

|   |     |
|---|-----|
| <b>Violante-González J, Monks S, Quiterio-Rendon G, García-Ibáñez S, Larumbe-Morán E, Rojas-Herrera AA</b><br>Life on the beach for a sand crab ( <i>Emerita rathbunae</i> ) (Decapoda, Hippidae):<br>parasite-induced mortality of females in populations of the Pacific<br>sand crab caused by <i>Microphallus nicolli</i> (Microphallidae) | 153 |
| <b>Conradie W, Bittencourt-Silva GB, Engelbrecht HM, Loader SP, Menegon M, Nanvonamuquitxo C, Scott M, Tolley KA</b><br>Exploration into the hidden world of Mozambique's sky island forests:<br>new discoveries of reptiles and amphibians   | 163 |
| <b>Maggioni T, Anabela Taverna A, Marcos Tatián M</b><br>Redescription of the deep-sea colonial ascidian <i>Synoicum molle</i> (Herdman, 1886):<br>first record since its original finding during the Challenger Expedition   | 181 |
| <b>Tomikawa K, Nakano T, Sato A, Onodera Y, Ohtaka A</b><br>A molecular phylogeny of <i>Pseudocrangonyx</i> from Japan, including<br>a new subterranean species (Crustacea, Amphipoda, Pseudocrangonyctidae)  | 187 |
| <b>Azevedo-Santos VM, Benine RC</b><br>A new species of <i>Moenkhausia</i> (Characiformes, Characidae) from the Içá River,<br>Amazon Basin, northern Brazil   | 203 |
| <b>Schmidt-Rhaesa A</b><br>The collection of Nematomorpha in the Zoological Museum Hamburg, including<br>description of a new species, <i>Chordodes jelkae</i> sp. n.   | 211 |
| <b>Lohrmann V, Waldren GC, Reiß M, Enge MS</b><br>An anocellar polistine wasp (Hymenoptera, Vespidae, Polistinae) from Texas  | 251 |
| <b>Miralles A, Köhler J, Glaw F, Vences M</b><br>Species delimitation methods put into taxonomic practice: two new <i>Madascincus</i><br>species formerly allocated to historical species names (Squamata, Scincidae)   | 257 |



# Zoosystematics and Evolution

## A Bulletin of Zoology since 1898

### Instructions for authors

#### Scope

**Zoosystematics and Evolution** (formerly *Mitteilungen aus dem Museum für Naturkunde in Berlin, Zoologische Reihe*) edited by the *Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity at the Humboldt University Berlin* is an international, peer-reviewed, life science journal, devoted to whole-organism biology. It mainly publishes original research and review articles in the field of Metazoan taxonomy, biosystematics, evolution, morphology, development and biogeography at all taxonomic levels. Its scope encompasses primary information from collection-related research, viz. taxonomic descriptions and discoveries, revisions, annotated type catalogues, aspects of the history of science, and contributions on new methods and principles of systematics. Entomological papers will also be accepted for review, but authors should first consider submission to the *Deutsche Entomologische Zeitschrift*. Articles whose main topic is ecology, functional anatomy, physiology, or ethology are only acceptable when of clear systematic or evolutionary relevance and perspective. Review articles and contributions to a discussion forum are welcome, but authors are asked to contact the editors beforehand.

#### Authors and submission

- Conflicts of interest: Authors must disclose relevant competing interests, both financial and personal.
- Ownership: Authors must declare that the submitted work is their own and that copyright has not been breached in seeking its publication.
- Originality: Authors must declare that the submitted work has not previously been published, and is not being considered for publication elsewhere.

#### Language and style

- The language of publication is English. There is no general limitation of the length of manuscripts, but please contact the editor before submitting papers exceeding 30 printed pages (approximately 60 manuscript pages including figures).
- Manuscripts should be written in a clear, straightforward style and must not have been published or submitted elsewhere.
- The text should be 12 pt, double-spaced, one-sided, left justified and with a margin of at least 3 cm.
- Use a standard typeface, e.g. Times New Roman as little formatted as possible (without tabulators, several blank spaces, etc.). Avoid footnotes.
- Divide the text into sections using headlines and sub-headlines. Do not number the headlines. Inline headers should be set in italics and followed by a full stop.
- The names of genera and species must be in italics.
- Taxonomic descriptions must comply with the rules of the 4th edition of the ICZN (see <http://www.iczn.org/>).
- Enter the page number on every page.
- Submit figures with a minimum resolution of 300 dpi.
- The preferred file formats are PSD (Photoshop) and TIFF for colour and grayscale illustrations, and EPS for vector graphics.
- JPG files are only accepted in high resolution.

#### General manuscript structure

If appropriate, the manuscript should be structured using headlines and sub-headlines, but without numbering, according to the following sections:

- Title page
- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- Acknowledgements
- References
- Tables with captions
- Figure captions

#### The publication process

##### Peer reviewing

Manuscripts are subject to peer review. All manuscripts submitted will be reviewed by at least two experts. Authors are welcome to make suggestions for competent reviewers.

##### Proofs

Prior to publication of your manuscript you will receive proofs in PDF format. Please correct and return the proofs within two weeks to the editorial office.

We recommend using the standard proofreading marks or – in the case of a few corrections – using page and line numbers. Do not change the contents of your article. Corrections extending beyond production errors will be carried out at the expense of the author.

The editorial office reserves the right to publish your article with only the editor's corrections, if your corrections do not reach us in time.

##### Publishing

The print and the online versions of your paper are published simultaneously. It is accessible in open access at Pensoft: <http://zse.pensoft.net>

#### COPE Membership

This journal endorses the COPE (Committee on Publication Ethics) guidelines and will pursue cases of suspected research and publication misconduct (e.g. falsification, unethical experimentation, plagiarism, inappropriate image manipulation, redundant publication). For further information about COPE, please see the website for COPE at <http://www.publicationethics.org.uk>

Zoosystematics

---

and Evolution

---

92 (2) 2016

# Zoosystematics and Evolution

## A Bulletin of Zoology since 1898

### Editor-in-Chief

**Matthias Glaubrecht**

Center of Natural History (CeNak)  
Universität Hamburg – Zoological Museum,  
Hamburg, Germany  
phone: +49 (0)40/42 838 2275  
e-mail: matthias.glaubrecht@uni-hamburg.de

### Managing Editor

**Lyubomir Penev**

Pensoft Publishers, Sofia, Bulgaria  
phone: +359-2-8704281  
fax: +359-2-8704282  
e-mail: penev@pensoft.net

### Editorial Secretary

**Yordanka Banalieva**

Pensoft Publishers, Sofia, Bulgaria  
phone: +359-2-8704281  
fax: +359-2-8704282  
e-mail: journals@pensoft.net

### Editorial Board

Vertebrata – Collection & Museum Research –  
Morphology & Development

**Peter Bartsch** – Museum für Naturkunde Berlin

Articulata – History of Science – Taxonomy & Systematics

**Michael Ohl** – Museum für Naturkunde Berlin

Mollusca – History of Science – Evolution & Biogeography

**Matthias Glaubrecht** – Center of Natural History (CeNak)  
Hamburg

Other Invertebrates

**Carsten Lüter** – Museum für Naturkunde Berlin

### Zoosystematics and Evolution

**2016. Volume 92. 2 Issues**

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

Abbreviated keys title: Zoosyst. Evol.

---

### In Focus

The cover picture shows a female of *Polistes (Fuscopolistes) dorsalis neotropicus* Bequaert, 1940 from Texas.

See paper of **Lohrmann V. et al.** An anocellar polistine wasp (Hymenoptera, Vespidae, Polistinae) from Texas

Publisher



Cover design

Pensoft

# Zoosystematics and Evolution

## A Bulletin of Zoology since 1898

### Content of volume **92 (2)** 2016

**Violante-González J, Monks S, Quiterio-Rendon G, García-Ibáñez S, Larumbe-Morán E, Rojas-Herrera AA**

Life on the beach for a sand crab (*Emerita rathbunae*) (Decapoda, Hippidae): parasite-induced mortality of females in populations of the Pacific sand crab caused by *Microphallus nicolli* (Microphallidae) 153

**Conradie W, Bittencourt-Silva GB, Engelbrecht HM, Loader SP, Menegon M, Nanvonamuquitxo C, Scott M, Tolley KA**

Exploration into the hidden world of Mozambique's sky island forests: new discoveries of reptiles and amphibians 163

**Maggioni T, Anabela Taverna A, Marcos Tatián M**

Redescription of the deep-sea colonial ascidian *Synoicum molle* (Herdman, 1886): first record since its original finding during the Challenger Expedition 181

**Tomikawa K, Nakano T, Sato A, Onodera Y, Ohtaka A**

A molecular phylogeny of *Pseudocrangonyx* from Japan, including a new subterranean species (Crustacea, Amphipoda, Pseudocrangonyctidae) 187

**Azevedo-Santos VM, Benine RC**

A new species of *Moenkhausia* (Characiformes, Characidae) from the Içá River, Amazon Basin, northern Brazil 203

**Schmidt-Rhaesa A**

The collection of Nematomorpha in the Zoological Museum Hamburg, including description of a new species, *Chordodes jelkae* sp. n. 211

**Lohrmann V, Waldren GC, Reiß M, Enge MS**

An anocellar polistine wasp (Hymenoptera, Vespidae, Polistinae) from Texas 251

**Miralles A, Köhler J, Glaw F, Vences M**

Species delimitation methods put into taxonomic practice: two new *Madascincus* species formerly allocated to historical species names (Squamata, Scincidae) 257

---

## Abstract & Indexing Information

Biological Abstracts® (Thompson ISI)

BIOSIS Previews® (Thompson ISI)

Cambridge Scientific Abstracts (CSA/CIG)

Web of Science® (Thompson ISI)

Zoological Record™ (Thompson ISI)



# Life on the beach for a sand crab (*Emerita rathbunae*) (Decapoda, Hippidae): parasite-induced mortality of females in populations of the Pacific sand crab caused by *Microphallus nicolli* (Microphallidae)

Juan Violante-González<sup>1</sup>, Scott Monks<sup>2</sup>, Guadalupe Quiterio-Rendon<sup>2</sup>, Sergio García-Ibáñez<sup>1</sup>, Edvino Larumbe-Morán<sup>3</sup>, Agustín A. Rojas-Herrera<sup>1</sup>

1 Unidad Académica de Ecología Marina, Universidad Autónoma de Guerrero, Gran Vía Tropical No. 20, Fracc. Las Playas, C.P. 39390, Acapulco, Guerrero, México

2 Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Hidalgo, Apartado Postal 1-10, C.P. 42001, Pachuca, Hidalgo, México

3 Centro de Estudios Tecnológicos del Mar No 16, Interior Isla del Cayacal s/n, Zona Industrial, Cd. Lázaro Cárdenas, C.P. 60950, Michoacán, México

<http://zoobank.org/499E17E0-C26D-47D1-A479-02B5077849B6>

Corresponding author: Scott Monks (monks.scott@gmail.com)

## Abstract

Received 24 February 2016

Accepted 23 August 2016

Published 8 September 2016

Academic editor:  
Michael Ohl

## Key Words

helminth parasite  
parasite-mediated mortality of hosts  
parasite population parameters  
Guerrero State  
Mexican coast

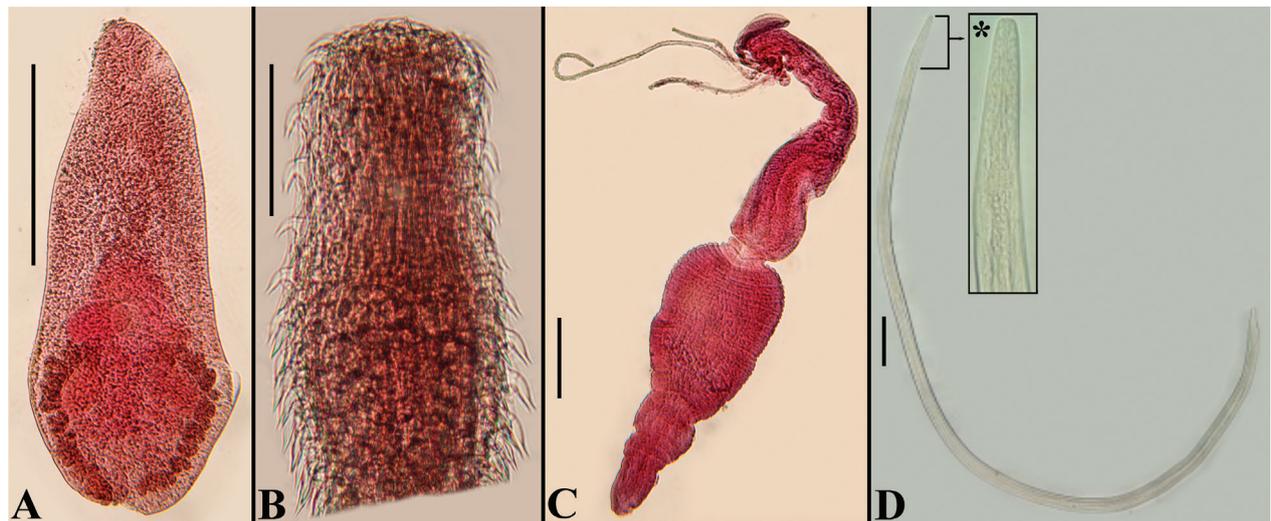
Parasites, by definition, can affect mortality of their host, making parasitism an important biotic determinant of animal population dynamics and community structure. Reduction in the number of larger, reproductive age females in populations of the Pacific sand crab, *Emerita rathbunae* (Decapoda, Hippidae), was observed in studies of the helminth community of this host. The aim of this study was to determine if high abundance of the metacercaria of the trematode, *Microphallus nicolli* (Microphallidae), causes mortality in this host. Females of *E. rathbunae* were collected from four sandy beaches in Guerrero State, Mexico, and helminths were collected from each crab. An analysis of variance (Anova) was applied to these data in order to identify differences in abundance between sizes of crabs, and an analysis of covariance (Ancova) was applied to identify differences in the abundance of metacercariae between locations. Parasite-related mortality was inferred by a decrease in abundance in older hosts. Linear and polynomial regressions of mean abundance of helminths ( $\log x+1$  transformed data) vs. cephalothorax length of crabs were significant for the four populations of *E. rathbunae*, indicating increased mortality of older, more heavily infected female crabs and resultant removal from the population. Encapsulation and melanization of cysts by crabs was observed, indicating that an immune response by crabs also killed a portion of the cysts from subsequent exposures. Mortality of hosts through behavioral modification favoring transmission of highly infected crabs was suggested as the driving force behind this process.

## Introduction

Parasitism is an important biotic determinant of animal population dynamics and community structure. Parasites can influence host numbers within a population by either decreasing recruitment rate (by reducing host fecundity) or increasing mortality rate. Host mortality attributable to parasites can occur as a reduction in host survival due to the pathological consequences of parasitic infection, or as alterations in a host's phenotype and/or behavior to facilitate parasite transmission to definitive hosts via

predation (Hansen and Poulin 2005; Koehler and Poulin 2010; Latham and Poulin 2002c; Poulin 1999; Thomas et al. 1995).

Several approaches based on statistical evidence have been used in a number of studies in an attempt to determine if infection with parasites affects host mortality rates in natural populations (Koehler and Poulin 2010; Latham and Poulin 2002c; Thomas et al. 1995). A frequent feature of many models is that host mortality rises as parasite load increases (Koehler and Poulin 2010; Latham and Poulin 2002c). In a system where the host continues



**Figure 1.** Helminth parasites of *Emerita rathbunae*. **A** *Microphallus nicolli*; **B** Everted proboscis of an excysted specimen of *Proflicolis* sp.; **C** Cestode larva; **D** *Proleptus* sp., inset (\*) = anterior end of worm. Scale bars: **A** = 500  $\mu$ m; **B** = 200  $\mu$ m; **C** = 100  $\mu$ m; **D** = 100  $\mu$ m.

to accumulate parasites over time, and assuming no density-dependent regulation of parasite numbers, older individuals can be expected to have higher average parasite loads than younger hosts. In some cases, however, older hosts have fewer parasites on average than intermediate age hosts, suggesting that more heavily infected individuals are removed from the host population by some effect of the parasites (Koehler and Poulin 2010; Latham and Poulin 2002c; Thomas et al. 1995).

The Pacific sand crab, or mole crab, *Emerita rathbunae* Schmitt, 1935 is common in the splash zone of sandy beaches in the intertidal zone (Hendrickx and Harvey 1999; Ríos-Elósegui and Hendrickx 2015). It is a good model for parasitological studies due to its wide geographical distribution, from the southeastern Gulf of California, Mexico, to the southern coast of Ecuador (Tam et al. 1996), and its importance in the macrofauna food chain of sandy beaches, where it can be very abundant (Oliva et al. 2008). A number of studies on the parasites of *E. analoga* have been made (Alvitres et al. 1999; Iannacone et al. 2007; Oliva et al. 2008; Oliva et al. 1992; Smith 2007), but only one study of the parasites of *E. rathbunae* (reported as *E. analoga*) has been made in Mexico (Violante-González et al. 2012; Violante-González et al. 2015). In that study, the authors (Violante-González et al. 2012; Violante-González et al. 2015) found that specimens of *E. rathbunae* inhabiting the coasts of Guerrero were parasitized by four species of larval helminth: one metacercariae [*Microphallus nicolli* (Cable and Hunninen, 1938)]; one cystacanth (*Proflicolis* sp.); one cestode (Trypanorhyncha); and one nematode (*Proleptus* sp.) (Fig. 1A–D). Infection levels of *M. nicolli* were significantly higher than reported for populations of *E. analoga* (hundreds to thousands per infected crab) from another North American location (Smith 2007). This suggested that these helminths have the potential to exert a substantial effect on populations of *E. rathbunae*. The objective

of the present study was to test that hypothesis and determine if high abundance of *M. nicolli* in populations of *E. rathbunae* on the coasts of Guerrero, Mexico, effects mortality rates of this species of host in a manner reported for other species of crustacean at other locations.

## Materials and methods

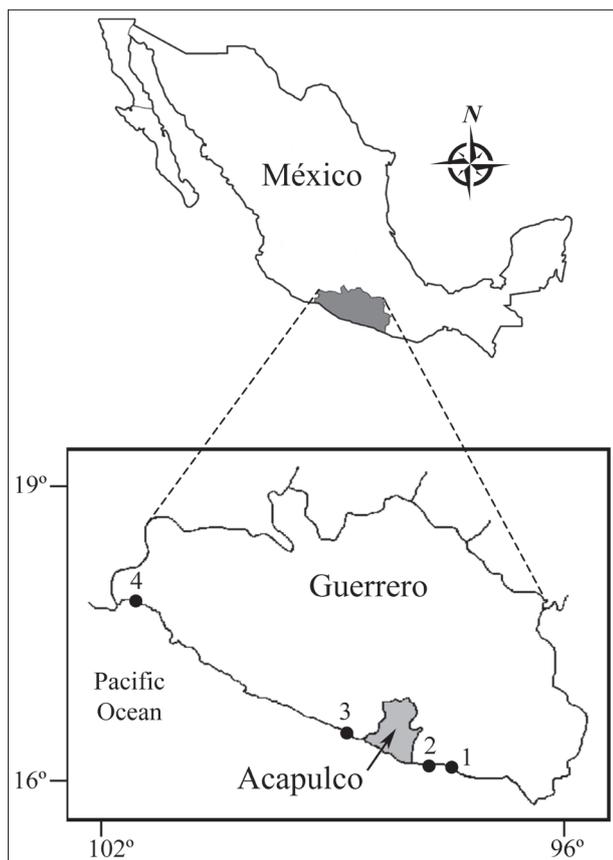
### Sampling

The Mexican Pacific coastal region experiences two distinct climatic seasons, a rainy period from June to November (precipitation  $\approx$  430 mm), and a dry season from December to May (precipitation < 70 mm). Adult females of *E. rathbunae* (Table 1) were collected from four sandy beaches on the Pacific Coast of Guerrero, Mexico, between August and December 2009: San Andrés (16°42' N, 99°40' W,  $n$  = 146); Revolcadero (16°47' N, 99°47' W,  $n$  = 114); Las Trancas (16°59' N, 100°13' W,  $n$  = 173); and Ixtapa (17°39' N, 101°36' W,  $n$  = 68) (Fig. 2). Males of *E. rathbunae* are much smaller than females, and measure from 2–14 mm (i.e., dwarf males), and they generally remain attached to the coxa of the female's pleopods, or they are located among the eggs (Ríos-Elósegui and Hendrickx 2015). Therefore, due to their small size they were not considered for this study.

