BI posterior probabilities (BPP) ≥ 0.95 were considered well supported.

Morphology

Our specimens of the putatively unnamed species were collected during opportunistic night searches. All specimens were euthanized, fixed in 5% buffered formalin for five hours, and then preserved in 70% ethanol, and subsequently deposited in The Museum of Biology, Sun Yat-sen University (SYS) and Chengdu Institute of Biology, Chinese Academy of Sciences (CIB), China.

Following Fei et al. (2009) and Rowley et al. (2013), measurements were taken with digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Calipers) to the nearest 0.1 mm. Measurements include: snout-vent length (SVL) from the tip of the snout to posterior margin of the vent; head length (HDL) from the tip of the snout

to the articulation of the jaw; head width (HDW) at the commissure of the jaws; snout length (SNT) from the tip of the snout to the anterior corner of the eye; eye diameter (EYE) from the anterior corner of the eye to posterior corner of the eye; internasal distance (IND); interorbital distance (IOD); horizontal diameter of tympanum (TMP); tympanum-eye distance (TEY) from the anterior edge of tympanum to posterior corner of the eye; tibial length (TIB) from the outer surface of the flexed knee to the heel; manus length (ML) from the tip of the third finger to the proximal edge of inner palmar tubercle; pes length (PE) from the tip of the fourth toe to the proximal edge of the inner metatarsal tubercle; length of lower arm and hand (LAHL) from the tip of the third finger to elbow; and hindlimb length (HLL) from the tip of the fourth toe to vent.

Sex was determined by direct observation of calling in life, the presence of internal vocal sac openings (males), or the presence of eggs, seen via external inspection of the abdomen (females). Comparative morphological data for other congeneric species of *Leptobranchella* were obtained from museum specimens (Appendix 1) and from the literature (Table 1).

Table 1. Data source of the currently known species of the genus *Leptobranchella*.

ID	<i>Leptobranchella</i> species	Literature obtained
1	<i>L. aerea</i> (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010c)	Rowley et al. 2010c
2	<i>L. aspera</i> Wang, Lyu, Qi & Wang, 2020	Wang et al. 2020
3	<i>L. alpina</i> (Fei, Ye & Li, 1990)	Fei et al. 1990; Fei et al. 2009, 2016
4	<i>L. applebyi</i> (Rowley & Cao, 2009)	Rowley and Cao 2009
5	<i>L. arayai</i> (Matsui, 1997)	Matsui 1997
6	<i>L. ardens</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
7	<i>L. baluensis</i> Smith, 1931	Dring 1983; Eto et al. 2016
8	<i>L. bashaensis</i> Lyu, Dai, Wei, He, Yuan, Shi, Zhou, Ran, Kuang, Guo, Wei & Yuan, 2020	Lyu et al. 2020a
9	<i>L. bijie</i> Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
10	<i>L. bidouensis</i> (Rowley, Le, Tran & Hoang, 2011)	Rowley et al. 2011
11	<i>L. bondangensis</i> Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
12	<i>L. botsfordi</i> (Rowley, Dau & Nguyen, 2013)	Rowley et al. 2013
13	<i>L. bourreti</i> (Dubois, 1983)	Ohler et al. 2011
14	<i>L. brevicrus</i> Dring, 1983	Dring 1983; Eto et al. 2015
15	<i>L. crocea</i> (Rowley, Hoang, Le, Dau & Cao, 2010)	Rowley et al. 2010a
16	<i>L. chishuiensis</i> Li, Liu, Wei & Wang, 2020	Li et al. 2020
17	<i>L. damingshanensis</i> Chen, Yu, Cheng, Meng, Wei, Zhou & Lu, 2021	Chen et al. 2021b
18	<i>L. dorsospina</i> Wang, Lyu, Qi & Wang, 2020	Wang et al. 2020
19	<i>L. dringi</i> (Dubois, 1987)	Inger et al. 1995; Matsui and Dehling 2012
20	<i>L. eos</i> (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
21	<i>L. feii</i> Chen, Yuan & Che, 2020	Chen et al. 2020
22	<i>L. firthi</i> (Rowley, Hoang, Dau, Le & Cao, 2012)	Rowley et al. 2012
23	<i>L. fritinniensis</i> (Dehling & Matsui, 2013)	Dehling and Matsui 2013
24	<i>L. fuliginosa</i> (Matsui, 2006)	Matsui 2006
25	<i>L. flaviglandulosa</i> Chen, Wang & Che, 2020	Chen et al. 2020
26	<i>L. fusca</i> Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
27	<i>L. gracilis</i> (Günther, 1872)	Günther 1872; Dehling 2012b
28	<i>L. hamidi</i> (Matsui, 1997)	Matsui 1997
29	<i>L. graminicola</i> Nguyen, Tapley, Nguyen, Luong & Rowley, 2021	Nguyen et al. 2021
30	<i>L. heteropus</i> (Boulenger, 1900)	Boulenger 1900
31	<i>L. isos</i> (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)	Rowley et al. 2015a
32	<i>L. itiokai</i> Eto, Matsui & Nishikawa, 2016	Eto et al. 2016
33	<i>L. juliandringi</i> Eto, Matsui & Nishikawa, 2015	Eto et al. 2015
34	<i>L. jinshaensis</i> Cheng, Shi, Li, Liu, Li & Wang, 2021	Cheng et al. 2021
35	<i>L. kjangensis</i> (Grismer, Grismer & Youmans, 2004)	Grismer et al. 2004
36	<i>L. kalonensis</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016