Female crabs were captured by hand, placed in labeled plastic bags and transported to the laboratory of the Unidad Académica de Ecología Marina, Universidad Autónoma de Guerrero, Acapulco. Before dissection, cephalothorax length (CL in mm) was measured to the nearest 0.1 millimeter using a digital caliper. This parameter was treated as the standard crab size in the analyses. Crabs were dissected by first removing the carapace, and then examining the internal structures, hepatopancreas, stomach, intestine, and muscle tissue, for helminths. Helminths were collected using standard practices and processed

**Table 1.** Infection parameters of metacercariae of *Microphallus nicolli* in *Emerita rathbunae* from four coastal locations in Guerrero, Mexico. Significant values for the relationship between crab size (cephalothorax length) and metacercariae abundance per infected host ( $r_s$ ) are in bold. P (%) = Prevalence of infection (% of infected crabs); Range of Intensity = minimum and maximum number of metacercariae;  $r_s$  = Spearman’s correlation coefficient.

| Date (2009) | Locality    | No. of crabs | Cephalothorax length (mm) | Total number of parasites | P (%) | Mean abundance of metacercariae | Range of Intensity | $r_s$        |
|-------------|-------------|--------------|---------------------------|---------------------------|-------|---------------------------------|--------------------|--------------|
| Sep.        | San Andrés  | 146          | 37.6 ± 2.9                | 40,3414                   | 100   | 2763.1 ± 1235.6                 | 300 · 6000         | <b>0.208</b> |
| Oct.        | Revolcadero | 114          | 34.2 ± 1.7                | 107,921                   | 100   | 946.7 ± 796.8                   | 103 · 4235         | <b>0.609</b> |
| Nov.        | Las Trancas | 173          | 38.2 ± 2.2                | 403,177                   | 100   | 2330.5 ± 1076.8                 | 1050 · 5800        | <b>0.444</b> |
| Dec.        | Ixtapa      | 68           | 32.9 ± 5.6                | 58,241                    | 100   | 856.5 ± 597.5                   | 15 · 2550          | <b>0.689</b> |



**Figure 2.** Map indicating the four collection localities along the Pacific coast of Guerrero. 1. San Andrés; 2. Revolcadero; 3. Las Trancas; 4. Ixtapa.

for identification (Pritchard and Kruse 1982). Infection parameters included prevalence (percent infected hosts); abundance (number of parasites per examined host) expressed as the mean ± standard deviation; range of intensity (Bush et al. 1997); and volumetric abundance (volume of live metacercariae of all development stages per host, in mm<sup>3</sup>) (see Violante-González et al. 2015 for discussion of these data). Vouchers of specimens were deposited in the Colección de Helmintos, Universidad Autónoma del Estado de Hidalgo, Mexico (CHE-F-0019).

**Statistical analyses**

Infection parameters included prevalence (percent of infected hosts); abundance (number of parasites per exam-

ined host) expressed as the mean ± standard deviation; range of intensity (Bush et al. 1997); and volumetric abundance (volume of live metacercariae of all developmental stages per host, in mm<sup>3</sup>). An analysis of variance (Anova) was applied to identify differences in sizes of the crabs, and an analysis of covariance (Ancova) was applied to identify differences in the abundance of metacercariae between locations. Data were log-transformed when significant deviations from normality were identified. Correlations were calculated using the Spearman range coefficient ( $r_s$ ). Significance for all the statistical analyses was established at  $P = 0.05$ , unless stated otherwise. The software SPSS (Version 20) was used for all statistical analyses.

Size varied among the encysted metacercariae of *M. nicolli*, and a number of melanized cysts were found in each infected crab, so samples of cysts were measured (length and maximum width in μm) using an ocular micrometer for two locations (San Andrés and Las Trancas;  $n = 866$ ). These measurements were used to calculate the volume of live cysts (mm<sup>3</sup>) using the volume formula for a scalene ellipsoid [ $V = (4/3) \pi (a/2) (b/2) (c/2)$  where  $a$  is cyst length,  $b$  is cyst width, and  $c$  is cyst height]. Melanized cysts were considered to contain dead metacercaria (Bryan-Walker et al. 2007; Koehler and Poulin 2010). Cysts also were classified into four categories based on size and characteristics (live or dead/melanized), following a modified version of the classification reported by Keeney et al. (2007): 1. = immature cyst (small); 2. = late immature (medium); 3. = mature (large); and 4. = melanized (dead). Crabs from San Andrés and Las Trancas were pooled and divided arbitrarily into 6 equal (2.1 mm) size-classes in order to describe cyst distribution by size (cephalothorax length = CL) of crabs.

Mean parasite load (total number of live metacercariae of all sizes/total number of crabs that were examined) was plotted versus size-class of crabs for the four localities in order to evaluate the affect of this parameter on host mortality; a close relationship was assumed between age and size (Latham and Poulin 2002b). The variance to mean abundance ratio, a measure of parasite aggregation (Bush et al. 1997), was calculated to determine variation in infection levels by host size-class. Differences in mean parasite abundance and the variance to mean abundance ratio were analyzed as a function of increasing size class. Linear and polynomial (curvilinear) regressions were

then fitted using the log-transformed data ( $\log x+1$ ) of mean parasite abundance (dependent variable) and CL (independent variable), by location. The regressions providing the best fit for the data were selected using the highest coefficient of determination ( $r^2$ ) value and a substantially low  $P$  value for each regression (Latham and Poulin 2002b).

## Results

The total sample size was 591 female crabs (*E. rathbunae*), ranging from 68 individuals at Ixtapa to 173 at Las Trancas (Table 1). Cephalothorax length was significantly different between collection locations: 32.9 mm  $\pm$  5.6 mm (Ixtapa) to 38.2 mm  $\pm$  2.2 mm (Las Trancas) (Anova,  $F = 80.8$ ,  $P < 0.0001$ ) (Table 1). Encysted metacercariae (Fig. 3) of *M. nicolli* were recovered largely from the hepatopancreas of infected crabs. Prevalence of *M. nicolli* was 100% at all four locations, although mean abundance varied from 857 metacercariae/crab  $\pm$  598 metacercariae (Ixtapa) to 2,763 metacercariae/crab  $\pm$  1,236 metacercariae (San Andrés, Table 1) (Ancova,  $F = 43.62$ ,  $P = 0.0001$ ).

Cephalothorax length was correlated positively with abundance of metacercariae at all locations ( $P < 0.05$ ), although correlation coefficient values ( $r_s$ ) were low in most cases (Table 1). The overall percentage of live (unmelanized) cyst decreased as the developmental stage progressed from immature to mature cysts (Table 2). The percentage of melanized cysts corresponded to the three previous development stages, although the general cyst

pattern remained unchanged, i.e., all crabs had a higher percentage of immature than mature but melanized cysts.

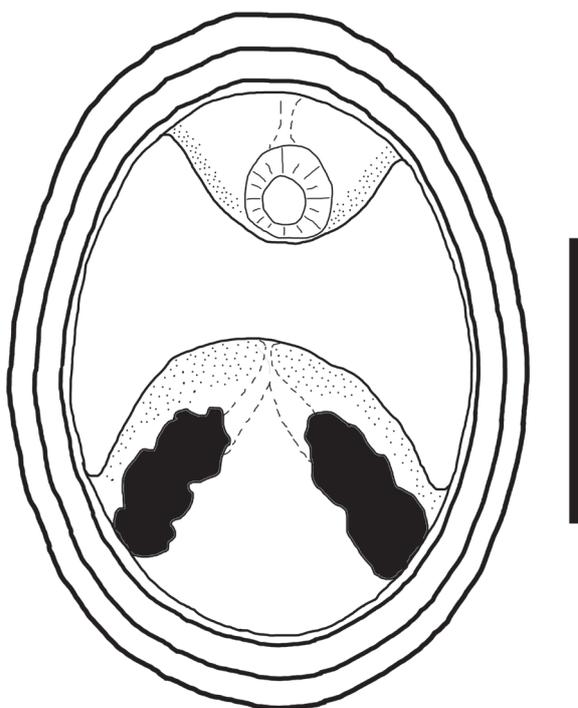
Distribution of cysts, based on developmental stage and crab size, did not differ at the two locations where this was analyzed (San Andrés and Las Trancas). The percentage of mature cysts decreased as crab size increased, and the percentage of melanized cysts increased with the increase (small to large) in the size of crabs (Fig. 4A, B). Mean volumetric abundance of metacercariae, considering the three developmental stages, decreased in the largest size classes of crabs at both locations (Fig. 5A, B).

Linear and polynomial regressions of the relationship between mean abundance of helminths ( $\log x+1$ ) and cephalothorax length of crabs were significant at all four beaches (Table 3). However, the coefficients of determination ( $r^2$ ) values for the polynomial regressions were higher, indicating that the curvilinear regressions provided a better fit to the data. Mean abundance of *M. nicolli* plotted against crab size-class produced a convex curve in the San Andrés and Ixtapa populations, indicating that cyst number tended to decline in the largest size-classes (Fig. 6A, D). In contrast, the curves generated for Revolcadero and Las Trancas were concave (Fig. 6B, C). The degree of parasite aggregation was lower in the largest size-classes at San Andrés and Ixtapa (Fig. 6A, D), which coincides with the convex curves produced by the polynomial regressions for these two locations.

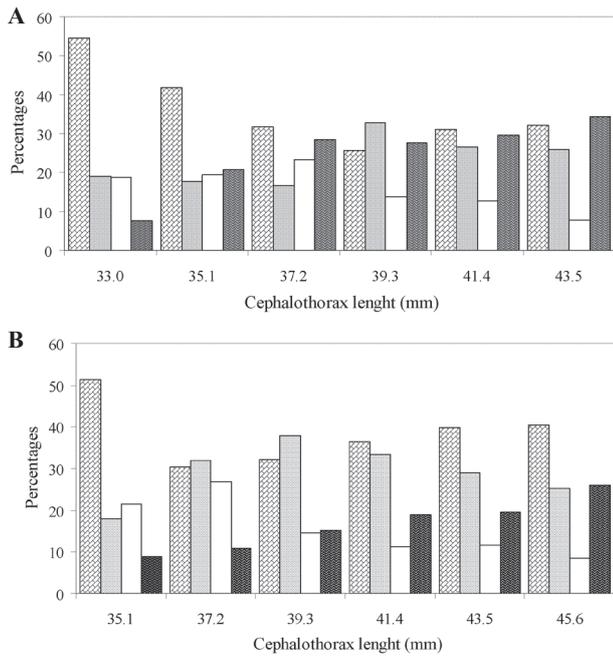
## Discussion

Species of *Microphallus* infect several species of marine crustaceans that function as second intermediate hosts; adult worms mature mainly in coastal birds (Hansen and Poulin 2005; Pina et al. 2011). The metacercariae exhibit high infection site-specificity and encyst mainly in the hepatopancreas of crabs, where they reach high prevalences (Hansen and Poulin 2005; Koehler and Poulin 2010; Pina et al. 2011; Robaldo et al. 1999). In crustaceans, the hepatopancreas is the main organ involved in carbohydrate metabolism (glucose and glycogen), which means that it provides greater availability of energy resources (nutrients) for establishment of helminths (Robaldo et al. 1999). All (100%) of the specimens of *E. rathbunae* from the four locations were infected with *M. nicolli*, recovered mainly from the hepatopancreas, confirming the expectation of a strong habitat specificity for members of this genus (Koehler et al. 2011).

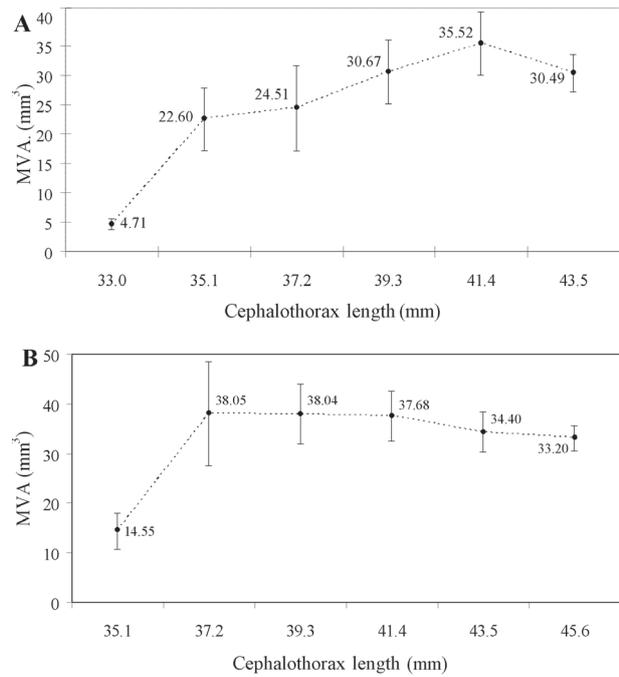
Although prevalence of *M. nicolli* did not differ between the four locations, mean abundance was significantly higher at San Andrés and Las Trancas (Table 1) than at the other two locations. Temporal and spatial variations in levels of infection in intermediate hosts are commonly observed patterns in studies of parasite ecology (Latham and Poulin 2002b; Oliva et al. 2008; Smith 2007; Studer and Poulin 2012). Spatial variation in the level of infection such as that observed here has been attributed by other authors to differences in abundances



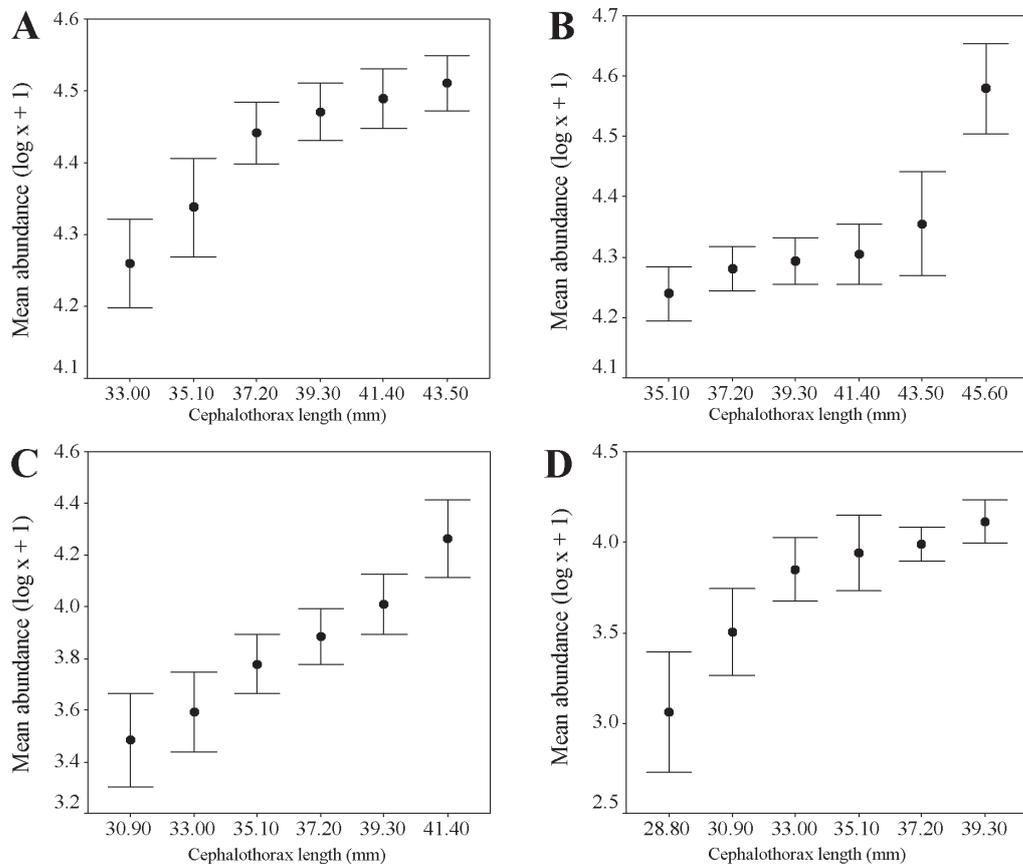
**Figure 3.** Cyst of an older metacercaria of *Microphallus nicolli*. Scale bar: 100  $\mu$ m



**Figures 4.** Percentages of cysts of *Microphallus nicolli* by development stage of cysts and size-classes of female crabs (*Emerita rathbunae*). Hatched bars = immature cysts; Shaded bars = late immature cysts; Open bars = mature cysts; Dark bars = melanized cysts. Data are only from San Andrés (A) and Las Trancas beaches (B). Stages of cyst maturity were modified from Keeney et al. (2007).



**Figure 5.** Relationship between mean abundance of cysts of *Microphallus nicolli* calculated from volumetric measurements and the cephalothorax length of females of *Emerita rathbunae*. Data are from only San Andrés (A) and Las Trancas beaches (B).



**Figure 6.** Relationship between mean abundance of cysts ( $\log x + 1$ ) of *Microphallus nicolli* and size-classes of females of *Emerita rathbunae* based on cephalothorax length. Data are for all four localities: San Andrés (A), Revolcadero (B), Las Trancas (C), and Ixtapa (D).

**Table 2.** Mean length and width measurements and the volume (mm<sup>3</sup>) of encysted metacercariae of *Microphallus nicolli* by development stage. Global percentage of melanized cysts corresponds to the sum of the percentages found in the three stages.

| Cyst stage     | No. of cysts | Length (mm)   | Width (mm)    | Volume (mm <sup>3</sup> ) | Global percentage of melanized cysts | Mean number of melanized cysts |
|----------------|--------------|---------------|---------------|---------------------------|--------------------------------------|--------------------------------|
| Immature       | 247          | 0.259 ± 0.040 | 0.221 ± 0.034 | 0.0066                    | 37%                                  | 91.4                           |
| Late immature  | 233          | 0.350 ± 0.038 | 0.301 ± 0.035 | 0.0166                    | 26%                                  | 86.2                           |
| Mature         | 151          | 0.591 ± 0.046 | 0.393 ± 0.038 | 0.0477                    | 16%                                  | 55.9                           |
| Melanized cyst | 235          | 0.317 ± 0.062 | 0.269 ± 0.044 | 0.0125                    | 21%                                  | ..                             |

of final and intermediate host (Iannacone et al. 2007; Latham and Poulin 2002b; Latham and Poulin 2002c; Oliva et al. 2008; Smith 2007). In this study area, a possible first intermediate host for *M. nicolli* is the snail *Agaronia testacea* (Lamarck, 1811), and the probable final hosts are coastal marine birds; species of Scolopacidae and Recurvirostridae (Charadriiformes); members of *Tringa* and *Numenius* are known to actively feed on *E. rathbunae* (Violante-González et al. 2012; Violante-González et al. 2015). However, to date, no studies have been made to identify precisely the definitive host of *M. nicolli* in Guerrero.

It is possible that variation in the abundance of *M. nicolli* between the four locations is the result of a factor or process related to the size of the crabs that might influence this infection parameter (Poulin 1999). A positive correlation between crab size and abundance was observed at all four locations (Table 1), with mean abundance highest in crabs from San Andrés and Las Trancas (Table 1). However, this tendency also is consistent with the hypothesis that largest crabs (i.e., older) have accumulated parasites for a longer period of time. Individuals of *E. analoga* can live up to 3.5 years (Oliva et al. 2008), so individuals of that species accumulate large quantities of helminths during their lifetime. In our study, it was not possible to determine the strength of a simple age-infrapopulation size effect because the maximum age/size of *E. rathbunae* has not been documented, but our results are similar to the mortality seen in studies of *E. analoga* (Alvitres et al. 1999; Iannacone et al. 2007; Oliva et al. 2008; Oliva et al. 1992; Smith 2007) and other shore crabs (Latham and Poulin 2002a; Latham and Poulin 2002b).

Metacercaria of *M. nicolli* continue to grow post-encystment (Peoples and Poulin 2011; Pina et al. 2011), and we found that size of cysts differed according to developmental stage (Table 2). Mean volume of immature (small) cysts was 0.0066 mm<sup>3</sup>, and the volume of mature cysts was 0.0477 mm<sup>3</sup>, representing an increase from initial volume of up to 700% during encystment inside the crabs. However, the mean number of cysts/crab decreased with developmental stage (247, 233, and 151, respectively). We interpret this as support for increased mortality of crabs as metacercariae develop. There are no studies of the histopathology caused by these helminths, but the increase in volume of metacercariae undoubtedly damages the tissues of the crabs. It also is not known if the size of metacercaria is linked directly to the size of

crabs (Ruiz-Daniels et al. 2013). These are areas for further research.

A high percentage of cysts had been encapsulated and melanized (Table 2). This process, encapsulation and subsequent cellular melanization of metacercariae, is a common response of the host's immune system in crustaceans, and it results in the death of the metacercaria (Keeney et al. 2007; Koehler and Poulin 2010). Immature cysts frequently were melanized more than were those of older stages, and the percentage of melanized immature cysts increased progressively from the smallest to the largest crab size classes (Fig. 4A, 4B). This suggests that the immunologic response in *E. rathbunae* is age-dependent; i.e., through this response larger (older) crabs can eliminate a large percentage of recently-introduced parasites, thus slowing greater accumulation of parasites and concurrent mortality (Keeney et al. 2007; Koehler and Poulin 2010). As well, the mean number of melanized cysts decreased with developmental stage; we cannot explain this decrease in number and there is no available evidence that the melanized cysts eventually were absorbed by the crabs. There also is no information on the potential for genetically mixed populations of *M. nicolli* (brought to these beaches by birds from completely different regions), although this is likely. This could confound our results if there are different immune responses to different strains, as was seen by Koehler et al. (2011). Molecular studies of individual helminths (DNA fingerprinting) should be carried out in the future to resolve this issue.

The overall percentage of mature (large) cysts was lower than the percentage of the two earlier stages (Table 2), which could be attributed to larger cysts requiring more nutrients for growth, thus causing death of immature stages, although there is no direct evidence for this. Intraspecific competition for resources at high cyst concentrations (800 to 2000 cysts in the present study) in the same structure (hepatopancreas) could be causing cyst death. Density-dependent effects acting on regulation of metacercariae growth are common in marine crustacean hosts (Fredensborg and Poulin 2005; Keeney et al. 2007), although more study is necessary to elucidate direct and indirect causes. This regulator effect on survival of parasites according to size may be very important for *M. nicolli* because, unlike many other microphallid larvae, it grows several hundred times its initial volume inside the second intermediate host (Fredensborg et al. 2004; Keeney et al. 2007; Koehler and Poulin 2010; Latham and Poulin 2002b).

Ample evidence exists of parasite-induced mortality in marine crustacean populations under field conditions (Fredensborg et al. 2004; Koehler and Poulin 2010; Latham and Poulin 2002b; Thomas et al. 1995). Parasite-induced host mortality can be deduced by means of a decrease in infection intensity in older hosts, which we observe in our data. Using an acanthocephalan/crab system, Latham and Poulin (2002) suggested that reductions in mean parasite numbers in the largest crab size-classes could be attributed to the loss of heavily-infected individuals; this also was observed in our data. With a similar approach, Koehler and Poulin (2010) reported host mortality in four species of crab from New Zealand, and they concluded that this was induced by two microphallids (*Maritrema novaezealandensis* and *Microphallus* sp.).

If an infrapopulation of a species of parasite does not induce host mortality, then a positive linear relationship can be expected between host size and parasite abundance due to parasite accumulation in the host over time (Koehler and Poulin 2010). In the data presented herein, polynomial (curvilinear) regressions had a better fit to mean abundance data per crab size-classes at all four locations (Table 3) than linear regressions. However, the curves generated were of two types; concave and convex. For Revolcadero and Las Trancas, the best-fit curves were concave (Fig. 6A, D), suggesting an increase in parasite load with crab size surpassing a linear relationship. The regression curves for San Andrés and Ixtapa were convex (Fig. 6B, C), indicating a reduction in parasite load in the largest (oldest) size classes. The latter could be attributed directly to death of those hosts harboring greater numbers of parasites and removal of both host and parasites from the population of crabs. It is interesting that these differences do not appear to be related to parasite intensity (Table 1).

In some studies of host mortality induced by helminth parasites, differences have been reported in the effect of parasites between host species and locations (Koehler and Poulin 2010; Latham and Poulin 2002b). These differences have been attributed to possible host manipulation by the parasites in order to increase the likelihood of predation by definitive hosts, and the presence of the final host (i.e., shorebirds) in locations where host population regulation was studied. Although several species of *Microphallus* can manipulate host behavior (Hansen and Poulin 2005; Ruiz-Daniels et al. 2013; Thomas et al. 1995), there have been no studies of the possibility of infected individuals of *E. rathbunae* being manipulated by *M. nicolli*. There is, however, a high abundance of marine coastal birds at San Andres (unpublished data) that could cause heavy predation of larger, highly-infected crabs, and, during this study, migratory birds were observed catching and eating crabs on these beaches. This might explain the possible host population regulation suggested by the regression analysis (Table 3) and the mean parasite abundance vs. crab size class graph (Fig. 6A). This is not the case for Ixtapa, although the statistical analyses also indicated a reduction in the parasite load in the largest size classes of crab (Fig. 6D). The number of birds

**Table 3.** Results of the regression analysis used to evaluate the relationship between number of cysts of *Microphallus nicolli* (log x+1) per crab and the cephalothorax length of crabs from four coastal locations in Guerrero, Mexico. n = number of crabs that were examined; R<sup>2</sup> = coefficient of determination; P = significance level. Note: 100% of the crabs examined were infected.

| Locality    | n   | Linear         |        | Polynomial     |        | Curve type |
|-------------|-----|----------------|--------|----------------|--------|------------|
|             |     | R <sup>2</sup> | P      | R <sup>2</sup> | P      |            |
| San Andrés  | 146 | 0.297          | 0.0001 | 0.330          | 0.0001 | Convex     |
| Revolcadero | 114 | 0.393          | 0.0001 | 0.395          | 0.0001 | Concave    |
| Las Trancas | 173 | 0.235          | 0.0001 | 0.300          | 0.0001 | Concave    |
| Ixtapa      | 68  | 0.508          | 0.0001 | 0.588          | 0.0001 | Convex     |

visiting that beach noticeably was less, probably due to constant tourist traffic, which would directly affect the parameters of the helminth population in *E. rathbunae*.

The most plausible explanation for the lower abundance of metacercaria in larger size crabs is the regulation of the population of *E. rathbunae* through elimination of a high proportion of metacercariae cysts, directly by mortality of crabs or by favoring the capture of the more highly-infected crabs (the larger, older hosts) (Keeney et al. 2007; Koehler and Poulin 2010). As we observed, *E. rathbunae* can eliminate/inactivate a considerable percentage of cysts through melanization, most of which are immature. The largest crabs may have a stronger immune response, allowing them to eliminate a greater number of cysts by encapsulation, followed by melanization, and eventual metacercariae death (Keeney et al. 2007; Koehler and Poulin 2010), but these would have to be absorbed by the crabs and disappear from the infrapopulation of metacercaria because the percentage decreases rather than increases. In that sense, the decline in aggregation levels of *M. nicolli* observed in the largest size-classes of crabs at most locations (Fig. 6A–D) would be attributed to the reduction in the abundance of metacercariae caused by higher cyst mortality in larger crabs. The reductions in mean volumetric metacercariae abundance in the largest size-classes at both San Andrés and Las Trancas (Fig. 5A, B) resulted from the number of large, mature cysts in these crab size-classes without overall increase in infrapopulation size. Interestingly, host population regulation by parasites is thought to be nearly impossible when parasites are highly aggregated in the host (Poulin and Morand 2000).

## Conclusions

Parasite-induced mortality in populations of a marine crustacean (*E. rathbunae*) under field conditions by infrapopulations of the metacercaria of *M. nicolli* is documented in four populations of sand crabs in Guerrero, Mexico. These results are consistent with previous studies of other Crustacea-Digenea host-parasite systems. Evidence that the population of *E. rathbunae* is affected, and possibly regulated, by the infrapopulations of

*M. nicolli* consists of five factors: 1. Mean volumetric abundance of metacercariae generally decreased as crabs aged (grew larger), but the proportion of mature cysts decreased, suggesting that the metacercariae were growing, but that crabs with a higher proportion of more mature cysts had been removed (preferentially?) from the population of crabs; 2. Linear and polynomial regressions of mean abundance of helminths ( $\log x+1$ ) vs. cephalothorax length of crabs were significant for the four populations of *E. rathbunae* (Table 3); 3. Although both the linear and polynomial regressions were highly significant, the polynomial regressions had a higher coefficient of determination ( $r^2$ ) values, indicating that curvilinear regressions provided a better fit to the data and confirming that selective mortality of hosts was occurring; 4. A positive correlation between crab size and abundance of helminths was observed at all four locations, confirming the potential for host population regulation and/or manipulation of host behavior (favoring transmission) by parasites; and, 5. The mean number of melanized (dead) cysts decreased with the age of the crabs, confirming that crabs either were eliminating substantial numbers of parasites throughout their lifetime or more heavily-infected crabs were being removed from the populations, but direct causes of the decrease could not be identified. The next steps in this study should be to evaluate the genetic composition of the infrapopulations and the metapopulations of *M. nicolli* in order to confirm or reject differential effects at some locations caused by genetic differences. As well, possibility of histopathology should be evaluated to determine if it, rather than behavior modification, is the cause of the changes in mean abundance between size classes of crabs and the decrease in melanized cysts in larger crabs.

## Acknowledgements

Funds for this study were provided to the authors by the Programa de Mejoramiento del Profesorado (PROMEP) as part of the collaborative project “Calidad ambiental y desarrollo sustentable: evaluación del impacto ambiental por actividades antropogénicas- alternativas de mitigación” (third year). Other funds (to JV-G) were provided by the project “Parásitos de rayas de importancia económica y ecológica en la Bahía de Acapulco, Gro.”, financed by the Universidad Autónoma de Guerrero, and the project “Fortalecimiento del Doctorado en Ciencias Ambientales de la Universidad Autónoma de Guerrero”, financed by Fondos Mixtos-Consejo Nacional de Ciencia y Tecnología (CONACyT)-Guerrero (2015). Guadalupe Quiterio-Rendón assisted with the processing of much of the material as part of her MS thesis project; she received a scholarship (No. 247514) from CONACyT during this study. The authors wish to thank students of the Marine Ecology Academic Unit (UAG) for their assistance with field and laboratory work, and the local residents who assisted with collection of the crabs.

## References

- Alvitre V, Chanamé J, Fupuy J, Chambergro A, Cortez M (1999) Cambios en la prevalencia de los helmintos parásitos de *Emerita análoga* [sic.] por efecto de “El Niño 1997-98”. Revista Peruana de Biología Volumen extraordinario Facultad de Ciencias Biológicas-UNMSM, Lima, Perú, December 1999: 69–76
- Bryan-Walker K, Leung TF, Poulin R (2007) Local adaptation of immunity against a trematode parasite in marine amphipod populations. Marine Biology 152(3): 687–695 doi: 10.1007/s00227-007-0725-x
- Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83(4): 575–583 doi: 10.2307/3284227
- Fredensborg BL, Mouritsen KN, Poulin R (2004) Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda: Crustacea) infected by a trematode: experimental infections and field observations. Journal of Experimental Marine Biology and Ecology 311(2004): 253–265 doi: 10.1016/j.jembe.2004.05.011
- Fredensborg BL, Poulin R (2005) Larval helminths in intermediate hosts: does competition early in life determine the fitness of adult parasites? International Journal for Parasitology 35(10): 1061–1070. doi: 10.1016/j.ijpara.2005.05.005
- Hansen EK, Poulin R (2005) Impact of a microphallid trematode on the behaviour and survival of its isopod intermediate host: phylogenetic inheritance? Parasitology Research 97(3): 242–246. doi: 10.1007/s00436-005-1435-2
- Hendrickx, ME, Harvey AW (1999) Checklist of Anomuran crabs (Crustacea: Decapoda) from the Eastern Tropical Pacific. Belgian Journal of Zoology 129(2): 363–389.
- Iannacone J, Alvaríño L, Bolognesi B (2007) Aspectos cuantitativos de los metazoos parásitos del muy muy *Emerita analoga* (Stimpson) (Decapoda: Hippidae) en Chorrillos, Lima, Peru. Neotropical Helminthology 1(2): 59–67.
- Keeney DB, Waters JM, Poulin R (2007) Diversity of trematode genetic clones within amphipods and the timing of same-clone infections. International Journal for Parasitology 37(3–4): 351–357 doi: 10.1016/j.ijpara.2006.11.004
- Koehler AV, Gonchar AG, Poulin R (2011) Genetic and environmental determinants of host use in the trematode *Maritrema novaezealandensis* (Microphallidae). Parasitology 138(01): 100–106. doi: 10.1017/S0031182010001022
- Koehler AV, Poulin R (2010) Host partitioning by parasites in an intertidal crustacean community. Journal of Parasitology 96(5): 862–868. doi: 10.1645/GE-2460.1
- Latham ADM, Poulin R (2002a) Effect of acanthocephalan parasites on hiding behaviour in two species of shore crabs. Journal of Helminthology 76(04): 323–326 doi: 10.1079/JOH2002139
- Latham ADM, Poulin R (2002b) Field evidence of the impact of two acanthocephalan parasites on the mortality of three species of New Zealand shore crabs (Brachyura). Marine Biology 141: 1131–1139. doi: 10.1007/s00227-002-0913-7
- Latham ADM, Poulin R (2002c) New records of gastrointestinal helminths from the southern black-backed gull (*Larus dominicanus*) in New Zealand. New Zealand Journal of Zoology 29(3): 253–257. doi: 10.1080/03014223.2002.9518309
- Oliva ME, Barrios I, Thatje S, Laudien J (2008) Changes in prevalence and intensity of infection of *Profilicollis altmani* (Perry, 1942) cystacanth (Acanthocephala) parasitizing the mole crab *Emerita analoga*

- (Stimpson, 1857): an El Niño cascade effect? *Helgoland Marine Research* 62(1): 57–62. doi: 10.1007/s10152-007-0082-7
- Oliva ME, Luque JL, Cevallos A (1992) Parásitos de *Emerita analoga* (Stimpson) (Crustacea): implicancias ecológicas. *Boletín de Lima* 79: 77–90.
- Peoples RC, Poulin R (2011) Encystment patterns and metacercarial size of an opecoelid trematode in two polychaete hosts. *Parasitology Research* 109(3): 865–870. doi: 10.1007/s00436-011-2313-8
- Pina S, Riussell-Pinto F, Rodrigues P (2011) Morphological and molecular study of *Microphallus primas* (Digenea: Microphallidae) metacercaria, infecting the shore crab *Carcinus maenas* from northern Portugal. *Folia Parasitologica* 58(1): 48–54. doi: 10.14411/fp.2011.005
- Poulin R (1999) Body size vs abundance among parasite species: positive relationships? *Ecography* 22(3): 246–250. doi: 10.1111/j.1600-0587.1999.tb00499.x
- Poulin R, Morand S (2000) Parasite body size and interspecific variation in levels of aggregation among nematodes. *Journal of Parasitology* 86(3): 642–647. doi: 10.1645/0022-3395(2000)086[0642:PBSAIV]2.0.CO;2
- Pritchard MH, Kruse GOW (1982) The collection and preservation of animal parasites, Technical Bulletin No. 1, The Harold W. Manter Laboratory. University of Nebraska Press, Lincoln, Nebraska.
- Ríos-Elósegui D, Hendrickx ME (2015) Abundance, relative growth and fecundity of *Emerita rathbunae* Schmitt, 1935 (Decapoda, Anomura, Hippidae) in the S.E. Gulf of California, Mexico. *Crustaceana* 88(2): 127–143. doi: 10.1163/15685403-00003400
- Robaldo RB, Monserrat J, Cousin JCB, Bianchini A (1999) Effects of metacercariae (Digenea: Microphallidae) on the hepatopancreas of *Chasmagnathus granulata* (Decapoda: Grapsidae). *Diseases of Aquatic Organisms* 37: 153–157. doi: 10.3354/dao037153
- Ruiz-Daniels R, Beltran S, Poulin R, Lagrue C (2013) Do parasites adopt different strategies in different intermediate hosts? Host size, not host species, influences *Coitocaecum parvum* (Trematoda) life history strategy, size and egg production. *Parasitology* 140(2): 275–283. doi: 10.1017/S0031182012001564
- Smith NF (2007) Associations between shorebird abundance and parasites in the sand crab, *Emerita analoga*, along the California coast. *Journal of Parasitology* 93(2): 265–273. doi: 10.1645/GE-1002R.1
- Studer A, Poulin R (2012) Seasonal dynamics in an intertidal mudflat: the case of a complex trematode life cycle. *Marine Ecology Progress Series* 455: 79–93. doi: 10.3354/meps09761
- Tam YK, Kornfield I, Ojeda FP (1996) Divergence and zoogeography of mole crabs, *Emerita* spp. (Decapoda: Hippidae), in the Americas. *Marine Biology* 125(3): 489–497. doi: 10.1007/bf00353262
- Thomas F, Renaud F, Rousset F, Cezilly F, de Meeüs T (1995) Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Royal Society of London (B)* 260(1359): 349–352. doi: 10.1098/rspb.1995.0103
- Violante-González J, Quiterio-Rendón G, Larumbe-Morán E, Gil-Guerrero S, Rojas-Herrera AA, Carbajal-Violante J (2012) Impacto del parasitismo en la mortalidad del “Chiquilique” *Emerita analoga* (Anomura: Hippidae), en 3 localidades del estado de Guerrero, México. *Tlamati* 4: 14–21.
- Violante-González J, Quiterio-Rendon G, Monks S, García-Ibañez S, Pulido-Flores G, Rojas-Herrera AA, Larumbe-Moran E (2015) Parasite communities of the Pacific mole crab, *Emerita rathbunae* (Anomura: Hippidae), in sandy beaches from Guerrero and Michoacán, Mexico. *Open Journal of Marine Science* 5(4): 468–476. doi: 10.4236/ojms.2015.54037



# Exploration into the hidden world of Mozambique's sky island forests: new discoveries of reptiles and amphibians

Werner Conradie<sup>1,2</sup>, Gabriela B. Bittencourt-Silva<sup>3</sup>, Hanlie M. Engelbrecht<sup>4,5</sup>, Simon P. Loader<sup>6</sup>, Michele Menegon<sup>7</sup>, Cristóvão Nanvonamuquitxo<sup>8</sup>, Michael Scott<sup>9</sup>, Krystal A. Tolley<sup>4,5</sup>

<sup>1</sup> Port Elizabeth Museum (Bayworld), P.O. Box 13147, Humewood 6013, South Africa

<sup>2</sup> South African Institute for Aquatic Biodiversity, P/Bag 1015, Grahamstown, 6140, South Africa

<sup>3</sup> University of Basel, Biogeography Research Group, Department of Environmental Sciences, Basel 4056, Switzerland

<sup>4</sup> South African National Biodiversity Institute, Private Bag X7, Claremont, 7735, South Africa

<sup>5</sup> Department of Botany and Zoology, Stellenbosch University, Matieland 7602, Stellenbosch, South Africa

<sup>6</sup> University of Roehampton, Department of Life Sciences, London, SW15 4JD, United Kingdom

<sup>7</sup> MUSE, Museo delle Scienze, Viale del Lavoro e delle Scienze, 3 Trento 38122, Italy

<sup>8</sup> Faculty of Natural Sciences, Lúrio University, Pemba, 958, Mozambique

<sup>9</sup> Khangela Safaris, www.khangelasafaris.com, Bulawayo, Zimbabwe

<http://zoobank.org/9DA068DA-B881-4091-99FE-252D31DDC7D1>

Corresponding author: Werner Conradie (werner@bayworld.co.za)

## Abstract

Received 27 July 2016

Accepted 9 September 2016

Published 26 September 2016

Academic editor:

Peter Bartsch

## Key Words

Amphibia

Reptilia

conservation

diversity

new species

We carried out a survey of reptiles and amphibians within Afromontane forest and woodland slopes of three inselbergs in northern Mozambique (Mount Mabu, Mount Namuli, and Mount Ribáuè). A total of 56 species (22 amphibians and 34 reptiles) were recorded during the current survey. Our findings substantially increase the number of herpetofaunal species recorded from these mountains (Mount Ribáuè 59%, Mount Mabu 37%, and Mount Namuli 11% of the total species), including one new country record and several putative new species. An updated checklist of the herpetofauna of these mountains is presented.

## Introduction

Northern Mozambique (north of the Zambezi River and south of the Rovuma River) is biologically one of the most poorly known areas in Africa (see Tolley et al. 2016). This is a consequence of the limited infrastructure in the region as well as the protracted civil war (1977-1992), which made travel and exploration problematic. In recent years, several biodiversity surveys have been conducted in northern Mozambique (e.g. Branch et al. 2005a & b, Timberlake et al. 2007, Bayliss 2008, Spottiswoode et al. 2008, Timberlake et al. 2009 & 2012, Bayliss et al. 2010,

Pascal 2011, Portik et al. 2013a, Bayliss et al. 2014, Ohler and Frétey 2014, Farooq and Conradie 2015, Farooq et al. 2015). These expeditions resulted in the discovery of several new species of reptiles (Branch and Bayliss 2009, Branch and Tolley 2010, Portik et al. 2013b, Branch et al. 2014), crustaceans (Daniels and Bayliss 2012, Daniels et al. 2014), butterflies (Congdon et al. 2010, Congdon and Bayliss 2012), and bats (Monadjem et al. 2010, Taylor et al. 2012, Taylor et al. 2015).

The topography of northern Mozambique is characterised by isolated mountains ('inselbergs'), mainly in the west closer to Mt. Mulanje in Malawi, where the highest

peaks are found, but also by chains of smaller inselbergs extending eastwards towards the coast. These montane isolates form an important link between the better studied Eastern Arc Mountains (EAM) and the East African Coastal Forest (EACF). White (1983) classified the vegetation from Mts. Chipirone, Mabu, and Namuli as being associated with the East African Coastal Mosaic (EACM; type 16b). However, Timberlake et al. (2007, 2009, 2012) disregard White's classification and describe the vegetation from these mountains (especially at above 1600 m altitude) as more similar to the moist montane forests from the EAM. The presence of species from both, EAM and EACM, in Mts. Namuli (e.g. Timberlake et al. 2009) and Mabu (e.g. Timberlake et al. 2012) suggests that these mountains form a mosaic of biodiversity from two different ecoregions.

Findings from previous surveys suggest the Mozambican montane sky island forests contain high levels of biodiversity, particularly in terms of single site endemics (e.g. Ryan et al. 1999, Congdon et al. 2010, Portik et al. 2013b, Branch et al. 2014). In particular, high diversity is predicted in less vagile groups because these forests are isolated, and forest species are likely to have speciated *in situ* resulting in a suite of narrow endemics per mountain (Congdon et al. 2010, Branch et al. 2014, Bittencourt-Silva et al. 2016). The biological diversity of these poorly sampled montane forests is expected to be underestimated. This is particularly worrying as these, as yet undescribed, narrow endemics are at risk of extinction because of the substantial land use changes in the region. It is therefore critical that these poorly sampled forests receive attention towards documenting their biological diversity before they disappear.