ID	<i>Leptobranchella</i> species	Literature obtained
37	<i>L. kecil</i> (Matsui, Belabut, Ahmad & Yong, 2009)	Matsui et al. 2009
38	<i>L. khasiorum</i> (Das, Tron, Rangad & Hooroo, 2010)	Das et al. 2010
39	<i>L. lateralis</i> (Anderson, 1871)	Anderson 1871; Humtsoe et al. 2008
40	<i>L. laui</i> (Sung, Yang & Wang, 2014)	Sung et al. 2014
41	<i>L. liui</i> (Fei & Ye, 1990)	Fei et al. 1990; Fei et al. 2009; Sung et al. 2014; this study
42	<i>L. macrops</i> (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)	Duong et al. 2018
43	<i>L. maculosa</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
44	<i>L. mangshanensis</i> (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018)	Hou et al. 2018; this study
45	<i>L. maoershanensis</i> (Yuan, Sun, Chen, Rowley & Che, 2017)	Yuan et al. 2017
46	<i>L. marmorata</i> (Matsui, Zainudin & Nishikawa, 2014b)	Matsui et al. 2014b
47	<i>L. maura</i> (Inger, Lakim, Biun & Yambun, 1997)	Inger et al. 1997
48	<i>L. melanoleuca</i> (Matsui, 2006)	Matsui 2006
49	<i>L. melica</i> (Rowley, Stuart, Neang & Emmett, 2010)	Rowley et al. 2010b
50	<i>L. minima</i> (Taylor, 1962)	Taylor 1962; Ohler et al. 2011
51	<i>L. mjobergi</i> Smith, 1925	Eto et al. 2015
52	<i>L. murphyi</i> Chen, Suwannapoom, Wu, Poyarkov, Xu, Pawangkhanant & Che, 2021	Chen et al. 2021a
53	<i>L. nahangensis</i> (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
54	<i>L. natunae</i> (Günther, 1895)	Günther 1895
55	<i>L. namdongensis</i> Hoang, Nguyen, Luu, Nguyen & Jiang, 2019	Hoang et al. 2019
56	<i>L. neangi</i> Stuart & Rowley, 2020	Stuart and Rowley 2020
57	<i>L. niveimontis</i> Chen, Poyarkov, Yuan & Che, 2020	Chen et al. 2020
58	<i>L. nokrekensis</i> (Mathew & Sen, 2010)	Mathew and Sen 2010
59	<i>L. nyx</i> (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
60	<i>L. oshanensis</i> (Liu, 1950)	Liu, 1950; Shi et al. 2021
61	<i>L. pallida</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
62	<i>L. palmata</i> Inger & Stuebing, 1992	Inger and Stuebing 1992
63	<i>L. parva</i> Dring, 1983	Dring 1983
64	<i>L. pelodytoides</i> (Boulenger, 1893)	Boulenger 1893; Ohler et al. 2011
65	<i>L. petrops</i> (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)	Rowley et al. 2017a
66	<i>L. picta</i> (Malkmus, 1992)	Malkmus 1992
67	<i>L. platycephala</i> (Dehling, 2012)	Dehling 2012a
68	<i>L. pluvialis</i> (Ohler, Marquis, Swan & Grosjean, 2000)	Ohler et al. 2000, 2011
69	<i>L. puhoatensis</i> (Rowley, Dau & Cao, 2017)	Rowley et al. 2017b
70	<i>L. purpurus</i> (Yang, Zeng & Wang, 2018)	Yang et al. 2018
71	<i>L. purpuraventra</i> Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
72	<i>L. pyrrops</i> (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)	Poyarkov et al. 2015
73	<i>L. rowleyae</i> (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)	Nguyen et al. 2018
74	<i>L. sabahmontana</i> (Matsui, Nishikawa & Yambun, 2014)	Matsui et al. 2014a
75	<i>L. serasanae</i> Dring, 1983	Dring 1983
76	<i>L. shangsiensis</i> Chen, Liao, Zhou & Mo, 2019	Chen et al. 2019
77	<i>L. sola</i> (Matsui, 2006)	Matsui 2006
78	<i>L. suiyangensis</i> Luo, Xiao, Gao & Zhou, 2020	Luo et al. 2020
79	<i>L. sungi</i> (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
80	<i>L. shiwandashanensis</i> Chen, Peng, Pan, Liao, Liu & Huang, 2021	Chen et al. 2021c
81	<i>L. tadungensis</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
82	<i>L. tamdil</i> (Sengupta, Sailo, Lalremsanga, Das & Das, 2010)	Sengupta et al. 2010
83	<i>L. tengchongensis</i> (Yang, Wang, Chen & Rao, 2016)	Yang et al. 2016
84	<i>L. tuberosa</i> (Inger, Orlov & Darevsky, 1999)	Inger et al. 1999
85	<i>L. ventripunctata</i> (Fei, Ye & Li, 1990)	Fei et al. 1990; Fei et al. 2009, 2016
86	<i>L. wuhuangmontis</i> Wang, Yang & Wang, 2018	Wang et al. 2018
87	<i>L. wulingensis</i> Qian, Xia, Cao, Xiao & Yang, 2020	Qian et al. 2020
89	<i>L. yingjiangensis</i> (Yang, Zeng & Wang, 2018)	Yang et al. 2018
90	<i>L. yunkaiensis</i> Wang, Li, Lyu & Wang, 2018	Wang et al. 2018
91	<i>L. yeae</i> Shi, Hou, Song, Jiang & Wang, 2021	Shi et al. 2021
92	<i>L. zhangyapingi</i> (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)	Jiang et al. 2013

Results

Essentially identical topologies were obtained by our ML and BI analyses (Fig. 2). In both phylogenetic estimates, all *Leptobranchella* samples formed a strongly-supported monophyletic clade. All *Leptobranchella* samples from populations from Shimentai and Luokeng nature reserves

form a monophyletic clade, without structure of within-population genetic divergence and with strong support (BPP = 1.00, BS = 100). The Shimentai and Luokeng lineage represents an independent lineage within *Leptobranchella*, relatively close to the lineages of *L. liui*, *L. mangshanensis*, *L. maoershanensis*, *L. bashaensis*, *L. laui*, and *L. yunkaiensis*. Nevertheless, support for the large clade