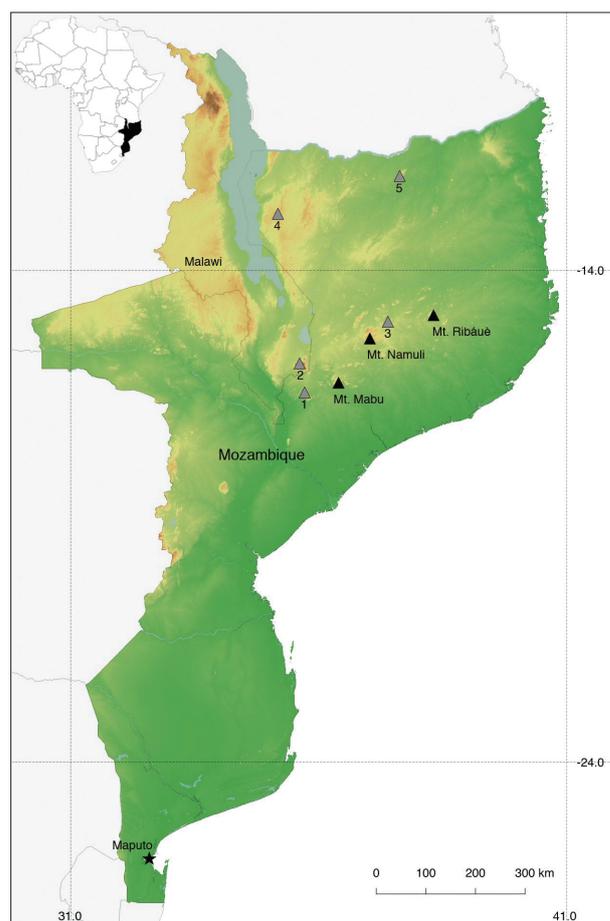
Through targeted fieldwork, we explored three poorly known montane isolates (Mts. Mabu, Namuli, and Ribáuè), to document their herpetofaunal diversity. We conducted intensive surveys on these mountains across several habitats and over an altitudinal gradient searching for amphibians and reptiles. Although some herpetological surveys have been done recently on Mt. Namuli (Timberlake et al. 2009, Portik et al. 2013a, Farooq and Conradie 2015) and Mt. Mabu (Timberlake et al. 2012, Bayliss et al. 2014), Mt. Ribáuè has not been explored for its herpetofauna since 1964 (Blake 1965). The Ribáuè massif consists of two adjacent inselbergs covered with dry miombo and separated by a narrow valley (ca. 3km) with miombo woodland. We only surveyed the eastern part of the massif known as Mt. M'páluwé. We suspect that Blake (1965, p.37 & 38) previously surveyed the M'páluwé section of Mt. Ribáuè and at that time only found seven species from the lower slopes and the forest was not visited. Poynton (1966) later reviewed the amphibians collected by Blake (1965) and recorded a total of ten amphibian species from M'páluwé section of Mt. Ribáuè. Herein we present an annotated checklist of reptiles and amphibians from Mts. Mabu, Namuli, and M'páluwé. Additionally, we provide a summary of all herpetological surveys carried out to date, on these sky island forests of northern Mozambique.

## Material and methods

### Study area

The study area comprises of two mountain blocks in the Zambezia province and one in the Nampula province in northern Mozambique (Fig. 1, Table 1). The landscape in this part of the country is scattered with inselbergs surrounded by miombo woodland forest. Some of these mountains are covered with dense moist forest (e.g. Mt. Chipirone and Mt. Mabu) whereas others are just exposed granitic rocks with isolated shrubs.

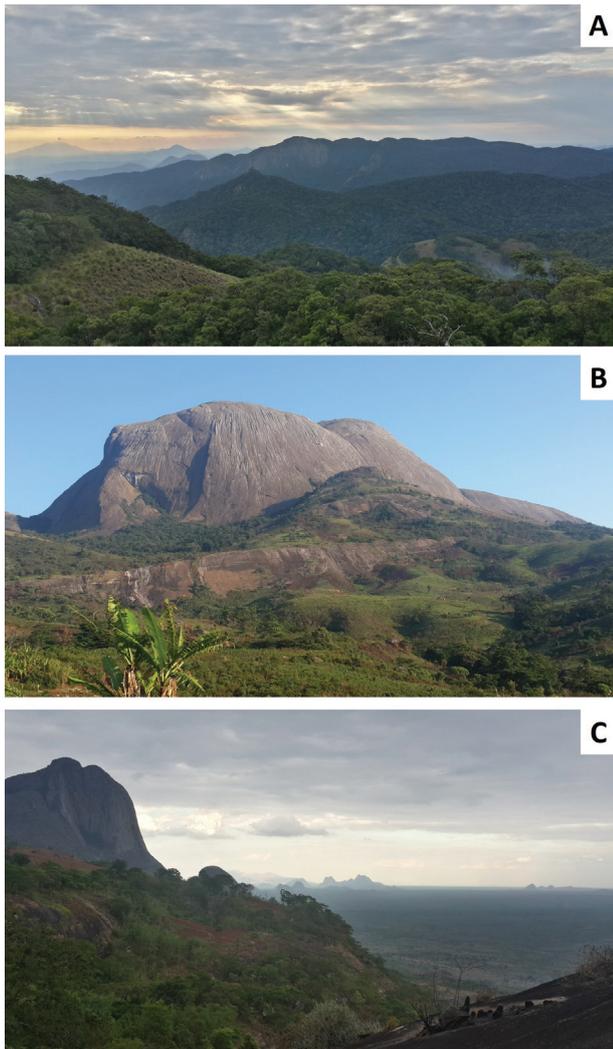
For both Mt. Mabu (Fig. 2A) and Mt. Namuli (Fig. 2B), the vegetation type at low and mid-elevation, i.e. below 800 m above sea level (asl), is dominated by cultivated areas and secondary forest, which gradually changes to denser and moister forests with the increase of elevation (>1000 m asl). On Mt. M'páluwé (Fig. 2C), cultivated areas (called 'shamba' or 'machamba' locally in East Africa) dominate the lower part of the mountain, and at mid and high elevation the forest is drier – possibly due to progressive deforestation in the area as a result of the



**Figure 1.** Map of localities surveyed in northern Mozambique. Black triangles indicate the mountains sampled during this study and grey triangles show other areas previously sampled in northern Mozambique and southern Malawi. Numbers indicate the following localities: (1) Mt. Chipirone, (2) Mt. Mulanje, (3) Mt. Inago, (4) Serra Jeci, (5) Serra Mecula.

**Table 1.** Localities surveyed in northern Mozambique.

| Mountain | Locality         | Province | Latitude   | Longitude | Elevation (m) |
|----------|------------------|----------|------------|-----------|---------------|
| Mabu     | River Camp       | Zambezia | -16.281528 | 36.443778 | 429           |
|          | Forest Base Camp | Zambezia | -16.286222 | 36.400056 | 987           |
|          | Summit Camp      | Zambezia | -16.296817 | 36.392433 | 1609          |
| Namuli   | Pese dome        | Zambezia | -15.386420 | 37.033520 | 1892          |
|          | Ukalini dome     | Zambezia | -15.369367 | 37.061417 | 1616          |
|          | Muretha Plateau  | Zambezia | -15.387194 | 37.044611 | 1892          |
|          | Satellite Camp   | Zambezia | -15.397972 | 37.019778 | 1633          |
| M'pàluwé | Oasis Water Camp | Nampula  | -14.915220 | 38.316450 | 588           |
|          | Miombo area      | Nampula  | -14.902583 | 38.323306 | 714           |
|          | Summit area      | Nampula  | -14.889306 | 38.316139 | 1366          |

**Figure 2.** Study area, northern Mozambique sky islands. **A** – Mount Mabu, **B** – Mount Namuli, **C** – Mount M'pàluwé to the left.

internal fragmentation of the forest due to slash and burn clearing. The summits are characterised by bare granitic rocks with isolated patches of montane heath vegetation. Temporary streams are found in all three mountains but no permanent stream was found in Mt. M'pàluwé.

In northern Mozambique, the average temperature in the warmest months (December–February) varies between 20–25 °C, and in the cooler months between 15–20 °C.

The wet season lasts from November–April when the average rainfall per month is 150–300 mm (McSweeney et al. 2010a & b). During our survey we experienced a total of 36 hours of non-stop heavy rain at Mt. Mabu from 17 to 18 November 2014. The remaining time of the survey was dry and warm with no further rain.

#### Data collection

We conducted herpetofaunal surveys in the study area, between 15 November and 4 December 2014, using a combination of visual encounter survey and standard drift fences with pitfalls (each trap array consisted of 3 × 10 m long and 50 cm high fences positioned in a Y-shape with four pitfall traps at the ends and middle, and two one-way funnels per fence – only employed at Mts. Mabu and Namuli). Diurnal searches were done by actively looking for specific microhabitats including underneath rocks and logs. Nocturnal surveys were carried out with the use of headlamps or flashlights. Specimens were captured by hand, hook stick, noosing or net (e.g. tadpoles), and euthanized, according to ethically approved methods (Conroy et al. 2009), after which they were fixed in 4% buffered formalin for 48 hours and transferred to 70% alcohol for long-term storage in the herpetological collections of the Port Elizabeth Museum (PEM), South Africa, Museo delle Scienze (MUSE), Italy, and Natural History Museum of Maputo, Mozambique. Prior to formalin fixing DNA samples were collected (thigh muscle or liver), and stored in 99% ethanol for future genetic analysis.

Specimens were identified using field guides (Broadley 1990, Branch 1998, Channing 2001, Marais 2004, Du Preez and Carruthers 2009) and morphological comparison to material in the Port Elizabeth Museum. A number of specimens collected were difficult to assign to currently recognised species. In some species, e.g. *Arthroleptis* spp., *Lygodactylus* spp., specimens presented a variety of characters shared with known species and the present assignment is provisional pending ongoing studies. In some cases, further genetic and morphometric analyses will be carried out to confirm species identifications and will be presented elsewhere. We follow Frost (2016) for taxonomy of amphibians, Uetz and Hošek (2016) for reptiles, and were further updated where appropriate. In

addition to our survey findings the following literature was consulted to compile historical records for the study area: Poynton (1996), Branch and Ryan (2001), Timberlake et al. (2009, 2012), Portik et al. (2013a), and Farooq and Conradie (2015). To compile species accounts, specimens were measured for body size: snout-urostyle length (SUL) for anurans and snout-vent length (SVL) and tail length (TL) for reptiles. Measurements were taken using a digital calliper to the nearest 0.1 mm.

## Results and discussion

A total of 27 amphibian species representing 10 families and 14 genera (Table 2), and 45 reptile species (19 snakes, 25 lizards, one tortoise) representing 12 families and 31 genera (Table 3) have been recorded from the three mountains. The current survey contributed numerous additional amphibian and reptile species to the checklist of northern Mozambique inselbergs (13 for Mt. Mabu, five for Mt. Namuli and 19 for Mt. M'páluwé).

During the current survey we recorded four species of reptiles endemic to Mozambique (*Nadzikambia baylissi*, *Rhampholeon maspictus*, *Rhampholeon tilburys*, and *Lygodactylus regulus*), and four putative new species of *Nothophryne* (Bittencourt-Silva et al. 2016). We also documented one new country record, the snake *Duberria shirana* (see account below). *Acanthocercus atricollis* was omitted from the final checklist of Mt. Namuli. It was erroneously reported by Portik et al. (2013a) to be recorded from Mt. Namuli, although they only listed specimens collected from Serra Jeci, more than 300 km to the northwest of Mt. Namuli. This record was followed in error by Farooq and Conradie (2015). Portik et al. (2013a) also reported a few species from the low-lying Gurué village and surroundings (*Breviceps mossambicus*, *Sclerophrys gutturalis*, *S. pusilla*, *Lygodactylus capensis*, *Naja melanoleuca*, and *Boaedon capensis*) and included them in their checklist for the inselbergs. As the focus of this study is to record the herpetological diversity of the sky island forests we omitted the above records, including *Agama mossambica* (current study – see species account).

**Table 2.** Updated species lists for amphibians based on historical records as well as data from the current surveys. Parentheses in the totals given denote the number of new species recorded during the current surveys. Literature records are indicated with L and new records with N.

| ORDER/Family             | Species                                    | Mount Mabu    | Mount Namuli  | Mount M'páluwé |
|--------------------------|--|---------------|---------------|----------------|
| <b>ANURA</b>             |  |               |               |                |
| <b>Arthroleptidae</b>    | <i>Arthroleptis francei</i>                | L,N           | L,N           |                |
|                          | <i>Arthroleptis stenodactylus</i>          | L,N           | N             | L,N            |
|                          | <i>Arthroleptis xenodactyloides</i>        | L,N           | L             | L,N            |
|                          | <i>Leptopelis broadleyi</i>                |               |               | L,N            |
|                          | <i>Leptopelis flavomaculatus</i>           | L,N           | N             |                |
| <b>Brevicipitidae</b>    | <i>Breviceps mossambicus</i>               |               | L,N           | N              |
| <b>Bufonidae</b>         | <i>Sclerophrys gutturalis</i>              |               | L,N           |                |
|                          | <i>Sclerophrys pusilla</i>                 | L,N           | L             | N              |
| <b>Hyperoliidae</b>      | <i>Afraxalus brachycnemis</i>              | L,N           | L             | N              |
|                          | <i>Afraxalus fornasini</i>                 | N             |               |                |
|                          | <i>Hyperolius nasutus</i> complex          |               | L             |                |
|                          | <i>Hyperolius marmoratus albofasciatus</i> |               | L,N           |                |
|                          | <i>Hyperolius spinigularis</i>             |               | L             |                |
| <b>Phrynobatrachidae</b> | <i>Hyperolius substriatus</i>              | L,N           | L,N           |                |
|                          | <i>Phrynobatrachus natalensis</i>          |               |               | L              |
|                          | <i>Phrynobatrachus acridoides</i>          |               |               | L,N            |
| <b>Pipidae</b>           | <i>Phrynobatrachus mababiensis</i>         |               | L             | L,N            |
|                          | <i>Xenopus laevis</i>                      | N             |               |                |
| <b>Ptychadenidae</b>     | <i>Ptychadena anchietae</i>                |               |               | L,N            |
|                          | <i>Ptychadena taenioscelis</i>             |               |               | N              |
|                          | <i>Ptychadena guibei</i>                   |               |               | L              |
|                          | <i>Ptychadena</i> cf. <i>porossisima</i>   |               | L,N           |                |
| <b>Pyxicephalidae</b>    | <i>Amietia queckettii</i>                  | N             | L,N           | L,N            |
|                          | <i>Nothophryne</i> cf. <i>broadleyi</i>    |               | L,N           | L,N            |
|                          | <i>Strongylopus fuelleborni</i>            | N             | L,N           |                |
| <b>Rhacophoridae</b>     | <i>Chiromantis xerampelina</i>             |               |               | L              |
| <b>GYMNOPHIONA</b>       |  |               |               |                |
| <b>Scolecophoridae</b>   | <i>Scolecophorus kirkii</i>                | N             | L             |                |
|                          |  | <b>12 (5)</b> | <b>18 (2)</b> | <b>15 (4)</b>  |

**Table 3.** Updated species lists for reptiles based on historical records as well as data from the current surveys. Parentheses in the totals given denote the number of new species recorded during the current surveys. Literature records are indicated with L and new records with N.

| ORDER/Family          | Species                                      | Mount Mabou   | Mount Namuli  | Mount M'pàluwé |
|-----------------------|--|---------------|---------------|----------------|
| <b>CHELONIA</b>       |  |               |               |                |
| <b>Testudinidae</b>   | <i>Kinixys belliana</i>                      |               | L             |                |
| <b>SQUAMATA</b>       |  |               |               |                |
| <b>Agamidae</b>       | <i>Agama kirkii</i>                          |               | L,N           | N              |
|                       | <i>Agama mossambica</i>                      | L             | L             |                |
| <b>Chamaeleonidae</b> | <i>Chamaeleo dilepis</i>                     |               | L,N           |                |
|                       | <i>Nadzikambia baylissi</i>                  | L,N           | N             |                |
|                       | <i>Rhampholeon tilburyi</i>                  |               | L,N           |                |
|                       | <i>Rhampholeon maspictus</i>                 | L,N           |               |                |
|                       | <i>Rhampholeon</i> sp.                       |               |               | N              |
|                       | <i>Trioceros melleri</i>                     | L             |               |                |
| <b>Cordylidae</b>     | <i>Platysaurus maculatus</i>                 |               |               | L,N            |
| <b>Gekkonidae</b>     | <i>Chondrodactylus turneri</i>               |               |               | N              |
|                       | <i>Hemidactylus mabouia</i>                  | N             | L             |                |
|                       | <i>Hemidactylus platycephalus</i>            | L             |               | N              |
|                       | <i>Lygodactylus grotei</i>                   | N             |               |                |
|                       | <i>Lygodactylus regulus</i>                  |               | L,N           |                |
|                       | <i>Lygodactylus</i> cf. <i>rex</i>           | N             |               |                |
|                       | <i>Lygodactylus</i> sp.                      |               |               | N              |
| <b>Lacertidae</b>     | <i>Holaspis laevis</i>                       | N             |               |                |
| <b>Scincidae</b>      | <i>Melanoseps</i> cf. <i>ater</i>            | L,N           | L             |                |
|                       | <i>Mochlus afer</i>                          | L             |               |                |
|                       | <i>Panaspis wahlbergi</i>                    |               | L             |                |
|                       | <i>Trachylepis boulengeri</i>                |               |               | N              |
|                       | <i>Trachylepis maculilabris</i>              | N             |               |                |
|                       | <i>Trachylepis margaritifer</i>              | L             | L             | N              |
|                       | <i>Trachylepis striata</i>                   |               | L,N           |                |
|                       | <i>Trachylepis varia</i>                     | L,N           | L,N           | N              |
| <b>Colubridae</b>     | <i>Dispholidus typus</i>                     |               | N             | L,N            |
|                       | <i>Dipsadoboa</i> cf. <i>shrevei shrevei</i> | L,N           |               | N              |
|                       | <i>Philothamnus angolensis</i>               |               | L,N           |                |
|                       | <i>Philothamnus</i> cf. <i>carinatus</i>     | L             |               |                |
|                       | <i>Philothamnus hoplogaster</i>              | N             |               | N              |
|                       | <i>Thelotornis mossambicanus</i>             | N             | L             | N              |
| <b>Elapidae</b>       | <i>Naja melanoleuca</i>                      | L,N           | L             |                |
|                       | <i>Naja mossambica</i>                       |               |               | N              |
| <b>Lamprophiidae</b>  | <i>Boaedon capensis</i>                      |               | L             | N              |
|                       | <i>Duberria shirana</i>                      |               | N             |                |
|                       | <i>Gonionotophis capensis</i>                | N             |               |                |
|                       | <i>Lycophidion acutirostre</i>               |               | L             |                |
|                       | <i>Psammophis orientalis</i>                 |               |               | N              |
|                       | <i>Psammophylax variabilis</i>               |               | L,N           |                |
| <b>Natricidae</b>     | <i>Natriciteres sylvatica</i>                | L             | L             |                |
| <b>Viperidae</b>      | <i>Atheris mabuensis</i>                     | L             | L             |                |
|                       | <i>Bitis arietans</i>                        |               | L             | N              |
|                       | <i>Bitis gabonica</i>                        | L             | L             |                |
|                       | <i>Causus defilippi</i>                      |               |               | L              |
|                       |  | <b>23 (8)</b> | <b>27 (3)</b> | <b>17 (15)</b> |

## Selected species accounts

### Amphibia

#### Anura

#### Arthroleptidae

##### *Arthroleptis francei* Loveridge, 1953

**Material.** Mt. Mabu (PEM A11180, male, 25.5 mm SUL; PEM A11181, male, 21.4 mm SUL; PEM A11182, male, 22.9 mm SUL; PEM A11183, male, 19.0 mm SUL; PEM A11192, female, 34.5 mm SUL; PEM A11193, female, 24.6 mm SUL; PEM A11194, male, 20.6 mm SUL; PEM A11195, juvenile, 12.1 mm SUL; PEM A11196, juvenile, 14.7 mm SUL; PEM A11197, juvenile, 17.3 mm SUL; PEM A11198, male, 23.16 mm SUL; PEM A11199, female, 34.7 mm SUL; PEM A11200, male, 24.7 mm SUL; PEM A11201, female, 21.5 mm SUL; PEM A11202, male, 21.4 mm SUL; PEM A11204, male, 25.7 mm SUL; PEM A11205, juvenile, 18.5 mm SUL; PEM A11206, female, 23.2 mm SUL; PEM A11207, male, 23.6 mm SUL; PEM A11208, male, 22.8 mm SUL; PEM A11209, female, 31.3 mm SUL; PEM A11268, female, 31.0 mm SUL; PEM A11292, female, 36.2 mm SUL; PEM A11294, juvenile, 15.1 mm SUL; PEM A11403, female, 31.8 mm SUL), Mt. Namuli (PEM A11296, juvenile, 13.0 mm SUL; PEM A11297, juvenile, 16.6 mm SUL; PEM A11298, female, 32.6 mm SUL; PEM A11299, male, 20.4 mm SUL; PEM A11303, female, 32.0 mm SUL; PEM A11304, male, 24.8 mm SUL; PEM A11305, juvenile, 17.4 mm SUL; PEM A11306, juvenile, 17.8 mm SUL; PEM A11307, female, 35.4 mm SUL; PEM A11308, juvenile, 18.4 mm SUL; PEM A11341, juvenile, 15.0 mm SUL; PEM A11398, male, 24.0 mm SUL; PEM A11404, female, 35.5 mm SUL). **Comments:** Specimens were collected from moist leaf litter in closed-canopy forest (Fig. 3A). Some individuals were found sitting on low vegetation (<1 m). All mature males exhibit extended third finger and dark throat.

##### *Arthroleptis stenodactylus* Pfeffer, 1893

**Material.** Mt. Mabu (PEM A11203, male, 25.5 mm SUL; PEM A11249, male, 31.6 mm SUL; PEM A11266, female, 30.4 mm SUL; PEM A11267, male, 23.4 mm SUL; PEM A11277, male, 22.1 mm SUL; PEM A11293, male, 23.0 mm SUL), Mt. M'páluwé (PEM female, 26.1 mm SUL). **Comments:** Found in both dry transitional miombo woodland and montane forest (Fig. 3C). This species is very similar to *A. francei*, which seems to be more abundant in these forests. One feature that helps to separate these two species is the hind limbs, being shorter in *A. stenodactylus*.