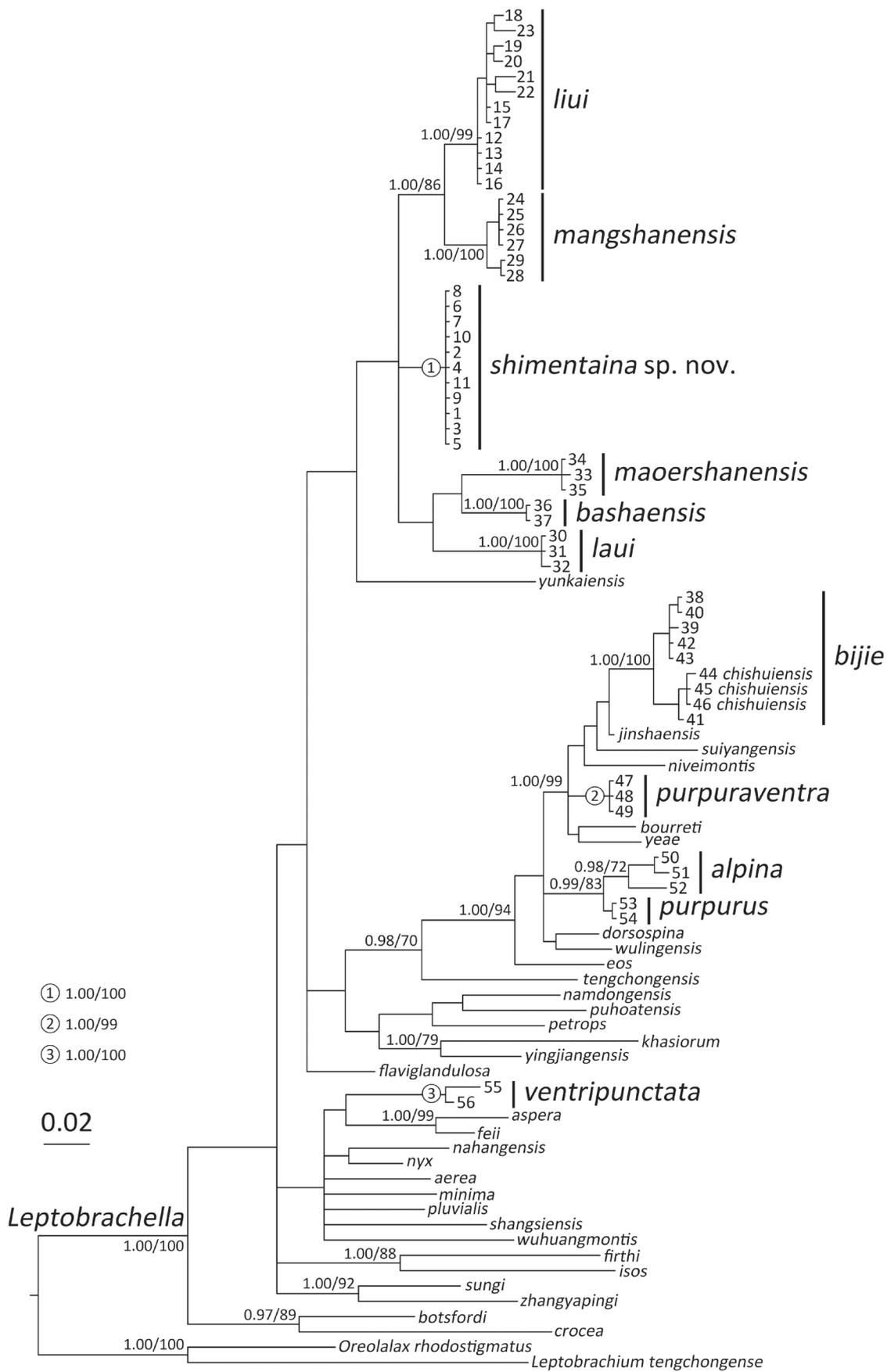


Figure 2. Bayesian inference tree derived from partial DNA sequences of the mitochondrial 16S r RNA gene. Numbers before slashes indicate Bayesian posterior probabilities and numbers after slashes are bootstrap support for maximum likelihood (1000 replicates) analyses. The number at the branch terminal corresponds to the ID in Suppl. material 1: Table S1.

including these species is insignificant, therefore, the relationships among these species are still unresolved. Detailed morphological examination has revealed discrete, diagnostic (non-overlapping ranges in traditional characters) differences among the specimens from this unnamed lineage and all other congeners. Our phylogenetic result and morphological comparison unanimously agree with the proposal that the Shimentai and Luokeng lineage represents an undescribed new species, i.e. “*Leptobranchella* sp. 6” in Chen et al. (2018). Therefore, based on the discrete and diagnostic morphological character differences, *Leptobranchella* populations from Shimentai and Luokeng nature reserves are described below as a new species.

Taxonomic account

Leptobranchella shimentaina J. Wang, Z.-T. Lyu & Y.-Y. Wang, sp. nov.

<http://zoobank.org/CF6EEF64-F539-415B-AD11-FA19C56BC3D5>
Shimentai Leaf Litter Toad (in English) / Shi Men Tai Zhang Tu Chan (石门台掌突蟾 in Chinese)
Figs 3–6

Chresonymy. “*Leptolalax* sp.6” (Chen et al. 2018).

Type material. *Holotype* ♂: SYS a007684, from Jiuzhou Yizhan (24°23'38.01"N, 113°06'35.38"E; ca. 300 m a.s.l.), Shimentai Nature Reserve, Yingde City, Guangdong Province, China, collected by Jian Wang and Yong-You Zhao on 23 April 2019.

Paratypes (n = 7). 5 ♂: SYS a007683/CIB116079, same collection place as holotype; SYS a007685–7686, collected by Yu-Long Li and Hong-Hui Chen on 23 April 2019 from Shijiao (24°26'35.31"N, 113°09'21.55"E; ca 400 m a.s.l.), Shimentai Nature Reserve; SYS a007687, collected by Jian Wang and Yong-You Zhao on 24 April 2019 from Longtankeng (24°26'31.38"N, 113°15'28.81"E; ca 310 m a.s.l.), Shimentai Nature Reserve; SYS a008329, collected by Jian Wang on 27 April 2020 from Luokeng Nature Reserve (24°31'42.3"N, 113°15'54.3"E; ca 590 m a.s.l.), Shaoguan City, Guangdong Province, China. 2 ♀: SYS a004711–4712, collected by Ying-Yong Wang, Jian Wang, and Zhi-Tong Lyu on 27 April 2016 from Hengshitang (24°26'14.92"N, 113°17'50.48"E; ca 380 m a.s.l.), Shimentai Nature Reserve.

Etymology. The specific epithet “*shimentaina*” is an adjective derived from “shimentai”, referring to the type locality of the new species, Shimentai Nature Reserve, “*ina*” is used as a feminine suffix which indicates the relationship of position.

Diagnosis. (1) small body size [SVL 26.4–28.9 mm in six adult males, SVL 30.1 and 30.7 mm in two adult females], (2) iris bicolored, upper half coppery orange and lower half silver, (3) tympanum distinct, (4) supratympanic line black, (5) fingers unwebbed, with distinct lateral fringes in males [absence in females], (6) toes with rudimentary webbing, lateral fringes wide in males [narrow in females], (7) longitudinal ridges under toes continuous, with constrictions at interphalangeal articulations, (8)

heels slightly overlapping when adpressed, tibial-tarsal articulation reaching mid-orbit, (9) relative lengths of fingers I = II = IV < III, and toe I < II < III = V < IV, (10) dorsal surface shagreened and granular, lacking enlarged warts, with some granules forming short longitudinal folds, (11) dorsum grayish brown to yellowish brown, with small light orange granules and distinct darker brown scattered markings with irregular light orange pigmentation, (12) flanks with several dark spots, (13) ventral surface grayish pink, with distinct hazy brown speckling on chest and ventrolateral flanks, (14) densely-distributed, small, raised pectoral and abdominal tubercles present; (15) dorsal surfaces of forelimbs and digits with dark transverse bars.

Description of holotype. Adult male. Body size small, SVL in 28.6 mm. Head length slightly larger than head width, HDW/HDL 0.91; snout slightly protruding, projecting slightly beyond margin of lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, internarial distance larger than interorbital distance, IND/IOD 1.10; pineal ocellus absent; pupil vertical; snout longer than eye diameter, SNT/EYE 1.19; tympanum distinct, rounded, diameter smaller than that of eye and larger than tympanum-eye distance, TMP/EYE 0.41, TEY/TMP 0.48; upper margin of tympanum in contact with supratympanic ridge; vomerine teeth absent; a single vocal sac; vocal sac openings slit-like, paired, located posterolaterally on floor of mouth, close to margins of mandible; tongue deeply notched posteriorly; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths I = II = IV < III; nuptial pad absent; subarticular tubercles absent; inner palmar tubercle large, rounded, distinctly separated from outer palmar tubercle; outer palmar tubercle small, rounded; fingers lacking interdigital webbing, with distinct lateral fringes. Tips of toes rounded, slightly swollen; relative toe length I < II < III = V < IV; longitudinal ridges under toes continuous, with constrictions at interphalangeal articulations; inner metatarsal tubercle large, oval; outer metatarsal tubercle absent; interdigital webbing between toes rudimentary; wide lateral fringes present on all toes. Tibia 47% of snout-vent length; tibiotarsal articulation reaches to middle of eye; heels slightly overlapping when thighs are adpressed at right angles with respect to body.