##### *Arthroleptis xenodactyloides* Hewitt, 1933

**Material.** Mt. Mabu (PEM A11210, female, 16.3 mm SUL; PEM A11211, male, 16.1 mm SUL; PEM

A11212, male, 16.8 mm SUL; PEM A11213, female, 18.3 mm SUL; PEM A11214, male, 15.3 mm SUL; PEM A11215, male, 12.1 mm SUL; PEM A11216, female, 15.2 mm SUL; PEM A11217, male, 13.9 mm SUL; PEM A11218, male, 13.2 mm SUL; PEM A11219, male, 13.1 mm SUL; PEM A11220, male, 15.4 mm SUL; PEM A11221, female, 17.4 mm SUL; PEM A11222, female, 17.1 mm SUL; PEM A11223, male, 15.8 mm SUL; PEM A11224, male, 12.9 mm SUL; PEM A11225, male, 14.2 mm SUL; PEM A11226, male, 15.9 mm SUL; PEM A11227, male, 13.5 mm SUL; PEM A11228, male, 17.7 mm SUL; PEM A11229, female, 15.0 mm SUL; PEM A11230, female, 16.7 mm SUL; PEM A11231, female, 17.4 mm SUL; PEM A11232, female, 16.3 mm SUL; PEM A11233, female, 15.2 mm SUL; PEM A11234, female, 17.6 mm SUL; PEM A11269, male, 15.6 mm SUL; PEM A11270, female, 18.8 mm SUL; PEM A11271, female, 22.1 mm SUL; PEM A11272, female, 19.9 mm SUL; PEM A11273, male, 17.4 mm SUL; PEM A11274, male, 18.3 mm SUL; PEM A11275, female, 19.0 mm SUL; PEM A11276, female, 16.8 mm SUL; PEM A11278, female, 17.5 mm SUL; PEM A11279, female, 17.6 mm SUL; PEM A11280, male, 14.8 mm SUL; PEM A11295, male, 15.4 mm SUL; PEM A11401, male, 17.8 mm SUL; PEM A11402, female, 17.2 mm SUL), Mt. M'páluwé (PEM A11377, male, 15.0 mm SUL; PEM A11378, male, 15.5 mm SUL; PEM A11379, female, 17.6 mm SUL; PEM A11380, female, 19.3 mm SUL; PEM A11386, female, 17.8 mm SUL; PEM A11387, female, 17.5 mm SUL; PEM A11388, female, 16.3 mm SUL; PEM A11389, male, 15.2 mm SUL). **Comments:** In Mt. Mabu it was common on the forest floor, and at Mt. M'páluwé it was found in moist leaf litter at lower elevations (Fig. 3B). Mature males exhibit extended third finger and dark throat.

##### *Leptopelis broadleyi* Poynton, 1985

**Material.** Mt. M'páluwé (PEM A11367, male, 30.9 mm SUL). **Comments:** Sub-adult male specimen was collected from mid-elevation slopes while calling from low vegetation. This species is part of the *L. argenteus* group. Some authors regard this as a valid species (Poynton and Broadley 1987, Schiøtz 1999, Channing and Howell 2006, Pickersgill 2007, Du Preez and Carruthers 2009, Mercurio 2011, Ohler and Frétey 2014), while Frost (2016) considers it as a junior synonym of *L. argenteus* based on similar calls. Herein we consider *L. broadleyi* as a valid species pending further investigation.

##### *Leptopelis flavomaculatus* (Günther, 1864)

**Material.** Mt. Mabu (PEM A11240, male, 50.3 mm SUL; PEM A11241, male, 38.1 mm SUL; PEM A11242, female, 72.0 mm SUL; PEM A11243, male, 48.1 mm

SUL; PEM A11244, male, 39.2 mm SUL; PEM A11245, female, 71.0 mm SUL; PEM A11246, female, 35.1 mm SUL; PEM A11247, male, 41.0 mm SUL; PEM A11290, male, 42.8 mm SUL), Mt. Namuli (PEM A11309, female, 65.2 mm SUL). **Comments:** Common along streams at both Mt. Mabu and Mt. Namuli. In most cases, the males of this species are much smaller and have a uniform lime-green dorsum with yellow spots. All the males have well developed pectoral glands. Females are much larger with dull brown dorsum with scattered green spots or patterns.

### Brevicipitidae

#### *Breviceps mossambicus* Peters, 1882

**Material.** Mt. Namuli (PEM A11310, male, 25.4 mm SUL), Mt. M'pàluwé (PEM A11362, male, 23.0 mm SUL). **Comments:** Both specimens are males, based on the presence of a very dark throat and small size. The specimen from Mt. M'pàluwé was collected at night in a shamba at mid-elevation (ca. 1000 m).

### Bufonidae

#### *Sclerophrys gutturalis* (Power, 1927)

**Material.** Mt. Namuli (PEM A11348, female, 68.8 mm SUL). **Comments:** One adult female collected from Muretha Plateau at night. No red infusions on the thighs or side of the body were observed, but the well-elevated parotid glands exclude it from being *S. pusilla*.

#### *Sclerophrys pusilla* (Mertens, 1937)

**Material.** Mt. Mabu (PEM A11260, male 64.6 mm SUL; PEM A11261, male 55.3 mm SUL; PEM A11262, male 63.1 mm SUL; PEM A11263, male 60.3 mm SUL; PEM A11264, male 54.8 mm SUL; PEM A11265, male 63.1 mm SUL), Mt. M'pàluwé (PEM A11364, male 63.1 mm SUL; PEM A11365, male, 53.2 mm SUL, PEM A11384, male 64.9 mm SUL). **Comments:** Specimens were found calling along low-elevation streams at dusk.

### Hyperoliidae

#### *Afrixalus brachynemis* (Boulenger, 1896)

**Material.** Mt. Mabu (PEM A11250, male, 19.5 mm SUL), Mt. M'pàluwé (PEM A11392, female, 317.8 mm SUL; PEM A11393, female, 21.2 mm SUL). **Comments:** Adult male was collected during the day sitting on a leaf in an open area at lower slopes of Mt. Mabu. Two females were collected sheltering in sugarcane fronds at the base of Mt. M'pàluwé. Species identification was based on the absence of dorsal patterning and transverse tibial bands (Pickersgill 2007). Others also reported this species to be present in northern Mozambique (Branch 2004, Portik et al. 2013a, Ohler and Frétey 2014).

#### *Afrixalus fornasinii* (Bianconi, 1849)

**Material.** Mt. Mabu (PEM A11251, female, 36.5 mm SUL; PEM A11252, female, 38.3 mm SUL; PEM A11253, female, 31.7 mm SUL). **Comments:** Three females were collected along a low-elevation forest stream. One female (PEM A11252) was gravid (collected 15 November 2014).

#### *Hyperolius marmoratus albofasciatus* (Hoffman, 1944)

**Material.** Mt. Namuli (PEM A11311, male, 27.8 mm SUL; PEM A11312, female, 26.5 mm SUL; PEM A11313, female, 33.6 mm SUL; PEM A11349, juvenile, 15.8 mm SUL; PEM A11350, juvenile, 18.8 mm SUL; PEM A11354, male, 30.6 mm SUL; PEM A11355, male, 33.7 mm SUL; PEM A11356, male, 34.2 mm SUL; PEM A11360, juvenile, 26.2 mm SUL). **Comments:** Found to be abundant on the grassland of the Muretha Plateau at Mt. Namuli. Males were found calling from very low vegetation close to water level. Adult males and females exhibit the same dorsal pattern indicating no sexual dichromatism. The *H. marmoratus* group consists of many regional colour patterns and this population conforms to that of *H. marmoratus albofasciatus* (Schiotz 1999). We believe that the two juvenile *H. marmoratus* reported from Mt. Namuli by Portik et al. (2013a) belongs to the same species group.

#### *Hyperolius substriatus* Ahl, 1931

**Material.** Mt. Mabu (PEM A11235, female, 27.7 mm SUL; PEM A11236, female, 30.4 mm SUL; PEM A11237, female, 28.0 mm SUL; PEM A11238, female, 27.3 mm SUL; PEM A11239, female, 29.0 mm SUL; PEM A11281, female, 29.7 mm SUL; PEM A11282, female, 29.6 mm SUL; PEM A11283, female, 23.4 mm SUL; PEM A11284, female, 35.3 mm SUL; PEM A11285, female, 22.5 mm SUL; PEM A11286, female, 22.1 mm SUL; PEM A11287, female, 29.1 mm SUL; PEM A11288, female, 27.2 mm SUL; PEM A11289, female, 26.9 mm SUL), Mt. Namuli (PEM A11314, female, 27.3 mm SUL; PEM A11315, female, 27.4 mm SUL; PEM A11316, female, 23.4 mm SUL; PEM A11317, female, 32.4 mm SUL; PEM A11318, female, 32.4 mm SUL; PEM A11361, female, 24.8 mm SUL). **Comments:** Found in abundance at low, mid (ca. 920 m asl) and high elevations along forested streams at both Mts. Mabu and Namuli. All material showed typical adult coloration, similar to that reported by Portik et al. (2013a) from Mt. Namuli.

### Phrynobatrachidae

#### *Phrynobatrachus acridoides* (Cope, 1867)

**Material.** Mt. M'pàluwé (PEM A11366, male, 26.6 mm SUL; PEM A1375, male, 26.4 mm SUL). **Comments:** Found moving near stagnant pools at a low-elevation

(544 m asl) streams. Species identification was based on extensive webbing and swollen toe tips. Poynton (1966) records both *P. natalensis* and *P. acridoides* from Mt. M'pàluwé area. We only found the latter.

### *Phrynobatrachus mababiensis* FitzSimons, 1932

**Material.** Mt. M'pàluwé (PEM A11381, male, 14.6 mm SUL; PEM A11382, male, 13.5 mm SUL; PEM A11383, male, 12.7 mm SUL; PEM A11390, male, 14.0 mm SUL; PEM A11391, male, 14.8 mm SUL; PEM A11394, female, 14.5 mm SUL). **Comments:** Specimens were found in dry leaf litter at low elevation, and two individuals were collected in the same microhabitat as *Nothophryne* cf. *broadleyi* (in water seepage over rocks).

### Pipidae

#### *Xenopus laevis* (Daudin, 1802)

**Material.** Mt. Mabu (PEM A11291, female, 45.8 mm SUL). **Comments:** One specimen was collected from a low-elevation stream below an old hydroelectricity dam. Poynton and Broadley (1991) report no records of *X. laevis* for northern Mozambique, but do report it from southern Malawi. The Malawi population has been confirmed by molecular analyses to be *X. laevis* (Furman *et al.* 2015). On the other hand, *X. muelleri* is common in lowland Mozambique. Ohler and Frétey (2014) reported the first record of *X. petersii* for Mozambique. Based on a low number of plaques (17 plaques around the eye and 20 from eye to vent) their assignment of the specimen to *X. petersii* is doubtful and most likely represents *X. laevis* (which shows the same level of variation in plaque counts).

### Ptychadenidae

#### *Ptychadena anchietae* (Bocage, 1868)

**Material.** Mt. M'pàluwé (PEM A11376, male, 35.0 mm SUL). **Comments:** Was found on dry leaf litter at low elevation.

#### *Ptychadena* cf. *porosissima* (Steindachner, 1867)

**Material.** Mt. Namuli (PEM A11351, female, 31.8 mm SUL). **Comments:** Collected from grassland on Muretha Plateau. This specimen is tentatively identified as *Ptychadena* cf. *porosissima* using the key provided in Poynton and Broadley (1985). The specimen lacks the characteristic tibial white line.

#### *Ptychadena taenioscelis* Laurent, 1954

**Material.** Mt. M'pàluwé (PEM A11368, female, 31.7 mm SUL). **Comments:** Found on dry leaf litter at low

slopes of Mt. M'pàluwé. Identification was based on the key provided in Poynton and Broadley (1985).

### Pyxicephalidae

#### *Amietia delalandii* (Duméril & Bibron, 1841)

**Material.** Mt. Mabu (PEM A11188, male, 77.2 mm SUL; PEM A11189, juvenile, 27.3 mm SUL; PEM A11190, male 58.3 mm SUL; PEM A11191, female, 75.5 mm SUL; PEM A11254, male, 52.8 mm SUL; PEM A11255, juvenile, 29.3 mm SUL; PEM A11256, female, 63.3 mm SUL; PEM A11257, female, 73.3 mm SUL; PEM A11258, female, 74.7 mm SUL; PEM A11259, juvenile, 32.5 mm SUL), Mt. Namuli (PEM A11300, female, 83.7 mm SUL; PEM A11301, female, 44.0 mm SUL; PEM A11302, male, 74.6 mm SUL; PEM A11319, male, 86.4 mm SUL; PEM A11397, female, 50.3 mm SUL, PEM T692-3), Mt. M'pàluwé (PEM A11363, male, 57.0 mm SUL; PEM A11373; male, 32.0 mm SUL; PEM A11374, juvenile, 29.8 mm SUL; PEM A11395, juvenile, 29.3 mm SUL; PEM A11396, juvenile, 26.4 mm SUL). **Comments:** Channing and Baptista (2013) revised southern African river frogs and restrict *A. angolensis* to Angola and assign southern populations of *A. angolensis* to either *A. quecketti* or *A. poyntoni*. Channing *et al.* (2016) re-instated *A. delalandii* as a senior synonym of *A. quecketti*. More recently, Larson *et al.* (2016) identified several well-supported cryptic lineages of river frogs previously assigned to *A. angolensis* in the Albertine Rift region, which refer that further cryptic diversity can be expected in East Africa. The phylogenetic affinities of our newly sampled population are unknown and additional work will be necessary to clarify its taxonomic status. Commonly found at both low and high elevation in forested streams (Fig. 3D).

#### *Nothophryne* cf. *broadleyi* Poynton, 1963

**Material.** Mt. Namuli (PEM A11320, male, 17.9 mm SUL; PEM A11321, female, 22.7 mm SUL; PEM A11322, male, 18.1 mm SUL; PEM A11322, PEM A11323, female, 23.4 mm SUL; PEM A11324, male, 18.0 mm SUL; PEM A11325, juvenile, 19.0 mm SUL; PEM A11326, male, 17.5 mm SUL; PEM A11327, male, 18.7 mm SUL; PEM A11328, female, 25.3 mm SUL; PEM A11329, male, 19.5 mm SUL; PEM A11330, female, 20.7 mm SUL; PEM A11331, male, 18.2 mm SUL; PEM A11332, juvenile, 17.1 mm SUL; PEM A11333, male, 17.4 mm SUL; PEM A11334, male, 18.2 mm SUL; PEM A11335, male, 17.5 mm SUL; PEM A11336, juvenile, 13.9 mm SUL; PEM A11337, juvenile, 13.3 mm SUL; PEM A11338, juvenile, 15.7 mm SUL; PEM A11339, female, 25.9 mm SUL; PEM A11340, male, 17.5 mm SUL; PEM A11342, male, 18.1 mm SUL; PEM A11343, female, 22.0 mm SUL; PEM A11344, male, 18.4 mm SUL; PEM A11345, male, 18.0 mm SUL; PEM A11346, male,

17.7 mm SUL; PEM A11347, male, 17.4 mm SUL), Mt. M'pàluwé (PEM A11369, female, 21.7 mm SUL; PEM A11370, female, 23.8 mm SUL; PEM A11371, male, 19.2 mm SUL; PEM A11372, male, 18.1 mm SUL). **Comments:** Specimens were collected both during the day and at night from rocky outcrops in water seepages (Fig. 3E, 3F). Historically, *Nothophryne* has only been reported from two localities: Mt. Mulanje, Malawi (type locality) and Mt. M'pàluwé in Mozambique (Poynton 1962 & 1966, Blake 1965), both higher than 1200 m asl. Timberlake et al. (2009) were the first to report this species from Mt. Namuli. Some calls similar to *Nothophryne* were heard from the summit of Mt. Mabu, but no frog was visually located to confirm the species occurrence in this mountain. Genetic analyses suggest a cryptic diversity in this genus (Bittencourt-Silva et al. 2016) and additional work is underway to update its taxonomy. Based on its small distribution this nominotypical species is listed as Endangered (Mazibuko and Poynton 2004). Referring to that the undescribed species will receive similar red list status.

#### *Strongylopus* cf. *fuelleborni* (Nieden, 1911)

**Material.** Mt. Mabu (PEM A11184, male, 35.5 mm SUL; PEM A1185, male, 36.3 mm SUL; PEM A11186, male, 35.3 mm SUL; PEM A11857, male, 37.3 mm SUL, PEM T691), Mt. Namuli (PEM 11352, juvenile, 27.9 mm SUL; PEM A11353, female, 49.8 mm SUL; PEM A11357, female, 49.1 mm SUL; PEM A11358, female, 49.9 mm SUL; PEM A11359, female, 43.6 mm SUL). **Comments:** Part of the *Strongylopus faciatus* group that has seen two newly described species in recent years (Channing and Davenport 2002, Clarke and Poynton 2005). Mercurio (2011) assign *Strongylopus* from Mt. Mulanje to *S. merumontanus*. While Poynton (2004) don't list them from southern Malawi and rather refer to them as *S. fuelleborni*. The phylogenetic affinities of our newly sampled population are unknown and additional work will be necessary to clarify its taxonomic status. For now, we assign them to *S. fuelleborni*. Adult specimens and tadpoles were found along a high-elevation stream in a patch of Afromontane forest at Mt. Mabu (Fig. 3G). Specimens from Mt. Namuli were collected along marshy areas in montane grassland.

#### Gymnophiona Scolecomorphidae

#### *Scolecomorphus* cf. *kirkii* Boulenger, 1883

**Material.** Mt. Mabu (PEM A11248). **Comments:** A single specimen was found under a log in the closed canopy forest of Mt. Mabu after 36 hours of heavy rain (Fig. 3H). The specimen (probably a young female) measures 210 mm total length, 6.3 mm body width and has 142 pri-

mary annuli. The olive-brown dorsal colouration of the preserved specimen is dorsally restricted and the venter is light yellowish (pinkish in life), agreeing with the description of Nussbaum (1985). This new record represents the southernmost distribution of *Scolecomorphus kirkii*, and for all African caecilians. Branch (2004) reported the first ever caecilian record for Mozambique from dry transitional miombo vegetation at the edge of Serra Mecula, while Farooq and Conradie (2015) recorded a second record from Mt. Namuli.

#### Reptilia Squamata Agamidae

#### *Agama kirkii* Boulenger, 1885

**Material.** Mt. Namuli (PEM R21169, male, 93.0 mm SVL; PEM R21171, female, 81.5 mm SVL), Mt. M'pàluwé (PEM R21159, female, 77.2 mm SVL; PEM R21211, male, 106.9 mm SVL). **Comments:** Collected at the lower slopes on the rocky outcrops of both Mt. Namuli and Mt. M'pàluwé. Both males brightly coloured, while females are dull in overall colouration.

#### *Agama mossambica* Peters, 1854

**Material.** Mt. Namuli (PEM R21114, female, 95.7 mm SVL). **Comments:** One specimen collected in Gurùè town. This record was omitted from our final checklist of the inselbergs, as it was collected from the low lying town (see Results and Discussion).

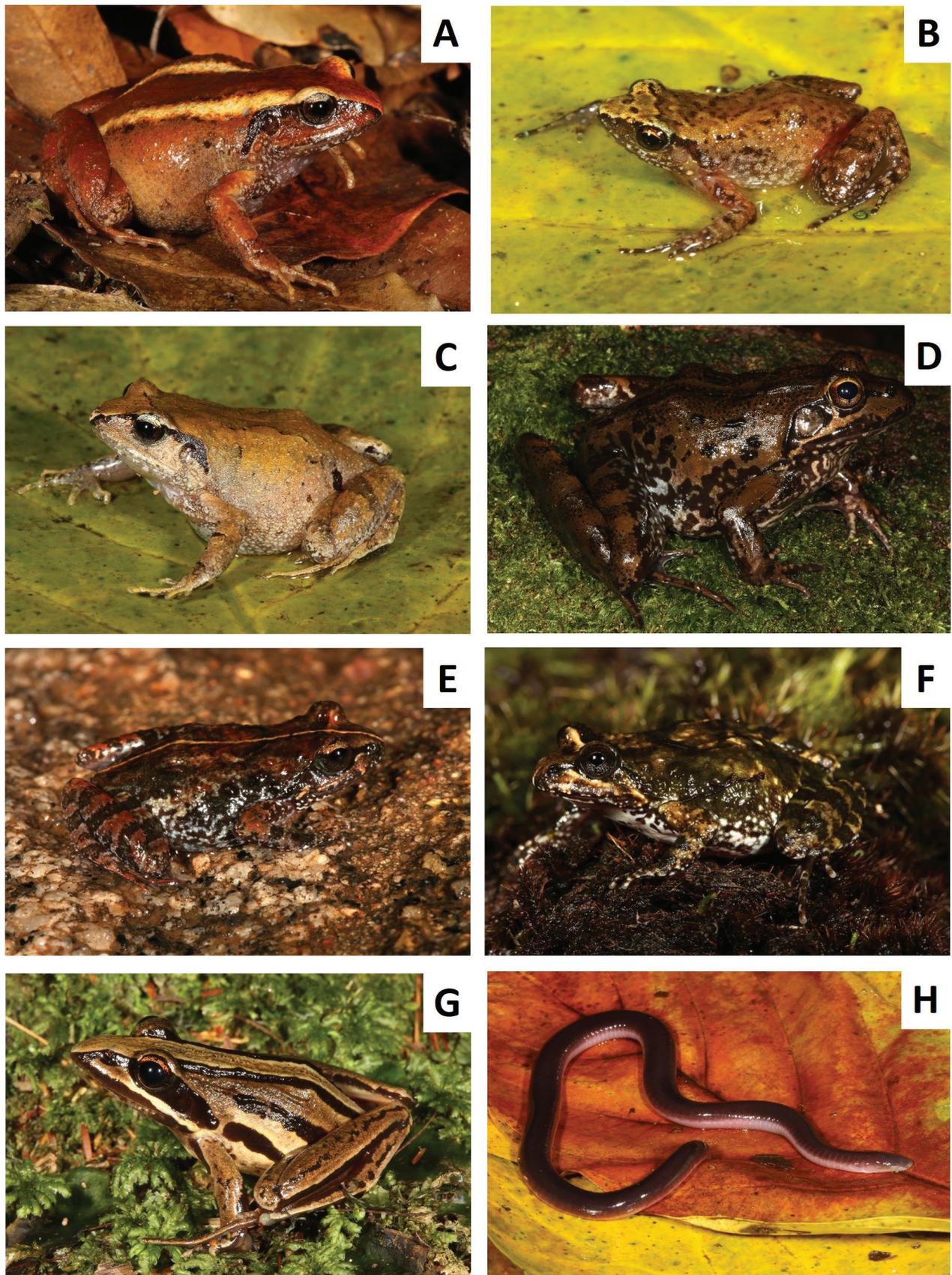
#### Chamaeleonidae

#### *Chamaeleo dilepis* Leach, 1819

**Material.** Mt. Namuli (PEM R21170, female, 104.7 mm SVL). **Comments:** One specimen donated by a local, presumably obtained from near the village just below the Muretha Plateau.

#### *Nadzikambia baylissi* Branch & Tolley, 2010

**Material.** Mt. Mabu (PEM R21128, male, 73.7 mm SVL; PEM R21129, female, 64.8 mm SVL; PEM R21130, female, 66.4 mm SVL; PEM R21131, male, 68.3 mm SVL; PEM R21132, female, 57.9 mm SVL), Mt. Namuli (PEM R21164; female, 79.9 mm SVL; PEM R21165, male, 71.4 mm SVL; PEM R21166, female, 75.5 mm SVL; PEM R21167, female, 79.3 mm SVL; PEM R21188, female, 74.3 mm SVL; PEM R21189, female, 72.0 mm SVL; PEM R21190, female, 80.1 mm SVL). **Comments:** Collected from canopy forest, in Afromontane forest above 600 m asl. Historically, *Nadzikambia* was considered a



**Figure 3.** A selection of amphibians from northern Mozambique inselbergs. **A** – *Arthroleptis francei* (Mt. Namuli, PEM A11303), **B** – *Arthroleptis xenodactyloides* (Mt. Mabu), **C** – *Arthroleptis stenodactylus* (Mt. Mabu), **D** – *Amietia delalandii* (Mt. Namuli, PEM A11319), **E** – *Nothophryne* cf. *broadleyi* (Mt. M'páluwé, PEM A11370), **F** – *Nothophryne* cf. *broadleyi* (Mt. Namuli), **G** – *Strongylopus* cf. *fuelleborni* (Mt. Mabu, PEM A11184), **H** – *Scolecomorphus* cf. *kirkii* (Mt. Mabu, PEM A11248).

monotypic genus endemic to Mt. Mulanje (Broadley 1965a), but recently Branch and Tolley (2010) described a second species from Mt. Mabu. We here report the first record for Mt. Namuli (Fig. 4A).

#### *Rhampholeon tilburyi* Branch, Bayliss & Tolley, 2014

**Material.** Mt. Namuli (PEM R21155, female, 58.7 mm SVL; PEM R21156, female, 53.2 mm SVL; PEM R21157, male, 62.2 mm SVL; PEM R21158, male, 65.4 mm SVL; PEM R21160, male, 62.7 mm SVL; PEM R21161, male, 61.4 mm SVL; PEM R21162, male, 66.5 mm SVL; PEM R21163, female, 69.7 mm SVL; PEM R21174, female, 61.1 mm SVL; PEM R21175; PEM R21176, female, 57.6 mm SVL; PEM R21177, juvenile, 26.6 mm SVL; PEM R21178, female, 52.3 mm SVL; PEM R21191, female, 60.6 mm SVL; PEM R21192, male, 45.4 mm SVL; PEM R21193, juvenile male, 28.2 mm SVL; PEM R21194, female, 58.2 mm SVL). **Comments:** This newly described species of pygmy chameleon was collected from Mt. Namuli at both the Ukalini forest and the forest patches on the Muretha Plateau (Fig. 4B). Considerable morphological differences, e.g. well-developed dorsal crenulations, continues and well developed temporal ridge, and reduced rostral and supraocular processes, were observed between the two sub-populations of Mt. Namuli.

#### *Rhampholeon maspictus* Branch, Bayliss & Tolley, 2014

**Material.** Mt. Mabu (PEM R21117, male, 53.8 mm SVL; PEM R21118, male, 62.4 mm SVL; PEM R21119, juvenile male, 31.7 mm SVL; PEM R21120, juvenile female, 30.6 mm SVL; PEM R21121, juvenile female, 35.8 mm SVL; PEM R21133, juvenile female, 36.7 mm SVL; PEM R21134, juvenile male, 41.9 mm S; PEM R21135, female, 39.0 mm SVL; PEM R21136, female, 60.5 mm SVL; PEM R21136, female, 60.5 mm SVL; PEM R21137, male, 64.5 mm SVL; PEM R21138, male, 65.8 mm SVL; PEM R21139, female, 47.0 mm SVL; PEM R21140, juvenile female, 36.5 mm SVL; PEM R2141, juvenile male, 42.0 mm SVL). **Comments:** Specimens were found in Afromontane forest above 1900 m asl. This species is restricted to Mt. Mabu.

#### *Rhampholeon* sp.

**Material.** Mt. M'pàluwé (PEM R21202, female, 45.8 mm SVL; PEM R21203, female, 57.3 mm SVL; PEM R21204, female, 44.1 mm SVL; PEM R21205, female, 65.1 mm SVL; PEM R21206, female, 56.2 mm SVL; PEM R21207, female, 40.7 mm SVL; PEM R21208, male, 34.0 mm SVL; PEM R21209, female, 61.9 mm SVL; PEM R21210, male, 37.5 mm SVL). **Comments:** This is the first record of a pygmy chameleon from Mt. Ribuáué insulates forests (Fig. 4C). Based on the isolation

of all the northern Mozambique inselbergs and high genetic differences reported by Branch et al. (2014), it is highly plausible that this new population represents an undescribed species. Most similar to *R. tilburyi* in external morphology.

#### Cordylidae

##### *Platysaurus maculatus* (Broadley, 1965)

**Material.** Mt. M'pàluwé (PEM R21220, subadult female, 62.7 mm SVL; PEM R21221, male, 79.5 mm SVL; PEM R21222, female, 62.9 mm SVL; PEM R21223, male, 81.7 mm SVL; PEM R21224, male, 75.8 mm SVL). **Comments:** Collected from the lower slopes of Mt. M'pàluwé where they were found in abundance running on rocky slopes. Previously, collected from Mt. M'pàluwé [=Ribuáué] by Blake (1965), which formed part of the type series of this species (Broadley 1965b). This species is now regarded as widespread in northern Mozambique (Broadley 1965b, Branch et al. 2005) and southern Tanzania (Broadley 1995).

#### Gekkonidae

##### *Chondrodactylus turneri* (Gray, 1864)

**Material.** Mt. M'pàluwé (PEM R21199, juvenile, 35.3 mm SVL; PEM R21200, juvenile, 39.5 mm SVL; PEM R21213, juvenile, 47.7 mm SVL). **Comments:** Collected at night on rock surface at lower slopes.

##### *Hemidactylus mabouia* (Moreau De Jonnés, 1818)

**Material.** Mt. Mabu (PEM R21124, female, 62.5 mm SVL; PEM R21143, male, 48.5 mm SVL; PEM R21144, male, 51.9 mm SVL), Mt. M'pàluwé (PEM R21215, female, 51.6 mm SVL; PEM R21216, male, 59.4 mm SVL). **Comments:** Collected at Mt. Mabu from rock surface and under tree bark in low to mid-elevation. At Mt. M'pàluwé specimens were collected in sympatry with *H. platycephalus* on derelict buildings of the Oasis Water Camp.

##### *Hemidactylus platycephalus* Peters, 1854

**Material.** Mt. M'pàluwé (PEM R21217, female, 81.4 mm SVL; PEM R21218, male, 72.9 mm SVL). **Comments:** Collected from derelict buildings and mango trees around the Oasis Water Camp.

##### *Lygodactylus grotei* Sternfeld, 1911

**Material.** Mt. Mabu (PEM R21125, female, 30.7 mm SVL). **Comments:** Collected at the base of a tree in dry miombo woodland.

***Lygodactylus regulus* Portik, Travers, Bauer & Branch, 2013**

**Material.** Mt. Namuli (PEM R21168, female, 33.8 mm SVL). **Comments:** Recently described gecko related to the larger *L. rex* from Mt. Mulanje (see Portik et al. 2013b). Only known from three specimens collected from Mt. Namuli. Our specimen was collected from a fallen tree in a shamba.

***Lygodactylus cf. rex* Broadley, 1963**

**Material.** Mt. Mabu (PEM R21147, male, 48.1 mm SVL; PEM R21148, male, 42.1 mm SVL). **Comments:** Specimens were collected from tree trunks in transitional miombo at lower slopes (Fig. 4D). These large geckos are morphologically similar to *L. rex* from Mt. Mulanje in that they share the large size of typical *L. rex* (up to 55 SVL: Portik et al. 2013b), the mental scale is very shallow with lateral slits, and the conspicuous ocellus (spot) above the shoulder. Given the high level of genetic diversity in montane species of this genus (Portik et al. 2013b, Travers et al. 2014) this could either represent an extension of its distribution or a new species. For that reason, we tentatively assign it to *L. rex*.

***Lygodactylus* sp.**

**Material.** Mt. M'pàluwé (PEM R21196, female, 41.0 mm SVL). **Comments:** One individual collected from a tree trunk in a newly cleared shamba at the top of Mt. M'pàluwé at night. Specimen from Mt. M'pàluwé differs from *L. cf. rex* from Mt. Mabu, and *L. regulus* from Mt. Namuli in that they lack the conspicuous ocellus above the shoulder. It closely resembles *L. angularis* in general throat markings and that the mental is entire and not split with shallow lateral slits as in the *L. rex* group.

**Lacertidae*****Holaspis laevis* Werner, 1895**

**Material.** Mt. Mabu (PEM R21145, male, 48.8 mm SVL; PEM R21146, male, 48.0 mm SVL). **Comments:** Found on tree trunks in transitional miombo woodland at lower slopes (Fig. 4E). This secretive species is only known from Amatongas, in central Mozambique (Cotts 1934) and numerous unpublished records from other localities in Mozambique: Marrumeu area (Branch pers. obs. in Branch 2000), Moebase (Branch pers. obs. in Branch 2000), Cavalo near Gorongosa Mountain (Broadley pers. obs. in Branch 2000), Dondo Inhamitanga Forests (Broadley pers. obs. in Branch 2000), Quiterajo (Conradie pers. obs.), Italthai (Verburgt pers. obs.), and Palma (Verburgt pers. obs.). It has also been recorded from southern Malawi from the Ruo River Gorge (Branch and Cunningham 2006).

**Scincidae*****Melanoseps cf. ater* (Günther, 1873)**

**Material.** Mt. Mabu (PEM R21126, 93 mm SVL, 28.6 mm TL; PEM R21127, 121 SVL, 39.9 mm TL). **Comments:** Broadley et al. (2006) assigned the northern Mozambique specimens to *M. loveridgei*, while specimens from southern Malawi are referred to as *M. ater*. We used the key from Broadley et al. (2006) to identify the specimens based on number mid-body scale rows (24). Timberlake et al. (2012) were the first to report this species from Mt. Mabu and this was only the second record for this genus in Mozambique. Farooq and Conradie (2015) recorded *M. cf. loveridgei* from Mt. Namuli. This identification was tentative, as material got lost in a motor vehicle accident and could not be examined. Based on the close proximity to Mt. Mabu the Mt. Namuli record should be regarded as *M. cf. ater*.

***Trachylepis boulengeri* (Sternfeld, 1911)**

**Material.** Mt. M'pàluwé (PEM R21225, male, 75.4 mm SVL). **Comments:** Only one specimen was collected in dry bamboo leaves from the eastern slopes of Mt. M'pàluwé. This species is known from only a handful of records from northern Mozambique, e.g. Niassa (Branch et al. 2005), and Marrupula (Blake 1965, Broadley 1974).

***Trachylepis maculilabris* (Gray, 1845)**

**Material.** Mt. Mabu (PEM R21150, female, 88.6 mm SVL). **Comments:** One adult female was collected from a transition woodland. Branch et al. (2005) were the first to record this species from northern Mozambique and indicate, based on material used from coastal northern Mozambique (Carranza et al. 2001) towards the presence of a cryptic species in the north of Mozambique. Our record is the first for Mt. Mabu and fills a large gap between the northern (Lipumbulo floodplain) and the Zambezi Valley records. Records from north of the Zambezi remains scattered to only a handful of records, e.g. Lipumbulo floodplain, Moebase, and Moma (Branch et al. 2005).

***Trachylepis margaritifera* (Peters, 1854)**

**Material.** Mt. M'pàluwé (PEM R21226, female, 97.1 mm SVL; PEM R21227, female, 101.7 mm SVL). **Comments:** Two adult females were collected from the Oasis Water Camp running among outbuildings and on rocky slopes. Broadley and Bauer (1998) recorded this species from a handful of records from the northern Mozambique, presumably based on records collected by Blake (1965). Our record from Mt. M'pàluwé just fills in the gap between Blake's (1965) Morrumbala and Nampula

records. Portik et al. (2013a) also recorded this species from Lichinga further north and Branch et al. (2005) recorded them from Niassa Game Reserve.

### *Trachylepis striata* (Peters, 1844)

**Material.** Mt. Namuli (PEM R21172, male, 71.1 mm SVL; PEM R21173, male, 67.7 mm SVL). **Comments:** Specimen was collected from the lower slopes of Mt. Namuli running on granite rocky outcrops. Common and widespread species in southern Africa, but only recorded from Niassa (Branch et al. 2005), Namuli and Lichinga (Portik et al. 2013a), Mossuril and Mozambique Island (Blake 1965) in northern Mozambique.

### *Trachylepis varia* (Peters, 1867)

**Material.** Mt. Mabu (PEM R21151, male, 58.6 mm SVL), Mt. Namuli (PEM R21179, female, 52.7 mm SVL; PEM R21180, female, 51.1 mm SVL; PEM R21181, female, 59.4 mm SVL; PEM R21182, female, 60.7 mm SVL; PEM R21183, male, 49.3 mm SVL; PEM R21185, male, 53.8 mm SVL), Mt. M'páluwé (PEM R21198, male, 57.6 mm SVL). **Comments:** Specimens from Mts. Mabu and M'páluwé represent the plain tan brown dorsum coloration with dark-edged white lateral line and uniform beige ventrum, while the montane grassland population of the Murteha Plateau of Mt. Namuli have the dorsum greenish with five finely striped white lines, and ventrally a bluish darker colouration. Broadley (2000) also refers to the fact that montane populations differs in size, scalation, and colour. Further taxonomical work is needed to resolve the status of these montane populations.

## Colubridae

### *Dispholidus typus* (Smith, 1828)

**Material.** Mt. M'páluwé (PEM R21214, a juvenile female, 518 mm SVL + 20 mm TL). **Comments:** Juvenile dislodged by a Southern Shrike (*Lanius* sp.) from the branches of a mango tree at the Oasis Water Camp. This specimen can be assigned to the northern race *D. typus viridis* (Smith, 1828).

### *Dipsadoboa cf. shrevei shrevei* (Loveridge, 1932)

**Material.** Mt. Mabu (PEM R21115, juvenile 312 mm SVL + 91 mm TL; PEM R21116, juvenile, 271 mm SVL + 77 mm SVL; PEM R21122, male, 802 mm SVL + 255 mm TL; PEM R21123, male, 681 mm SVL + 243 mm TL), Mt. M'páluwé (PEM R21195, juvenile, 381 mm SVL + 106 mm SVL). **Comments:** Specimens collected from forest floor or in low growing trees along streams (Fig. 4F). Except for Mt. M'páluwé specimen, which was

collected from low growing scrubs in the forest, far from any water. Based on the identification key provided by Spawls et al. (2002) the Mozambique species keys out as *D. shrevei shrevei*, from which it differs on ventral and subcaudal counts. First collected in 2008 (Timberlake et al. 2012, Bayliss et al. 2014) from Mt. Mabu.

### *Philothamnus angolensis* Bocage, 1882

**Material.** Mt. Namuli (PEM R21187, female, 641 mm SVL + 254 mm TL). **Comments:** One specimen collected from mid-elevation forested stream, swimming across the stream at the base of the two Namuli granite domes when disturbed.

### *Philothamnus hoplogaster* (Günther, 1863)

**Material.** Mt. Mabu (PEM R21154, female, 390 mm SVL 165 mm TL), Mt. M'páluwé (PEM R21219, male, 406 mm SVL + 188 TL). **Comments:** Specimen collected from Mt. Mabu has 10 black spots anterior on the dorsum, while the Mt. M'páluwé specimen has uniform lime-green colouration. The Mt. Mabu specimen was collected from an overhanging tree along a well vegetated low-elevation stream at night, while the Mt. M'páluwé was collected around a water tank at the Oasis Water Camp.

### *Thelotornis mossambicanus* (Bocage, 1895)

**Material.** Mt. Mabu (PEM R21142, juvenile, 312 SVL+163 mm TL; PEM R21149, male, 650 mm SVL + 400 mm TL), M'páluwé (PEM R21228, female, 706 mm SVL +419 mm TL). **Comments:** At Mt. Mabu one specimen was collected from closed-canopy forest while the other was collected in transitional miombo woodland. A male and female specimen donated by a local presumably obtained from near Ribáuè town. The juvenile collected from closed-canopy forest had a juvenile *Rhampholeon maspictus* in its stomach.

## Elapidae

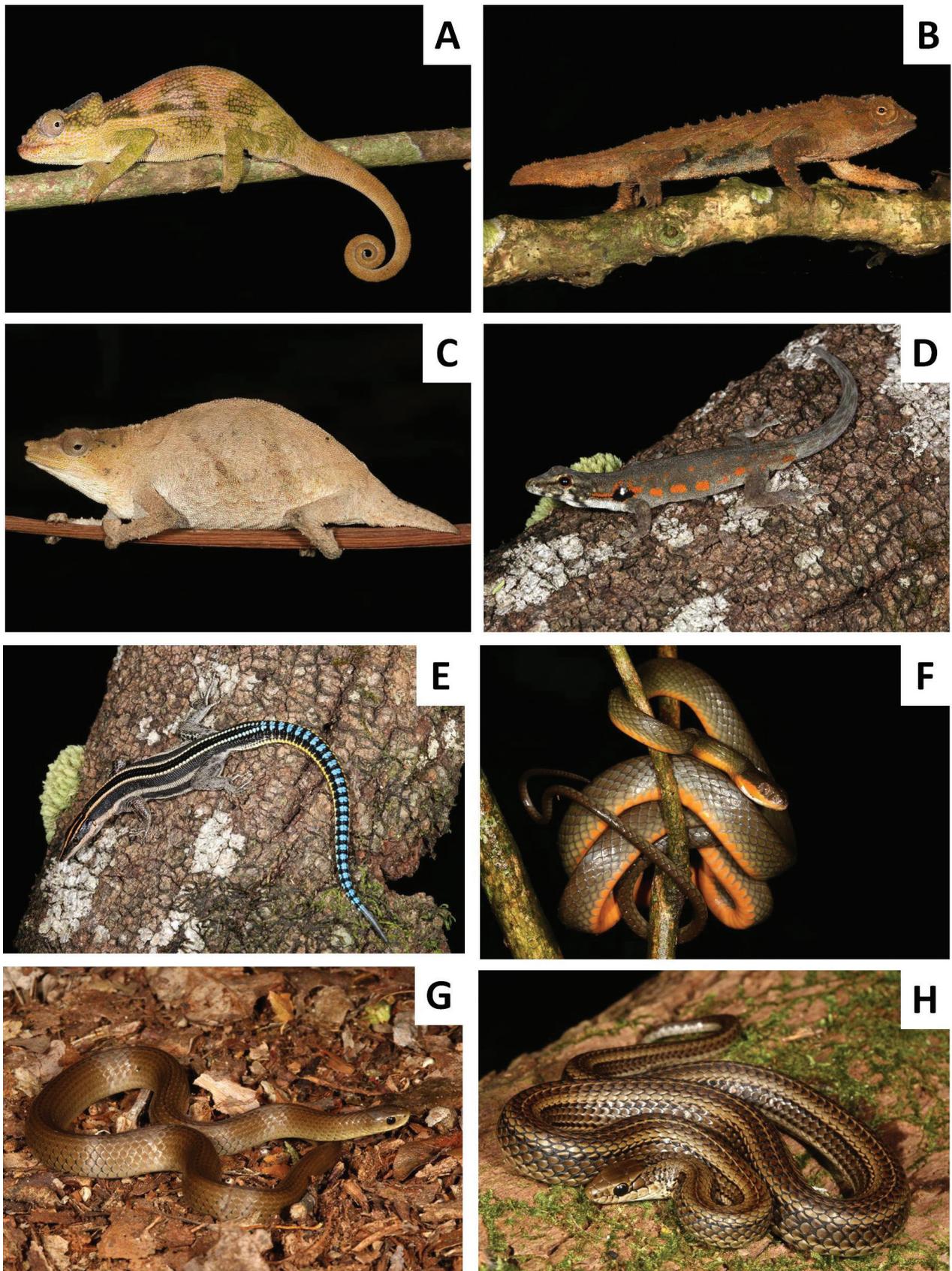
### *Naja mossambica* Peters, 1854

**Material.** Mt. M'páluwé (PEM R21201, female, 715 mm SVL + 146 mm TL). **Comments:** Collected at night on the lower slopes near the shambas.

## Lamprophiidae

### *Boaedon capensis* Duméril & Bibron, 1854

**Material.** Mt. M'páluwé (PEM R21229, male 147 SVL + 98 mm TL). **Comments:** A female specimen donated by a local, presumably obtained from near Ribáuè town.