Dorsal surface shagreened and granular, lacking enlarged tubercles or warts, some granules forming short longitudinal folds; ventral skin smooth, densely-distributed small raised scapular and abdominal tubercles present; pectoral and femoral glands oval; both larger in diameter than tip of fingers; femoral glands larger in diameter than tip of toes; femoral gland situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary gland raised. Ventrolateral gland distinctly visible, forming an longitudinal uncontinuous series.

Coloration of holotype in life. Dorsal background color yellowish-brown, with small orange granules, distinct dark brown markings, and rounded spots, and scattered

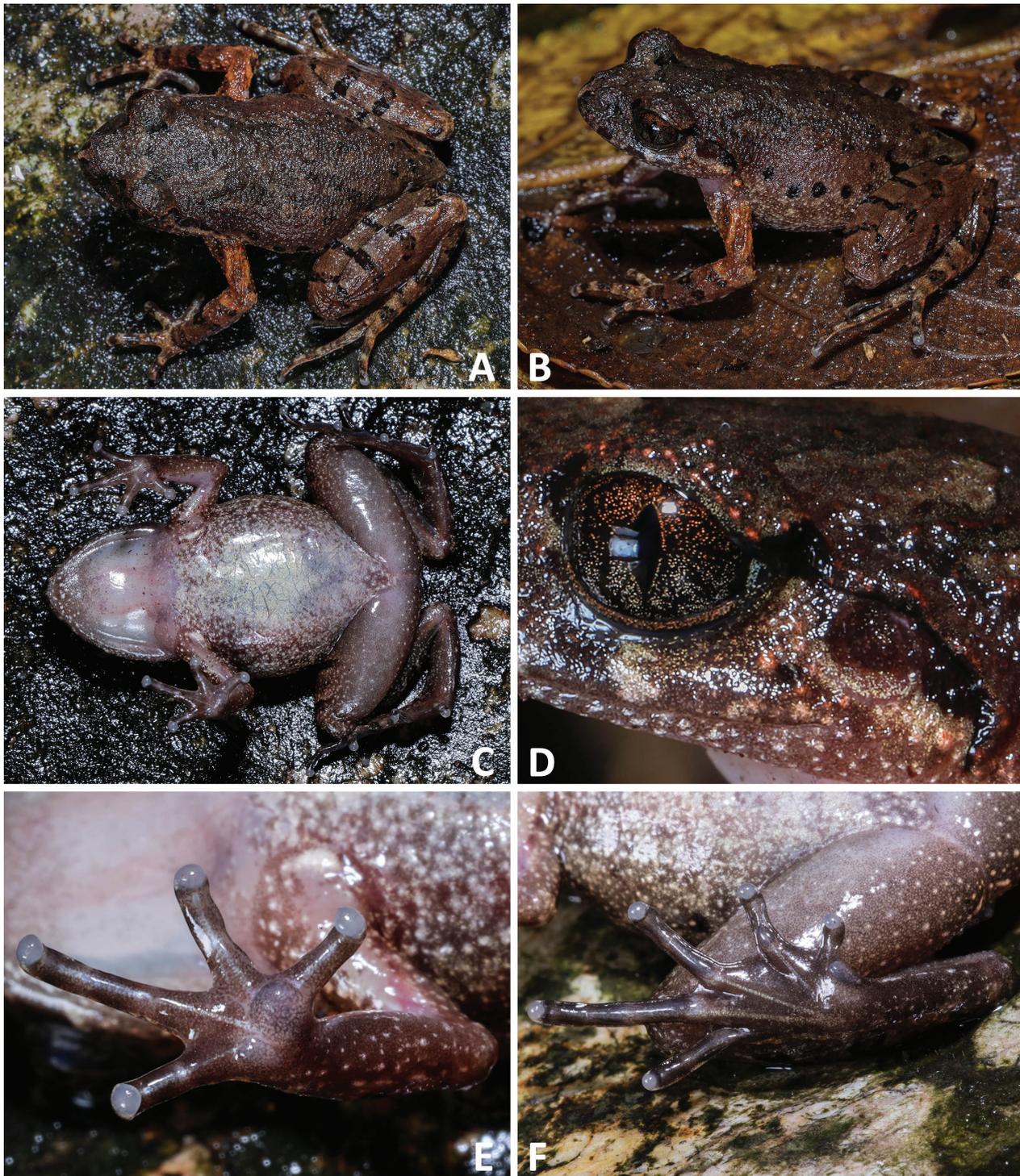


Figure 3. Morphological features of the holotype of *Leptobrachella shimentaina* sp. nov., SYS a007684 in life: **A.** Dorsal view; **B.** Dorsolateral view; **C.** Ventral view; **D.** Character of eye and temporal region; **E.** Ventral view of hand; **F.** Ventral view of foot.

with irregular light grayish-brown pigmentation. A dark brown inverted triangular marking present between anterior corners of eyes, connecting to dark brown W-shaped marking in interorbital region. This W-shaped marking is in connection to the other W-shaped marking on occipital region. Tympanum dark brown, lower margin grayish yellow. Supratympanic line black. Dorsal surfaces of body and limbs with small orange granules; a pair of dark brown vertical bars present under eyes; transverse dark

brown bars on dorsal surfaces of distal limbs and digits; distinct dark brown blotches on flanks; surfaces of elbows and upper arms coppery orange, without dark bars.

Surface of throat, chest, and belly grayish pink, with distinct hazy brown speckling on chest and ventrolateral flanks, without black spots; surface of chin and lower lip brown with grayish white spots and patches; ventral surface of limbs brown with pink hues. Supra-axillary gland coppery orange, pectoral glands grayish white, femoral



Figure 4. The holotype of *Leptobrachella shimentaina* sp. nov., SYS a007684 in preservative.

glands coppery orange, ventrolateral glands brown. Iris bicolored, upper half coppery orange, lower half silver.

Coloration of holotype in preservative (Fig. 4). Dorsal background color grayish brown, scattered with irregular light grayish-brown pigmentation. All markings, bars and spots become more distinct. Tympanum dark brown, lower margin gray.

Ventral surface yellowish brown; speckling on chest and ventrolateral flanks become more distinct; ventral surface of limbs dark brown. Supra-axillary, pectoral, and ventrolateral glands greyish yellow.

Variation. Measurements and body proportions are listed in Table 2. All male paratypes from Shimentai Nature Reserve (SYS a007683/CIB116079, SYS a007685 (Fig. 5B), 7686 (Fig. 5C), 7687) show very similar morphological characters to holotype SYS a007684; paratype SYS a008329 (Fig. 6) from Luokeng Nature Reserve shows a darker coloration on dorsum, and brighter coloration on ventrum. Moreover, female paratypes, SYS a004711 (Fig. 5A) and SYS a004712 show lighter background coloration and more obvious dark brown marking orange tubercles on back.

Table 2. Measurements (minimum–maximum (mean ± SD); in mm), and body proportions of *Leptobrachella shimentaina* sp. nov.

Vocher	SYS a007683	SYS a007684	SYS a007685	SYS a007686	SYS a007687	SYS a008329	Range	Vocher	SYS a004711	SYS a004712	Range
Sex	Male	Male	Male	Male	Male	Male	Males (n = 6)	Sex	Female	Female	Females (n = 2)
SVL	28.9	28.6	28.4	28.7	28.4	26.4	26.4–28.9 (28.2 ± 0.9)	SVL	30.1	30.7	30.1–30.7 (30.4 ± 0.4)
HDL	11.7	10.7	10.8	10.9	10.8	9.8	9.8–11.7 (10.8 ± 0.6)	HDL	10.1	10.4	10.1–10.4 (10.3 ± 0.2)
HDW	9.7	9.7	9.7	9.7	9.7	9.2	9.2–9.7 (9.6 ± 0.2)	HDW	9.7	9.9	9.7–9.9 (9.8 ± 0.2)
SNT	4.3	4.2	4.1	4.3	4.2	3.3	3.3–4.3 (4.1 ± 0.4)	SNT	4.0	4.3	4.0–4.3 (4.1 ± 0.2)
IND	2.9	2.7	2.9	2.8	2.7	2.9	2.7–2.9 (2.8 ± 0.1)	IND	2.7	2.8	2.7–2.8 (2.7 ± 0.1)
IOD	2.6	2.5	2.5	2.5	2.5	2.6	2.5–2.6 (2.5 ± 0.1)	IOD	2.7	2.8	2.7–2.8 (2.7 ± 0.0)
EYE	3.5	3.5	3.4	3.4	3.4	3.3	3.3–3.5 (3.4 ± 0.1)	EYE	3.5	3.6	3.5–3.6 (3.5 ± 0.1)
TMP	1.5	1.5	1.5	1.5	1.5	1.5	1.5–1.5 (1.5 ± 0.0)	TMP	1.5	1.8	1.5–1.8 (1.7 ± 0.2)
TEY	0.7	0.7	0.7	0.7	0.7	0.6	0.6–0.7 (0.7 ± 0.0)	TEY	0.6	0.9	0.6–0.9 (0.8 ± 0.2)
ML	7.4	7.2	7.2	7.4	7.3	6.9	6.9–7.4 (7.2 ± 0.2)	ML	7.2	7.8	7.2–7.8 (7.5 ± 0.4)
LAHL	14.4	14.0	14.4	14.5	14.4	12.5	12.5–14.5 (14.0 ± 0.8)	LAHL	14.1	15.0	14.1–15.0 (14.6 ± 0.6)
PL	12.6	12.6	13.0	12.2	12.6	11.9	11.9–13.0 (12.5 ± 0.4)	PL	13.1	12.5	12.5–13.1 (12.8 ± 0.4)
TIB	14.2	13.3	13.3	13.3	13.4	12.4	12.4–14.2 (13.3 ± 0.6)	TIB	14.1	14.6	14.1–14.6 (14.3 ± 0.4)
HLL	45.1	44.5	44.5	45.1	44.9	39.61	39.6–45.1 (43.9 ± 2.1)	HLL	44.4	45.6	44.4–45.6 (45.0 ± 0.9)
HDL/ SVL	0.40	0.37	0.38	0.38	0.38	0.37	0.37–0.40 (0.38 ± 0.01)	HDL/ SVL	0.34	0.34	0.34–0.34 (0.34 ± 0.00)
HDW/ SVL	0.34	0.34	0.34	0.34	0.34	0.35	0.34–0.35 (0.34 ± 0.00)	HDW/ SVL	0.32	0.32	0.32–0.32 (0.32 ± 0.00)
HDW/ HDL	0.83	0.91	0.90	0.89	0.90	0.94	0.83–0.94 (0.90 ± 0.03)	HDW/ HDL	0.95	0.95	0.95–0.95 (0.95 ± 0.00)
SNT/ HDL	0.37	0.39	0.38	0.39	0.39	0.34	0.34–0.39 (0.38 ± 0.02)	SNT/ HDL	0.39	0.41	0.39–0.41 (0.40 ± 0.02)
IND/ HDW	0.30	0.28	0.29	0.28	0.28	0.31	0.28–0.31 (0.29 ± 0.01)	IND/ HDW	0.28	0.28	0.28–0.28 (0.28 ± 0.01)
IOD/ HDW	0.27	0.25	0.26	0.25	0.25	0.29	0.25–0.29 (0.26 ± 0.01)	IOD/ HDW	0.28	0.28	0.28–0.28 (0.28 ± 0.00)
EYE/ HDL	0.30	0.33	0.31	0.31	0.32	0.34	0.30–0.34 (0.32 ± 0.01)	EYE/ HDL	0.34	0.35	0.34–0.35 (0.34 ± 0.00)
TMP/ EYE	0.42	0.41	0.43	0.43	0.43	0.45	0.41–0.45 (0.43 ± 0.01)	TMP/ EYE	0.44	0.50	0.44–0.50 (0.47 ± 0.04)
ML/SVL	0.25	0.25	0.25	0.26	0.26	0.26	0.25–0.26 (0.26 ± 0.00)	ML/ SVL	0.24	0.25	0.24–0.25 (0.25 ± 0.01)
LAHL/ SVL	0.50	0.49	0.51	0.50	0.51	0.47	0.47–0.51 (0.50 ± 0.01)	LAHL/ SVL	0.47	0.49	0.47–0.49 (0.48 ± 0.01)
PL/SVL	0.43	0.44	0.46	0.42	0.44	0.45	0.42–0.46 (0.44 ± 0.01)	PL/ SVL	0.43	0.41	0.41–0.43 (0.42 ± 0.02)
TIB/SVL	0.49	0.47	0.47	0.46	0.47	0.47	0.46–0.49 (0.47 ± 0.01)	TIB/ SVL	0.47	0.47	0.47–0.47 (0.47 ± 0.01)
HLL/SVL	1.56	1.55	1.57	1.57	1.58	1.50	1.50–1.58 (1.55 ± 0.03)	HLL/ SVL	1.47	1.48	1.47–1.48 (1.48 ± 0.01)

Distribution and ecology. *Leptobrachella shimentaina* sp. nov. is known from its type locality Shimentai Nature Reserve, Yingde City, and the adjacent Luokeng Nature Reserve, Shaoguan City, Guangdong, China (Fig. 1; ca. 300–600 m a.s.l.). In the Hengshitang Protection Station of the Shimentai Nature Reserve, the new species is sympatric with *L. mangshanensis*. The new species inhabits primitive clear-water rocky streams surrounded by broad-leaved forest in karst landforms or granite landforms. From April to June, males were observed calling while perched on the rocks or under rocks in flowing streams, and females possessed mature oocytes.