**Figure 4.** A selection of reptiles from northern Mozambique inselbergs. **A** – *Nadzikambia baylissi* (Mt. Namuli, PEM R21165), **B** – *Rhampholeon tilburyi* (Mt. Namuli, PEM R21157), **C** – *Rhampholeon* sp. (Mt. M'páluwé, PEM R21209), **D** – *Lygodactylus* cf. *rex* (Mt. Mabu, PEM R21147), **E** – *Holaspis laevis* (Mt. Mabu, PEM R21146), **F** – *Dipsadoboa* cf. *shrevei shrevei* (Mt. Mabu, PEM R21123), **G** – *Duberria shirana* (Mt. Namuli, PEM R21184), **H**– *Psammophylax variabilis* (Mt. Namuli, PEM R21186).

We follow Portik et al. (2013a) and refer to this specimen as *capensis*, rather than *fuliginosus*, based on general colouration.

### ***Duberria shirana* (Boulenger, 1894)**

**Material.** Mt. Namuli (PEM R21184, male, 193 mm SVL + 49 mm TL). **Comments:** Collected in a funnel trap situated in montane grassland of the Muretha Plateau (Fig. 4G). This represents the first known record of this species for the whole of Mozambique.

### ***Gonionotophis capensis* (Smith, 1847)**

**Material.** Mt. Mabu (PEM R21152, male, 965 mm SVL + 160 mm TL; PEM R21153, female, 993 mm SVL + 143 mm TL). **Comments:** Two specimens were collected on the same night (15 November 2014) from the same locality near a stream at the base of Mt. Mabu. It is possible that the male was following the female, as they were collected minutes apart.

### ***Psammophis orientalis* Broadley, 1977**

**Material.** Mt. M'pàluwé (PEM R21197, female, 475 mm SVL + 243 mm TL). **Comments:** One specimen collected at night, one meter from the ground in a low growing shrub in transitional miombo.

### ***Psammophylax variabilis* Günther, 1893**

**Material.** Mt. Namuli (PEM R21186, female, 329 mm SVL + 77 mm TL). **Comments:** Collected in montane grassland on the Muretha Plateau (Fig. 4H). This represents only the third record of this species for the whole of Mozambique (Timberlake et al. 2009; Farooq and Conradie 2015).

## **Viperidae**

### ***Bitis arietans* Merren, 1820**

**Material.** Mt. M'pàluwé (PEM R21212, female, 573 mm SVL + 51 mm TL). **Comments:** One specimen collected at night on the path near the shambas.

## **Conclusions**

We have found eight putative new species through field identification, added additional species known from northern inselbergs (13 species to Mt. Mabu, five species to Mt. Namuli, and 19 species to Mt. M'pàluwé), and one new

country record. Additional analyses are necessary, including barcoding and phylogenetic analyses, to determine whether these mountains are exceptionally high in species richness. We now know there are at least 30–40 species of reptiles and amphibians on each of these sky islands, many of which are montane endemics. Although the state of knowledge is growing for Mt. Mabu and Mt. Namuli and can be considered to be relatively well sampled, it is clear that Mt. Ribáuè isolates requires more work given the brevity of our survey. In addition, several other sky islands in the area have received little or no attention in terms of the herpetofaunal survey (e.g. Mt. Inago and Mt. Chipirone).

The present collection is essentially a preliminary assessment of amphibian and reptile diversity in the region and does not account for seasonal variation in activity of herpetofauna. Future surveys that are more comprehensive in space and over time should considerably increase our understanding of the regional diversity, endemism, and richness of these inselbergs. Although the state of biodiversity knowledge has grown for Mts. Mabu and Namuli, there is an urgent need for a clear understanding of the nature of threats, and mitigation measures that will directly improve protection of habitat. At Mt. Ribáuè additional surveys are imperative, given the comparatively limited exploration on that mountain coupled with the apparent high rate of forest clearing. Overall, the sky islands of Mozambique clearly require additional surveys to quantify species richness and endemism for a broad range of taxonomic groups. Ultimately, a better understanding of the threats to biodiversity will allow for prioritisation of conservation interventions.

## **Acknowledgments**

We thank the Natural History Museum of Maputo who endorsed and provided permits to carry out this work, National Geographic Society Committee for Research and Exploration (CRE 9281-13), the South African National Biodiversity Institute, and the National Research Foundation of South Africa (Grant #92776) for providing funding and logistical support for the 2014 survey, and Khangela Safaris for camp logistics in 2014. We also thank Bill Branch and Harith Farooq for their support for this survey, and the Swiss-African Kick-Start Funding, the Freiwillige Akademische Gesellschaft and the University of Basel for funding contributions to SPL and GBBS. We thank the two reviewers and editor whose comments improved the quality of this paper.

## **References**

- Bayliss J (2008) Danger and discoveries in northern Mozambique. *Lep-soc News Africa* 4(2008): 3–6.
- Bayliss J, Monteiro J, Fishpool L, Congdon C, Bampton I, Bruessow C, Matimele H, Banze A, Timberlake J (2010) Biodiversity and

- Conservation of Mount Inago, Mozambique. Report produced under Darwin Initiative Award 15/036. Royal Botanic Gardens, Kew, London, UK, 32 pp. Available from: [http://www.kew.org/sites/default/files/Inago%20report%20Nov%202010\\_low.pdf](http://www.kew.org/sites/default/files/Inago%20report%20Nov%202010_low.pdf) [accessed 22 August 2016]
- Bayliss J, Timberlake J, Branch WR, Bruessow C, Collins S, Congdon C, Curran M, De Sousa C, Dowsett R, Dowsett-Lemaire F, Fishpool L, Harris T, Herrmann E, Georgiadis S, Kopp M, Liggitt B, Monadjem A, Patel H, Ribeiro D, Spottiswoode C, Taylor P, Willcock S, Smith P (2014) The discovery, biodiversity and conservation of Mabu forest—the largest mid-elevation rainforest in southern Africa. *Oryx* 48: 177–185. doi: 10.1017/S0030605313000720
- Bittencourt-Silva G, Conradie W, Siu-Ting K, Tolley KA, Channing A, Cunningham M, Farooq HM, Menegon M, Loader SP (2016) The phylogenetic position and diversity of the enigmatic mongrel frog *Nothophryne* Poynton, 1963 (Amphibia, Anura). *Molecular Phylogenies and Evolution* 99: 89–102. doi: 10.1016/j.ympev.2016.03.021
- Blake DK (1965) The fourth Umtali Museum expedition to Mozambique: November–December 1964. *The Journal of the Herpetological Association of Rhodesia* 23: 31–46. doi: 10.1080/0440730X.1965.9650549
- Branch WR (1998) Field guide to the snakes and other reptiles of Southern Africa. Struik Publishers, Cape Town, 399 pp.
- Branch WR (2000) Survey of the reptiles and amphibians of the Zambezi Delta. In: Timberlake JR (Ed.) Biodiversity of the Zambezi Basin Wetlands, Consultancy report for IUCN ROSA. Biodiversity Foundation of Africa, Bulawayo/The Zambezi Society, Harare, 377–392.
- Branch WR (2004) Herpetological survey of the Niassa Game Reserve. Technical report prepared for Sociedade para a Gestão e Desenvolvimento da Reserva do Niassa Moçambique, 59 pp.
- Branch WR, Bayliss J (2009) A new species of *Atheris* (Serpentes: Viperidae) from northern Mozambique. *Zootaxa* 2113: 41–54. doi: 10.11646/zootaxa.3710.5.2
- Branch WR, Bayliss J, Tolley KA (2014) Pygmy chameleons of the *Rhampholeon platyceps* complex (Squamata: Chamaeleonidae): Description of four new species from isolated ‘sky islands’ of northern Mozambique. *Zootaxa* 3814: 1–36. doi: 10.11646/zootaxa.3814.1.1
- Branch WR, Cunningham M (2006) Herpetological survey of Mount Mulanje, Malawi. Report for Mount Mulanje Conservation Trust, Blantyre, Malawi, 74 pp.
- Branch WR, Rödel M-O, Marais J (2005a) Herpetological survey of the Niassa Game Reserve, northern Mozambique – Part I: Reptiles. *Salamandra* 41: 195–214.
- Branch WR, Rödel M-O, Marais J (2005b) A new species of rupicolous *Cordylus* Laurenti 1768 (Sauria: Cordylidae) from Northern Mozambique. *African Journal of Herpetology* 54: 131–138. doi: 10.1080/21564574.2005.9635526
- Branch WR, Ryan PG (2000) Additions to the Mozambique Herpetofauna: Two new lizards from the Namuli Massif, Mozambique. *Herpetological Review* 32: 281–282.
- Branch WR, Tolley KA (2010) A new species of chameleon (Sauria: Chamaeleonidae: *Nadzikambia*) from Mount Mabu, central Mozambique. *African Journal of Herpetology* 59: 157–172. doi: 10.1080/21564574.2010.516275
- Broadley DG (1965a) A new chameleon from Malawi. *Arnoldia* 32: 1–3.
- Broadley DG (1965b) A new species of *Platysaurus* from northern Mozambique. *Arnoldia* 33: 1–4.
- Broadley DG (1974) A review of the *Mabuya maculilabris* group in southeastern Africa (Sauria: Scincidae). *Arnoldia* 6: 1–15.
- Broadley DG (1990) FitzSimons’ Snakes of Southern Africa (Revised Edition). 84 colour pl. + Addendum. Jonathan Ball and Ad. Donker, Parklands, 387 pp.
- Broadley DG (1995) Geographical Distribution. Sauria: Cordylidae: *Platysaurus maculatus maculatus* Broadley, 1965. *African Herp News* 22: 52–53.
- Broadley DG (2000) A review of the genus *Mabuya* in southeastern Africa (Sauria: Scincidae). *African Journal of Herpetology* 49: 87–110. doi: 10.1080/21564574.2000.9635437
- Broadley DG, Bauer AM (1998) A review of the *Mabuya quinquetaeniata* complex in East Africa (Sauria: Scincidae). *African Journal of Herpetology* 47: 43–58. doi: 10.1080/21564574.1998.9650002
- Broadley DG, Whiting SA, Bauer AM (2006) A revision of East Africa species of *Melanoseps* Boulenger (Sauria: Scincidae: Feylininae). *African Journal of Herpetology* 55: 95–112. doi: 10.1080/21564574.2006.9635543
- Carranza S, Arnold EN, Mateo JA, Lopez-Jurado LF (2001) Parallel gigantism and complex colonisation patterns in the Cape Verde scincid lizards *Mabuya* and *Macrosclincus* (Reptilia: Scincidae) revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society of London* 268: 1595–1603. doi: 10.1098/rspb.2001.1699
- Channing A (2001) Amphibians of Central and Southern Africa. Cornell University Press, Ithaca, New York, 470 pp.
- Channing A, Baptista N (2013) *Amietia angolensis* and *A. fuscigula* (Anura: Pyxicephalidae) in southern Africa: A cold case reheated. *Zootaxa* 3640: 501–520. doi: 10.11646/zootaxa.3640.4.1
- Channing A, Dehling JM, Lötters S, Ernst R (2016) Species boundaries and taxonomy of the African river frogs (Amphibia: Pyxicephalidae: *Amietia*). *Zootaxa* 4155(1): 001–076. doi: 10.11646/zootaxa.4155.1.1
- Channing A, Davenport TRB (2002) A new stream frog from Tanzania (Anura: Ranidae: *Strongylopus*). *African Journal of Herpetology* 51: 135–142. doi: 10.1080/21564574.2002.9635469
- Channing A, Howell KM (2006) Amphibians of East Africa. Cornell University Press, New York, 418 pp.
- Clarke BT, Poynton C (2005) A new species of stream frog, *Strongylopus* (Anura: Ranidae) from Mount Kilimanjaro, Tanzania, with comments on a ‘northern volcanic mountains group’ within the genus. *African Journal of Herpetology* 54: 53–60. doi: 10.1080/21564574.2005.9635517
- Congdon TCE, Bayliss JL (2012) Butterflies of Mt Mecula and Mt Yao, Niassa Province, Northern Mozambique. *Metamorphosis* 23: 26–34.
- Congdon TCE, Collins S, Bayliss J (2010) Butterflies of south east Africa’s mountains (Mozambique and Malawi). *Metamorphosis* 21(2): 45–107.
- Conroy CJ, Papenfuss T, Parker J, Hahn NE (2009) Use of Tricaine Methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the American Association for Laboratory Animal Science* 48: 28–32.
- Cott HN (1934) The Zoological Society’s expedition to the Zambesi, 1927: No.5. On a collection of lizards, mainly from Portuguese East Africa, with description of new species of *Zonurus*, *Monopeltis* and *Chirindia*. *Proceedings of the Zoological Society of London* 1934: 145–173. doi: 10.1111/j.1469-7998.1934.tb06228.x
- Daniels SR, Bayliss J (2012) Neglected refugia of biodiversity: mountainous regions in Mozambique and Malawi yield two novel fresh-

- water crab species (Potamonautidae: *Potamonautes*). Zoological Journal of the Linnean Society 164: 498–509. doi: 10.1111/j.1096-3642.2011.00773.x
- Daniels S, Phiri E, Bayliss J (2014) Renewed sampling of inland aquatic habitats in southern Africa yields two novel freshwater crab species (Decapoda: Potamonautidae: *Potamonautes*). Zoological Journal of the Linnean Society 171: 356–369. doi: 10.1111/zoj.12139
- Du Preez LH, Carruthers VC (2009) A Complete Guide to the Frogs of Southern Africa. Struik Nature, Cape Town. doi: 10.1111/j.1096-3642.2011.00773.x
- Farooq HOM, Conradie W (2015) A second record of *Scolecophorus kirkii* Boulenger, 1883 (Gymnophiona: Scolecophoridae) for Mozambique. Herpetology Notes 8: 59–62.
- Farooq H, Liedtke HC, Bittencourt-Silva G, Conradie W, Loader S (2015) The distribution of *Mertensophryne anotis* with a new record in Northern Mozambique. Herpetology Notes 8: 305–307.
- Frost DR (2016) Amphibian Species of the World: an Online Reference, Version 6.0. American Museum of Natural History, New York. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> [accessed 1 April 2016]
- Furman BJS, Bewick AJ, Harrison TL, Greenbaum E, Gvoždík V, Kusamba C, Evans BJ (2015) Pan-African phylogeography of a model organism, the African clawed frog '*Xenopus laevis*'. Molecular Ecology 24: 909–925. doi: 10.1111/mec.13076
- Larson TR, Catrso D, Behangana M, Greenbaum E (2016) Evolutionary history of the river frog genus *Amietia* (Anura: Pyxicephalidae) reveals extensive diversification in Central African highlands. Molecular Phylogenetics and Evolution 99: 168–181. doi: 10.1016/j.ympev.2016.03.017
- Marais J (2004) A complete guide to the snakes of Southern Africa. Struik, Cape Town, 312 pp.
- Mazibuko L, Poynton J (2004) *Nothophryne broadleyi*. The IUCN Red List of Threatened Species 2004: e.T58077A11714410. doi: 10.2305/IUCN.UK.2004.RLTS.T58077A11714410.en
- McSweeney C, New M, Lizcano G (2010a) UNDP climate change country profiles: Mozambique. <http://country-profiles.geog.ox.ac.uk/> [accessed 15 May 2016]
- McSweeney C, New M, Lizcano G, Lu X (2010b) The UNDP climate change country profiles improving the accessibility of observed and projected climate information for studies of climate change in developing countries. Bulletin of the American Meteorological Society 91: 157–166. doi: 10.1175/2009BAMS2826.1
- Mercurio V (2011) Amphibians of Malawi, an analysis of their richness and community diversity in a changing landscape. Edition Chimaira, Frankfurt/M.
- Monadjem A, Schoeman MC, Reside A, Pio DV, Stoffberg S, Bayliss J, Cotterill FPD, Curran M, Kopp M, Taylor PJ (2010) A recent inventory of the bats of Mozambique with documentation of seven new species for the country. Acta Chiropterologica 12(2): 371–391. doi: 10.3161/150811010X537963
- Nussbaum RA (1985) Systematics of caecilians (Amphibia: Gymnophiona) of the family Scolecophoridae. Occasional Papers of the Museum Zoology, University of Michigan 713: 1–49.
- Ohler A, Frétey T (2014) Going back to Rovuma: the frog fauna of a coastal dry forest, and a checklist of the amphibians of Mozambique. Journal of East African Natural History 103: 73–124. doi: 10.2982/028.103.0203
- Pascal O (2011) The coastal forests of northern Mozambique, 2008–2009 expeditions. Our Planet Reviewed. Programme report n°1. Pro-Natura international / Muséum national d'Histoire naturelle, Paris. [http://www.laplaneterevisitee.org/ressources/pdfs/Rapport\\_de\\_Mission\\_Mozambique\\_23.pdf](http://www.laplaneterevisitee.org/ressources/pdfs/Rapport_de_Mission_Mozambique_23.pdf) [accessed 20 July 2016]
- Pickersgill M (2007) Frog search. Results of expeditions to Southern and Eastern Africa from 1993–1999. Frankfurt Contributions to Natural History Volume 28. Edition Chimaira, Frankfurt am Main.
- Portik DM, Mulungu EA, Sequeira D, Mcentee JP (2013a) Herpetological surveys of the Serra Jeci and Namuli massifs, Mozambique, and an annotated checklist of the southern Afromontane archipelago. Herpetological Review 44: 394–406.
- Portik DM, Travers SL, Bauer AM, Branch WR (2013b) A new species of *Lygodactylus* (Squamata: Gekkonidae) endemic to Mount Namuli, an isolated 'sky island' of northern Mozambique. Zootaxa 3710: 415–435. doi: 10.11646/zootaxa.3710.5.2
- Poynton JC (1963) Descriptions of southern African amphibians. Annals of the Natal Museum 15: 319–332.
- Poynton JC (1966) Amphibia of northern Mozambique. Memoirs de Instituto de Investigação Científica de Moçambique Ser. A. Ciências B 8: 13–34.
- Poynton JC (2004) Stream frogs in Tanzania (Ranidae: *Strongylopus*): The case of *S. merumontanus* and *S. fuelleborni*. African Journal of Herpetology 53: 29–34. doi: 10.1080/21564574.2004.9635496
- Poynton JC, Broadley DG (1985) Amphibia Zambeziaca 2. Ranidae. Annals of the Natal Museum 27: 115–181.
- Poynton JC, Broadley DG (1987) Amphibia Zambeziaca 3. Rhacophoridae and Hyperoliidae. Annals of the Natal Museum 28: 161–229.
- Poynton JC, Broadley DG (1991) Amphibia Zambeziaca 5. Zoogeography. Annals of the Natal Museum 32: 221–277.
- Ryan PG, Bento C, Cohen C, Graham J, Parker V, Spottiswoode C (1999) The avifauna and conservation status of the Namuli Massif, northern Mozambique. Bird Conservation International 9: 315–331. doi: 10.1017/S0959270900003518
- Schiötz A (1999) Treefrogs of Africa. Edition Chimaira, Frankfurt am Main, 350 pp.
- Spawls S, Howell K, Drewes R, Ashe J (2002) A Field Guide to the Reptiles of East Africa. Academic Press, London, 543 pp.
- Spottiswoode CN, Patel IH, Herrmann E, Timberlake J, Bayliss J (2008) Threatened bird species on two little-known mountains (Chiperone and Mabu) in northern Mozambique. Ostrich 79(1): 1–7. doi: 10.2989/OSTRICH.2008.79.1.1.359
- Taylor PJ, Richards LR, Bayliss J, Cotterill FPD (2015) Rediscovery of the Transvaal free-tailed bat (*Tadarida ventralis africana*) in South Africa, with an additional record of the Malagasy free-tailed bat (*T. fulminans*) from Northern Mozambique. Durban Natural Science Museum Novitates 38: 50–55.
- Taylor PJ, Stoffberg S, Monadjem A, Schoeman MC, Bayliss J, Cotterill FPD (2012) Four new bat species (*Rhinolophus hildebrandtii* complex) reflect Plio-Pleistocene divergence of dwarfs and giants across an afromontane archipelago. PLoS ONE 7: e41744. doi: 10.1371/journal.pone.0041744
- Timberlake J, Bayliss J, Alves T, Baena S, Francisco J, Harris T, da Sousa C (2007) Biodiversity and Conservation of Mount Chiperone, Mozambique. Technical Report: Darwin Initiative Award 15/036: Monitoring and Managing Biodiversity Loss in South-East Africa's Montane Ecosystems. Available from: [http://www.kew.org/sites/default/files/kppcont\\_046092\\_0.pdf](http://www.kew.org/sites/default/files/kppcont_046092_0.pdf) [accessed 20 July 2016]
- Timberlake J, Dowsett-Lemaire F, Bayliss J, Alves T, Baena S, Bento C, Cook K, Francisco J, Harris T, Smith P, de Sousa C (2009) Mt.