Comparisons. In our phylogenetic analyses (Fig. 2, Table 3) *Leptobrachella shimentaina* sp. nov. clusters with *L. bashaensis*, *L. laui*, *L. liui*, *L. mangshanensis*, *L. maoershanensis*, and *L. yunkaiensis*, thus, these six species constitute the most important comparisons, for the purpose of diagnosis and recognition of the new species. In having wide fringes along lateral surfaces of toes in males, it can be distinguished from *L. bashaensis*, *L. mangshanensis* and *L.*

maoershanensis (vs. lateral fringes narrow); by ventral coloration, grayish pink with distinct hazy brown speckling on chest and ventrolateral flanks; it can be distinguished from *L. bashaensis* (vs. creamy-white chest and belly with irregular black spots), *L. laui* (vs. creamy white with dark brown dusting on ventrolateral flanks), *L. liui* (vs. creamy white with dark brown spots on chest and ventrolateral flanks), *L. maoershanensis* (vs. creamy white chest and belly with irregular black spots), and *L. yunkaiensis* (vs. belly pink with distinct or indistinct speckling); by having dorsal skin with round granular tubercles and glandular folds, the new species can be further distinguished from *L. laui* (vs. dorsal skin lacking glandular folds), *L. mangshanensis* (vs. dorsal skin almost smooth with tiny transparent spines), and *L. yunkaiensis* (vs. dorsal skin shagreened with short skin ridges and raised warts); by dense small raised tubercles present on surface of chest and both sides of abdomen; it can be further distinguished from *L. mangshanensis*, *L. liui*, *L. yunkaiensis*, *L. maoershanensis* and *L. laui* (vs. ventral surface smooth); by having longitudinal ridges under toes



Figure 5. Morphological features of the paratypes of *Leptobrachella shimentaina* sp. nov.: **A1.** A female (SYS a004711 in situ; ventral view of the same specimen's hand [A2] and foot [A3]; **B.** Male SYS a007685 in situ; **C.** Male SYS a007686 in situ.

Table 3. Comparisons of selected diagnostic characters for the new species and its phylogenetically related congeners.

<i>Leptobrachella</i> spp.	Male SVL (mm)	Toes webbing	Fringes on toes	Longitudinal ridges under toes (interrupt + / not interrupt -)	Ventral coloration	Dorsal skin texture
<i>L. shimentaina</i> sp. nov.	26.4–28.9	Rudimentary	Wide in males	-	Grayish pink with distinct hazy brown speckling on chest and ventrolateral flanks	Round granular tubercles with glandular folds
<i>L. laui</i>	24.8–26.7	Rudimentary	Wide	-	Creamy white with dark brown dusting on ventrolateral flanks	Round granular tubercles
<i>L. liui</i>	23.0–28.7	Rudimentary	Wide	+	Gray white to creamy white with dark brown spots on chest and ventrolateral flanks	Shagreened with numerous small tubercles, round warts and sparse short skin ridges
<i>L. mangshanensis</i>	22.2–27.8	Rudimentary	Narrow	-	Creamy white belly, scattered with white speckles	Almost smooth with tiny transparent spines, small tubercles and sparse short skin ridges
<i>L. maoershanensis</i>	25.2–30.4	Rudimentary	Narrow	-	Creamy white chest and belly with irregular black spots	With longitudinal folds
<i>L. bashaensis</i>	22.9–25.6	Rudimentary	Narrow	-	Creamy-white chest and belly with irregular black spots	Slightly shagreened with small tubercles
<i>L. yunkaiensis</i>	25.9–29.3	Rudimentary	Wide	-	Belly pink with distinct or indistinct speckles	Shagreened with short skin ridges and raised warts

continuous, with constrictions at interphalangeal articulations, it can be distinguished from *L. laui* (vs. longitudinal ridges without constrictions at interphalangeal articulations), *L. liui* (vs. longitudinal ridges interrupted at inter-

phalangeal articulations), *L. maoershanensis* (vs. longitudinal ridges indistinct and not interrupted at interphalangeal articulations), and *L. yunkaiensis* (vs. longitudinal ridges without constrictions at interphalangeal articulations).

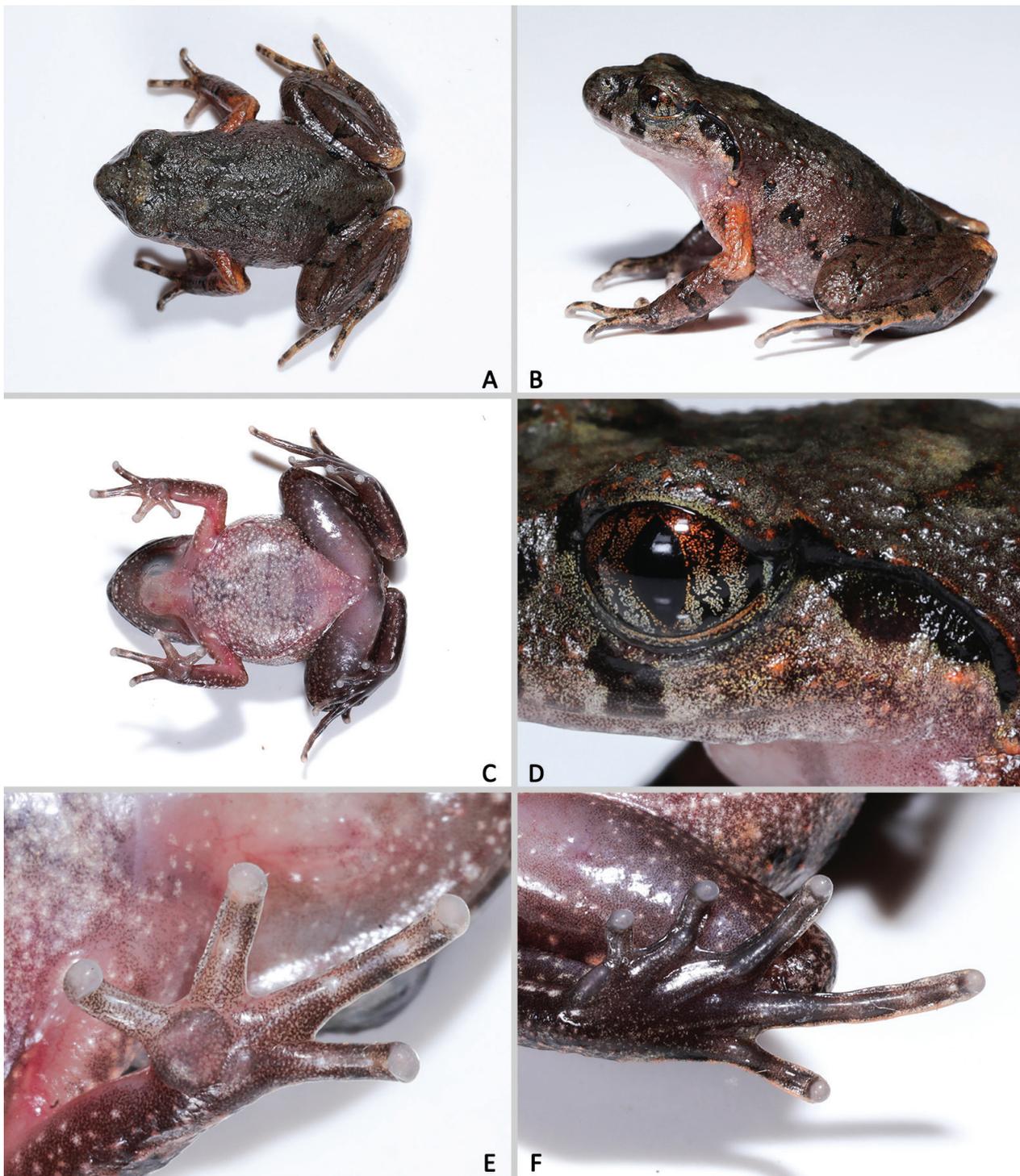


Figure 6. Morphological features of a paratype (SYS a007687) of *Leptobranchella shimentaina* sp. nov. in life.

Compared with the 26 known congeners of the genus *Leptobranchella* occurring south of the Isthmus of Kra, by the presence of supra-axillary and ventrolateral glands, *L. shimentaina* sp. nov. can easily be distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kjangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola*, all of which lack supra-axillary and ventrolateral glands; and by its distinctly larger male body size, SVL 26.4–28.9 mm, *L. shimentaina* sp. nov. differs from the smaller *L. baluensis* (14.9–15.9),

L. brevicrus (17.1–17.8), *L. bondangensis* (17.8), *L. fusca* (16.3), *L. itiokai* (15.2–16.7s), *L. juliandringi* (17.0–17.2), *L. mjobergi* (15.7–19.0), *L. natunae* (17.6), *L. parva* (15.0–16.9), and *L. palmata* (14.4–16.8); the female of *L. serasanae* (16.9), is also smaller than *L. shimentaina* sp. nov. (females 30.1–30.7).

For the remaining 60 members of the genus *Leptobranchella*, males *L. shimentaina* sp. nov. (SVL 26.4–28.9 mm) differs from males of the larger *L. chishuiensis* (30.8–33.4), *L. damingshanensis* (33.6–34.4), *L. eos* (33.1–34.7), *L. graminicola* (23.1–24.6), *L. neangi*

(30.9), *L. nahangensis* (40.8), *L. pyrrhops* (30.8–34.3), *L. sungi* (48.3–52.7), *L. tamdil* (32.3), and *L. zhangyapingi* (45.8–52.5); and from the smaller *L. aspera* (22.4), *L. applebyi* (19.6–22.3), *L. ardens* (21.3–24.7), *L. bidoupensis* (18.5–25.4), *L. feii* (21.5–22.8), *L. melica* (19.5–22.7), *L. murphyi* (23.2–24.9), *L. niveimontis* (22.5–23.6), *L. pluvialis* (21.3–22.3), and *L. rowleyae* (23.4–25.4). Through its possession of toes with rudimentary webbing and with wide lateral fringes in males, the new species can be distinguished from *L. bijie*, *L. dorsospina*, *L. flaviglandulosa*, *L. jinshaensis*, *L. purpuraventra*, *L. puhoatensis*, *L. shangsiensis*, *L. suiyanensis*, *L. tengchongensis*, *L. wuhuangmontis* and *L. yeae* (lateral fringes narrow), *L. bourreti*, *L. fuliginosa* (lateral fringes weak), *L. jinshaensis*, *L. kalonensis*, *L. maculosa*, *L. oshanensis*, *L. shiwandashan*, *L. tadungensis*, *L. ventripunctata* (no webbing or lateral fringes), *L. lateralis*, *L. namdongensis*, *L. macrops*, *L. minima*, *L. nyx* (no lateral fringes), and *L. pelodytoides* (extensive webbing and narrow lateral fringes), and additionally from *L. alpina*, *L. khasiorum*, *L. nokrekensis*, *L. yingjiangensis* (ventral coloration creamy white) and *L. purpurus* (ventral coloration dull white) by having greyish pink ventral coloration. By having black spots on flanks, it further differs from *L. aerea*, *L. botsfordi*, *L. crocea*, *L. firthi*, *L. isos*, *L. pallida*, *L. petrops*, and *L. tuberosa*, all of which lack black spots on the flanks.

Discussion

Studies of taxonomy and species diversity of the family Megophryidae have been challenged by morphological conservativeness among the majority of species in this clade (Rowley et al. 2015b; Wang et al. 2019). In recent years, the approach of integrative taxonomy has made substantial progress with this species-rich group, particularly with regards to our understanding of species boundaries in the genera *Leptobranchella* Smith, 1925 and *Boulenophrys* Fei, Ye & Jiang, 2016 (Lyu et al. 2021; Qi et al. 2021; AmphibiaWeb 2022; Frost 2022). Nonetheless, as more and more new species have been proposed, primarily based on molecular data (Chen et al. 2017, 2018; Mahony et al. 2017; Liu et al. 2018), detailed analyses of morphological characteristics, bioacoustics data, ecological information or other natural history data must also be provided to independently substantiate these claims (Lyu et al. 2020b, 2021). It is important that researchers remain vigilantly conservative and avoid taxonomic inflation within this species-rich family, particularly in light of geographically sparse, inadequate, poorly sampled surveys and increasingly atomized taxonomic partitioning in the absence of new data.

Leptobranchella bijie was described based on a single population from Bijie, northwestern Guizhou (Wang et al. 2019). Li et al. (2020) subsequently proposed *L. chishuiensis* from Chishui, northwestern Guizhou, and most recently, Wang et al. (2021) reported *L. bijie* from

Huagaoxi Nature Reserve, southeastern Sichuan (Fig. 1). In our analysis, we found *Leptobranchella* populations from these three neighboring localities to cluster together with only very minor divergences and with strong support for their monophyly (BPP = 1.00 and BS = 100; Fig. 2). Diagnostic morphological character differences are quite subtle between *L. bijie* and *L. chishuiensis* (Li et al. 2020), and slight apparent “differences” may amount from human measurement error, such as snout-vent length of males (29.0–30.4 mm in *L. bijie* vs. SVL 30.8–33.4 in *L. chishuiensis*) and the internasal versus interorbital distances (IND = IOD in *L. bijie* vs. IND > IOD in *L. chishuiensis*). The specimen SYS a004955 (sample ID 41 in our Figs 1, 2) from Chishui is clustered with the type specimens of *L. chishuiensis* (without appreciable genetic divergence), however, its morphological characters inversely match those of *L. bijie*. For instance, its heels are just meeting when thighs are positioned at right angles to the body (vs. heels overlapping in *L. chishuiensis*), the tibia-tarsal articulation reaches forward to the middle of the eye (vs. tympanum to eye in *L. chishuiensis*). Li et al. (2020) performed bioacoustic analysis to support proposed taxonomic validity of *L. chishuiensis*, however, this study confused note and call intervals (comparing note interval in *L. chishuiensis* to call interval of *L. bijie*), which resulted in one apparently erroneous stated diagnostic “difference” (a shorter call interval in *L. chishuiensis*). The other difference (2 notes for *L. bijie* vs. 1–4 notes for *L. chishuiensis*) has been suggested to be only differences between populations rather than between species (Weaver et al. 2020). Geographically, the newly discovered population of *L. bijie* from southeastern Sichuan is much closer to the type locality of *L. chishuiensis* and any distinct barriers in geography (which might promote divergence) are absent. Therefore, we suspect *L. chishuiensis* should most likely be considered conspecific (and be synonymized) with *L. bijie*.

We also noticed the close relationship between the congeners *Leptobranchella alpina* and *L. purpurus*, leading to the problem of the taxonomic validity of *L. purpurus*. The latter species was described by Yang et al. (2018) based on two specimens, without comparison to the molecular data of *L. alpina*. However, whereas molecular data corresponding to *L. alpina* were provided (Chen et al. 2018), corresponding data for *L. purpurus* were not included. In our phylogenetic analysis, both “species” clustered together, with minimal “interspecific” divergences, and with inconsistent support for their monophyly (BPP = 0.99, BS = 83; Fig. 2). Accessible voucher material for these two species is limited (two males for *L. purpurus* and three males for *L. alpina*), and reported type localities of the two species are distant from each other with a distance over 340 km (Fig. 1). Therefore, we refrain from making taxonomic changes to *L. purpurus* until further studies, based on geographically comprehensive surveys, with robust, statistically-valid sample sizes, are conducted throughout southwestern Yunnan Province, to clarify or refute species’ boundaries involving these two taxa.

Acknowledgements

We would like to thank Guangdong Shimentai Nature Reserve, Guo-Xin Guo, Yang-Jin Zeng, Ming Kong, Ming-Ke Xu, Ming-Zhu Huang, Tai-Yang Zhou, Gui-Li Fan, Tian-Du Zhang, Di-Hao Wu, Run-Lin Li, and Hai-Long He, for their help with fieldwork, and Yao Li for her help in the lab work. We thank Rafe M. Brown, Kin Onn Chan, and the anonymous reviewer for their constructive comments on the manuscript. This work was supported by the Project of Fauna of Guangdong (DFGP202115), the National Animal Collection Resource Center, China, and the Project of Animal Diversity Survey and Monitoring System Construction of Guangdong Shimentai National Nature Reserve.

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Appendix 1

Specimens examined:

- Leptobranchella alpina* (n = 3):** China: Yunnan: Jingdong County: Mt. Wuliang: CIB 24353–24354; SYS a 003927.
- Leptobranchella bijie* (n = 9):** China: Guizhou: Bijie City: SYS a007313–7320; China: Guizhou: Chishui City: SYS a004955.
- Leptobranchella laui* (n = 26):** China: Hong Kong: SYS a002057 (Holotype), SYS a002058; China: Guangdong: Shenzhen City: SYSa 001505–1507, 1515–1521, 3471–3472, 5644–5645.
- Leptobranchella liui* (n = 32):** China: Fujian: Mt. Wuyi: SYS a001572, 1596, 2478, 2479, 5925, 5926; China: Fujian: Mt. Daiyun: SYS a001736, 6010; China: Fujian: Mt. Longqi: SYS a002505, 2506; China: Guangdong: Mt. Tongguzhang: SYS a004733–4735; China: Guangdong: Mt. Fenghuang: SYS a003698–3699; China: Guangdong: Mt. Motianshi: SYS a007610–7613; China: Guangdong: Mt. Nankun: SYS a002020, 4497; China: Guangdong: Gutian Nature Reserve: SYS a002650; China: Jiangxi: Mt. Jiulian: SYS a002104–2105; China: Jiangxi: Mt. Tongbo: SYS a001702, 2059; China: Jiangxi: Mt. Yangjifeng: SYS a006667, 6672; China: Zhejiang: Jingning: SYS a002732–2735.
- Leptobranchella mangshanensis* (n = 11):** China: Hunan: Mangshan Nature Reserve: SYS a008366; China: Guangdong: Nanling Nature Reserve: SYS a002828–2830, 5754; China: Guangdong: Shimentai Nature Reserve: SYS a005763, 6880; China: Guangdong: Mt. Tianjing: SYS a002806, 2809; China: Guangdong: Mt. Dadong: SYS a002847–2848.
- Leptobranchella purpuraventra* (n = 15):** China: Guizhou: Bijie City: SYS a007277–7284, 7300–7306.
- Leptobranchella yunkaiensis* (n = 8):** China: Guangdong: Maoming City: Dawuling Forestry Station: SYS a004664/CIB107272, SYS a004663, 4665–4669, 4690.

Supplementary material 1

Table S1

- Authors: Jian Wang, Shuo Qi, Ke-Yuan Dai, Zhi-Tong Lyu, Zhao-Chi Zeng, Hong-Hui Chen, Yuan-Qiu Li, Yong-You Zhao, Yun-Ze Wang, Ying-Yong Wang
- Data type: excel file
- Explanation note: Collection localities, voucher data, and Genbank numbers (16S rRNA) for all *Leptobranchella* samples used in this study.
- Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: <https://doi.org/10.3897/zse.98.73162.suppl1>

Supplementary material 2

Table S2

- Authors: Jian Wang, Shuo Qi, Ke-Yuan Dai, Zhi-Tong Lyu, Zhao-Chi Zeng, Hong-Hui Chen, Yuan-Qiu Li, Yong-You Zhao, Yun-Ze Wang, Ying-Yong Wang
- Data type: excel file
- Explanation note: Uncorrected P-distance of 16S gene among 45 *Leptobranchella* species in this study.
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- Link: <https://doi.org/10.3897/zse.98.73162.suppl2>