- Namuli. Mozambique: Biodiversity and Conservation. Technical Report: Darwin Initiative Award 15/036: Monitoring and Managing Biodiversity Loss in South-East Africa's Montane Ecosystems. [http://www.kew.org/science/directory/projects/annex/Namuli\\_report\\_FINAL.pdf](http://www.kew.org/science/directory/projects/annex/Namuli_report_FINAL.pdf) [accessed 20 July 2016]
- Timberlake JR, Bayliss J, Dowsett-Lemaire F, Conghan C, Branch WR, Collins S, Curran M, Dowsett RJ, Fishpool L, Francisco J, Harris T, Kopp M, de Sousa C (2012) Mt. Mabu, Mozambique: Biodiversity and Conservation. Report produced under the Darwin Initiative Award 15/036. Royal Botanic Gardens, Kew, London. [http://www.kew.org/sites/default/files/kppcont\\_067897.pdf](http://www.kew.org/sites/default/files/kppcont_067897.pdf) [accessed on 26 January 2015]
- Travers SL, Jackman TR, Bauer AM (2014) A molecular phylogeny of Afrotropical dwarf geckos (*Lygodactylus*) reveals a single radiation and increased species diversity in a South African montane center of endemism. *Molecular Phylogenetics and Evolution* 80: 31–42. doi: 10.1016/j.ympev.2014.07.017
- Tolley KA, Alexander GJ, Branch WR, Bowles P, Maritz B (2016) Conservation status and threats for African reptiles. *Biological Conservation*, In Press. doi: 10.1016/j.biocon.2016.04.006
- Uetz P, Hošek J (Eds) (2016) The Reptile Database. <http://www.reptile-database.org> [accessed on 17 April 2016]
- White F (1983) The Vegetation of Africa. A Descriptive Memoir to Accompany the Unesco/AETFAT/UNSO Vegetation Map of Africa. UNESCO, Paris.

# Redescription of the deep-sea colonial ascidian *Synoicum molle* (Herdman, 1886): first record since its original finding during the Challenger Expedition

Tamara Maggioni<sup>1</sup>, Anabela Taverna<sup>1</sup>, Marcos Tatián<sup>1</sup>

<sup>1</sup> Instituto de Diversidad y Ecología Animal (CONICET-UNC) and Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Avenida Vélez Sarsfield 299, (5000), Córdoba, Argentina

<http://zoobank.org/3D64E3D8-308C-4C44-A40B-83CA30F06171>

Corresponding author: Tamara Maggioni (tamaramaggioni@gmail.com)

## Abstract

Received 12 June 2016  
Accepted 4 September 2016  
Published 14 October 2016

Academic editor:  
Matthias Glaubrecht

## Key Words

Ascidacea  
continental slope  
Southwestern Atlantic

The colonial ascidian *Synoicum molle* (Herdman, 1886) was recorded for the first time after its original description. The slope area where *S. molle* was discovered during the Challenger Expedition (1872–1876), located at the mouth off Río de La Plata (Argentine Sea), still remains highly underexplored. During a series of deep-sea prospections along the SW Atlantic (A.R.A. Puerto Deseado, August 2012), 7 colonies of *S. molle* were collected few km from the type locality. The finding permitted to perform, for the first time, a complete morphological description of this species. The proper identification of marine species, especially deep-sea organisms, has implications for the general knowledge of biodiversity, a necessary tool for the conservation and study of ecosystem benefits that marine environments provide.

## Introduction

Ascidians (Tunicata) are important components of marine benthic communities, even in the deep-sea. In relation to other invertebrates, ascidians reach their maximum richness at greater depths (Monniot and Monniot 1978). The deep waters of the SW Atlantic have been scarcely explored. Few research campaigns have circumnavigated the area, thus, the ascidiofauna remains highly undersampled. The Challenger Expedition (1872–1876) (Station 320: 37°17'S, 53°52'W, 1097 m), collected a total of 10 ascidian species, all of them new to science (Herdman, 1886). In a neighboring area, the U.S. Atlantis II 60<sup>th</sup> Campaign recovered 22 ascidian species, of which 7 were reported new (Monniot and Monniot 1976). The taxonomical status of 4 ascidian species collected during the Challenger Expedition lacks a proper description and thus requires a revision: these are *Aplidium incrustans* Herdman, 1886; *Psammoplidium* (*Aplidium*) *flavum* Herdman, 1886; *Hypobythius moseleyi* Herdman, 1882; *Styela flava* Herdman, 1881 and *Polyclimun* (*Synoicum*) *molle* Herdman, 1886.

The colonial ascidian *Synoicum molle* (Herdman, 1886), originally named *Polyclinum molle* Herdman 1886, constituted the second deepest register in the genus. The original description performed by W. Herdman lacks several characters, which are essential for a proper species identification: the disposition of the zooids around the cloacal aperture, the number of stigmata rows (and number of stigmata per row), the shape and position of the atrial aperture, the number and disposition of the muscle bands, the level of row of stigmata at which the border of the anus culminates, and the description and number of larvae incubated in the atrial cavities. Hartmeyer (1912) doubted the inclusion of this species under *Polyclinum* due to the location and depth where it was found. Van Name (1945) noticed the absence of branchial papillae, a reliable character to diagnose *Polyclinum*. He proposed, instead, the genus *Synoicum* in consideration of the latter and also based on the presence of a rounded and almost globular stomach and the shape of the post-abdomen.

We analyzed a total of 7 colonies that match the characters listed by Herdman for *S. molle* in a close area from

the type locality of that species. These include: the form and texture of the colony; the color of the tunic; the average size of the zooids; the form of the atrial languet; the shape and characteristics of the stomach; the general form of the post-abdomen; and the elongated body of the larvae. We provide a more comprehensive and detailed description of the species including illustrations of the entire colony and of the individual zooids.

## Materials and methods

The examined specimens were collected by the research vessel A.R.A Puerto Deseado during the first Continental Slope Campaign, within the Argentinean Exclusive Economic Zone, on August 2012. The prospections comprised a bathymetric gradient between 200 and 3,000 m close to 38°S, including the Mar del Plata submarine canyon (Fig. 1). The sampling devices used consisted of a fishing net and two epibenthic trawls (mesh sizes of 3×3 cm and 1×1 mm).

Onboard, the colonies were photographed, relaxed under seawater with menthol crystals for 2 hours, and then fixed in formalin-seawater 4%. In the laboratory, a minimum of 10 zooids per colony were removed and studied under a stereo microscope. Each zooid was drawn, measured and photographed. The 7 colonies were deposited in the collection of the Museo de Zoología, Universidad Nacional de Córdoba (MZUCVIO192).

The actual status of the different species was verified through the World Register of Marine Species (WoRMS) webpage ([www.marinespecies.org](http://www.marinespecies.org)).

## Results

### *Synoicum molle* (Herdman, 1886)

*Polyclinum molle* Herdman, 1886: 194, pl. XXV, figs 7-9; non Rocha and Costa 2005: 59, fig 2-4.

*Synoicum molle*; Van Name 1945: 84, fig. 20.

**Material examined.** Station 2: 37°58'S, 55°12'W; 7 colonies; 308 m; Ago/17/2012.

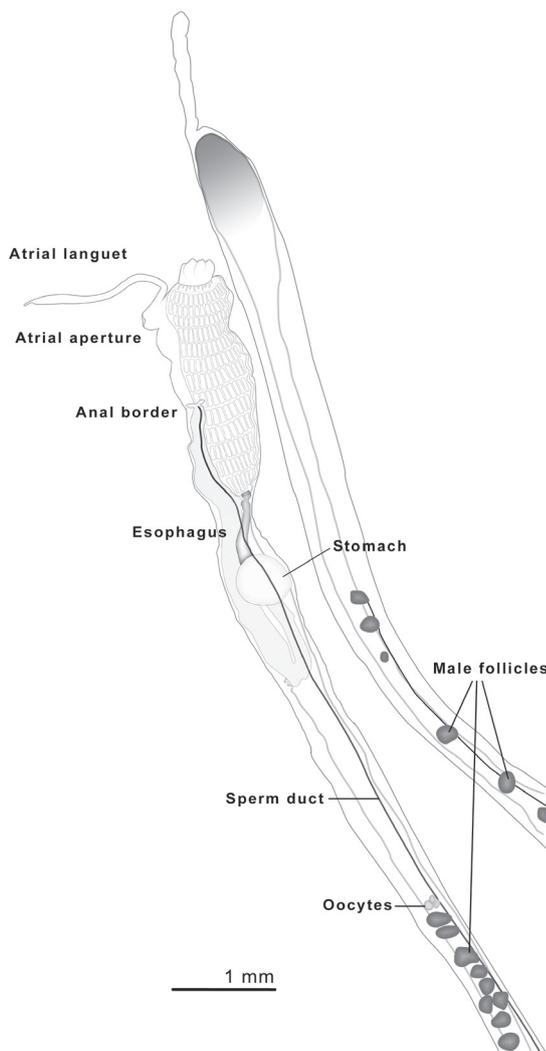
**Description.** The colonies are almost identical in shape: globular, nearly spherical (Fig. 2). The biggest colony measures 9.1 cm in diameter by 3.2 cm in height, while the smallest one reaches 2.5 cm in diameter. The tunic is grayish with variation in intensity, the smallest colonies being the darkest. The zooids, when alive, are white. When fixed in formalin, they turn pale yellow. The test is soft and free of foreign material. Only in two small specimens, some grains of sand and a few epibiotic foraminifera were detected. The tunic is consistent and rigid. The zooids, in variable numbers, are arranged in irregular rosette-like systems around common, although not visible to the naked eye, cloacal apertures. One colony shows zooids with no arrangement at all in the uppermost area,



**Figure 1.** Study area in the SW Atlantic. The asterisk shows the sampling location.



**Figure 2.** Colony of *Synoicum molle* photographed in vivo.



**Figure 3.** Zooid of *Synoicum molle*. Only the initial and last sections of the post-abdomen are shown.

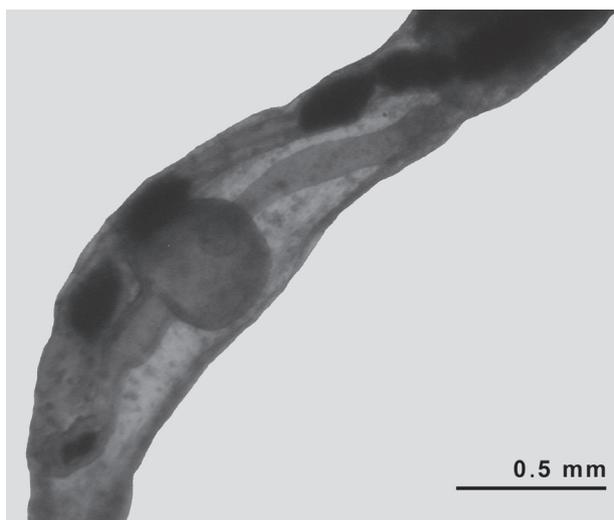
while the area nearest to the base maintains the irregular rosette-like configuration.

All colonies present zooids with a marked variation in size and also of sexual maturation. When fully developed, zooids are large, with an average length of 13 mm (Fig. 3). Zooids with empty thoraces and no digestive systems but fully developed gonads were also found in the biggest colony. These reach a maximum length of 29 mm. The oral siphon bears 6 distinct lobes. The atrial aperture is small, most of the times completely surrounded by an extension of the tunic that forms a small ring. It extends between the 4<sup>th</sup> and 8<sup>th</sup> rows of stigmata. The atrial languet is generally thin, long and simple, though it can also be bifid. It extends as long as the entire length of the thorax, or long enough to cover the atrial aperture. The margins are smooth or slightly serrated.

The thorax bears 8 to 10 thin longitudinal muscle bands on each side, running along the entire body and joining at the end of the post-abdomen. There are between 14 and 16 simple and stout oral tentacles, alternating in size and placed in a circle. The dorsal tubercle is small and rounded.

There are from 12 to 14 rows of stigmata. Rarely, zooids may bear only 9 or 10. Nonetheless, they never exceed 14 rows. Each row contains 10 longitudinal rectangular-shaped stigmata. These vary slightly in size, being thinner and longer towards the center of the thorax. Branchial papillae are not present.

The straight and thin-walled esophagus connects with the stomach vertically. The stomach wall is smooth, although some striations -never folds- might occur randomly (Fig. 4). The stomach shows two alternate shapes: almost spherical or dome-shaped with a straight base. The intestine turns to the dorsal and anterior end vertically. The anus shows two lobes and is located at the level of the 8<sup>th</sup> row of stigmata.



**Figure 4.** Abdomen of *Synoicum molle* showing the rounded smooth-walled stomach.

The gonads are situated in a long post-abdomen, either directly adjacent to the abdominal region or at some distance (0.3 to 4.7 mm) away from it. The ovary contains from one to 2–3 or 6–8 small oocytes. The male follicles are found just below or surrounding the oocytes, disposed in clusters or arranged in a straight line.

On average, a dozen of immature larvae are present in the atrial cavities of a few zooids of one colony. They are arranged in double rows along the entire length and half the width of the thorax. In that immature stage, larvae had only developed a small and stout tail but lacked sensory organs, papillae and vesicles.

## Discussion

Most characters today considered of taxonomic validity were deemed not important or could not be studied in detail in the XIX century, either because of the difficulties regarding the techniques used or due to the limited number of available specimens. Out of 14 ascidian species collected and originally described from Station 320 (Challenger Expedition), 10 are currently accepted. After taxonomical revision of the types, various specimens were placed under a different species: *Styela oblonga* Herdman, 1881 was synonymized with *Styela squamosa* Herdman, 1881 (Monniot and Monniot 1982, 1983); *Ascidia tenera* Herdman, 1880 was synonymized with *Ascidia meridionalis* Herdman, 1880 (Van Name 1945, Monniot and Monniot 1976); *Psammaplidium effrenatum* Herdman, 1886 was synonymized with *Aplidium effrenatum* (Herdman, 1886) (Van Name 1945) and *Leptoclinium tenue* Herdman, 1886 with *Didemnum tenue* (Herdman, 1886) (Van Name 1945). Still, other ascidians from the same station remain uncertain: the status of *Psammaplidium* (*Aplidium*) *flavum* is unknown (*nomen dubium*) (Van Name 1945, Rocha and Lambert pers. com. 2013); *Hypobythius moseleyi* might be closer to *Dicopia* or *Situla*

(Monniot and Monniot 1976); *Styela flava* remains a dubious species (Rodrigues 1966) and *Aplidium incrustans* was proposed to be a synonym of *Synoicum molle* (Van Name 1945). The latter is not *Psammaplidium incrustans* Herdman, 1891 with type locality in Port Stephens, New South Wales, Australia, a synonym of *Aplidium solidum* (Ritter & Forsyth, 1917) (Kott 2005).

According to Herdman (1886), *S. molle* and *A. incrustans* are alike in external appearance and structure of the test. However, they differ in two essential aspects: the shape of the stomach and the structure of the branchial sac. Van Name (1945) does not agree with this characterization. First, he doubts the existence of true folds on the stomach of *A. incrustans*, suggesting the condition observed by Herdman could be caused by collapse or muscular squeeze. Second, he was impeded to compare between branchial sacs, since the description of this organ in *S. molle* lacks the number of stigmata rows as well as the number of stigmata per row. But a deeper inspection of the specimens of *S. molle* collected during the Continental Slope Campaign, granted us the possibility of comparing in detail the structures of the branchial sac. While we cannot give any statement about the nature of the folds of *A. incrustans* unless a revision of the type is made, we can certainly assure that neither the number of rows nor the stigmata per row of both species coincide: *A. incrustans* is described as having “at least 15 rows of stigmata, with nearly twenty in each row” (Van Name 1945, p. 59). The colonies of *S. molle* recovered showed from 12 to 14 rows of stigmata (never exceeding 14) and only 10 stigmata per row. Thus, we propose to maintain both entities as separate species, rejecting the synonymy proposed by Van Name (1945).

The maximum species richness for many invertebrate zoological groups is located between 2,000 and 3,000 m. However, for ascidians, this boundary is located between 4,000 and 4,500 m (Monniot and Monniot 1978, Vinogradova 1962). Colonial ascidians are frequently found in shallow waters. But their presence diminishes with depth, being replaced by solitary forms (Monniot and Monniot 1978). The genus *Synoicum* follows this trend. Only 7 species out of the 84 listed under the genus *Synoicum* have been reported at depths below 500 m: *S. adareanum* (Herdman, 1902), *S. daucum* Monniot C. & Monniot F., 1977, *S. georgianum* Sluiter, 1932, *S. molle* (Herdman, 1886), *S. pererratum* (Sluiter, 1912), *S. ramulosum* Kott, 1969 and *S. tentaculatum* Kott, 1969. *S. georgianum* shows the widest distribution range, presenting registers in the SO and the SW Atlantic (Patagonian shelf and off La Plata River). *S. adareanum* has a circumantarctic register (Kott 1969). The other 5 species, on the contrary, have more restricted distribution ranges. All are reported from only one location: *S. daucum* from the NE Atlantic (Monniot and Monniot 1974), *S. pererratum*, *S. ramulosum* and *S. tentaculatum* from the Southern Ocean (Sluiter 1912, Van Name 1945, Kott 1969), and *S. molle* from the SW Atlantic off Mar del Plata (Herdman 1886, Van Name 1945). Additionally, *S. molle* can be characterized as eurybathic. This is due to the fact that we found the

species for the second time at 308 m depth, 700 m shallower than the original observation (1,097 m).

An increase of bathymetrical gradient studies would help to test the proposed hypothesis of the establishment of a pattern of reduction of colonial forms with increasing depths as well as to elucidate the mechanisms governing this process. Moreover, the increment of the range of the bathymetrical gradient at least up to 5,000 m depth would be especially useful in consideration of the maximum species richness registered for this group of benthic animals (Monniot and Monniot 1978, Vinogradova 1962).

## Acknowledgments

We want to acknowledge Mariano Martinez for his cooperation in regard to the map figure. Special thanks to Martín Brogger and all scientists and students involved in the Argentinean BOPD Slope Campaigns. We thank an anonymous reviewer and reviewer Andreas Schmidt-Rhaesa for their constructive comments on the manuscript. We also thank Maximiliano Maggioni for his assistance and opinion in the preparation of the ascidian figures. The study was partially supported by Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET (PIP CONICET N° 20130100508) and Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba (SECyT30720130100645CB).

## References

- Hartmeyer R (1912) Die Ascidien der Deutschen Tiefsee Expedition. Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer Valdivia, 1898–1899, 16(3): 225–392, figs 1–10, pls. 37–46.
- Herdman WA (1886) Report on the Tunicata collected during the voyage H.M.S. Challenger during the years 1873–1876. Part II, Ascidiaceae. Report on the scientific results of the voyage of H.M.S. Challenger during the years 1873–1876, Zool. 14(38): 1–432.
- Kott P (1969) Antarctic Ascidiacea. Antarctic Research Series, 13, 1–239.
- Kott P (2005) Catalogue of Tunicata in Australian waters. Australian Biological Resources Study, Department of the Environment and Heritage, Australia. <http://www.environment.gov.au/system/files/pages/81f491f3-4fc8-42d0-a92e-5dce256fd340/files/catalogue-tunicata-australian-waters.pdf>
- Monniot C, Monniot F (1974) Ascidiées abyssales de l'Atlantique récoltées par le «Jean Charcot» (campagnes Noratlante, Walda, Polygas A). Bulletin du Muséum National d'Histoire Naturelle, 3<sup>e</sup> série n°226, Zoologie 154: 721–786.
- Monniot C, Monniot F (1976) Tuniciers abyssaux du bassin argentin récoltées par l'Atlantis II. Bulletin du Muséum National d'Histoire Naturelle, 3<sup>e</sup> série n°387, Zoologie 269: 629–662.
- Monniot C, Monniot F (1978) Recent work on the deep-sea Tunicates. Oceanographic Marine Biological Annual Review, vol. 18: 181–228.
- Monniot C, Monniot F (1982) Some Antarctic deep-sea Tunicates in the Smithsonian collections. Biology of the Antarctic Seas X, Antarctic Research Series 32: 95–128. doi: 10.1029/AR032p0095
- Monniot C, Monniot F (1983) Ascidiées Antarctiques et Subantarctiques: morphologie et biogéographie. Mémoires du Muséum National d'Histoire Naturelle, série A, Zoologie 215: 1–135.
- Rodrigues SA (1966) Notes on Brazilian ascidians. Papéis Avulsos do Departamento de Zoologia, vol. 19: 95–115.
- Sluiter CP (1912) Les Ascidiens de l'Expédition Antarctique Française du “Pourquoi-pas?” commandée par le Dr J Charcot, 1908–1909. Bulletin du Muséum National d'Histoire Naturelle, vol. 18: 452–460.
- Van Name WG (1945) The North and South American Ascidiaceans. Bulletin of the American Museum of Natural History, vol. 84: 1–476.
- Vinogradova NG (1962) Vertical zonation in the distribution of deep-sea benthic fauna in the ocean. Deep-Sea Research, vol. 8: 245–250. doi: 10.1016/0146-6313(61)90025-9
- WoRMS Editorial Board (2016) World Register of Marine Species, Available from <http://www.marinespecies.org> [at VLIZ, accessed 2016-06-01]



# A molecular phylogeny of *Pseudocrangonyx* from Japan, including a new subterranean species (Crustacea, Amphipoda, Pseudocrangonyctidae)

Ko Tomikawa<sup>1</sup>, Takafumi Nakano<sup>1</sup>, Anna Sato<sup>2,4</sup>, Yutaka Onodera<sup>3</sup>, Akifumi Ohtaka<sup>2</sup>

<sup>1</sup> Department of Science Education, Graduate School of Education, Hiroshima University, Higashi-Hiroshima 739-8524, Japan

<sup>2</sup> Faculty of Education, Hirosaki University, Hirosaki 036-8560, Japan

<sup>3</sup> Kurosawajirikita High School, Kitakami 024-0012, Japan

<sup>4</sup> Aomori Akenohoshi Junior and Senior High School, Aomori 030-0961, Japan

<http://zoobank.org/3AA1E1BC-87C3-4E0E-AA66-408368320F64>

Corresponding author: Ko Tomikawa (tomikawa@hiroshima-u.ac.jp)

## Abstract

Received 15 August 2016  
Accepted 3 October 2016  
Published 14 October 2016

Academic editor:  
Michael Ohl

## Key Words

Crangonyctoidea  
interstitial  
biogeography  
cryptic diversity

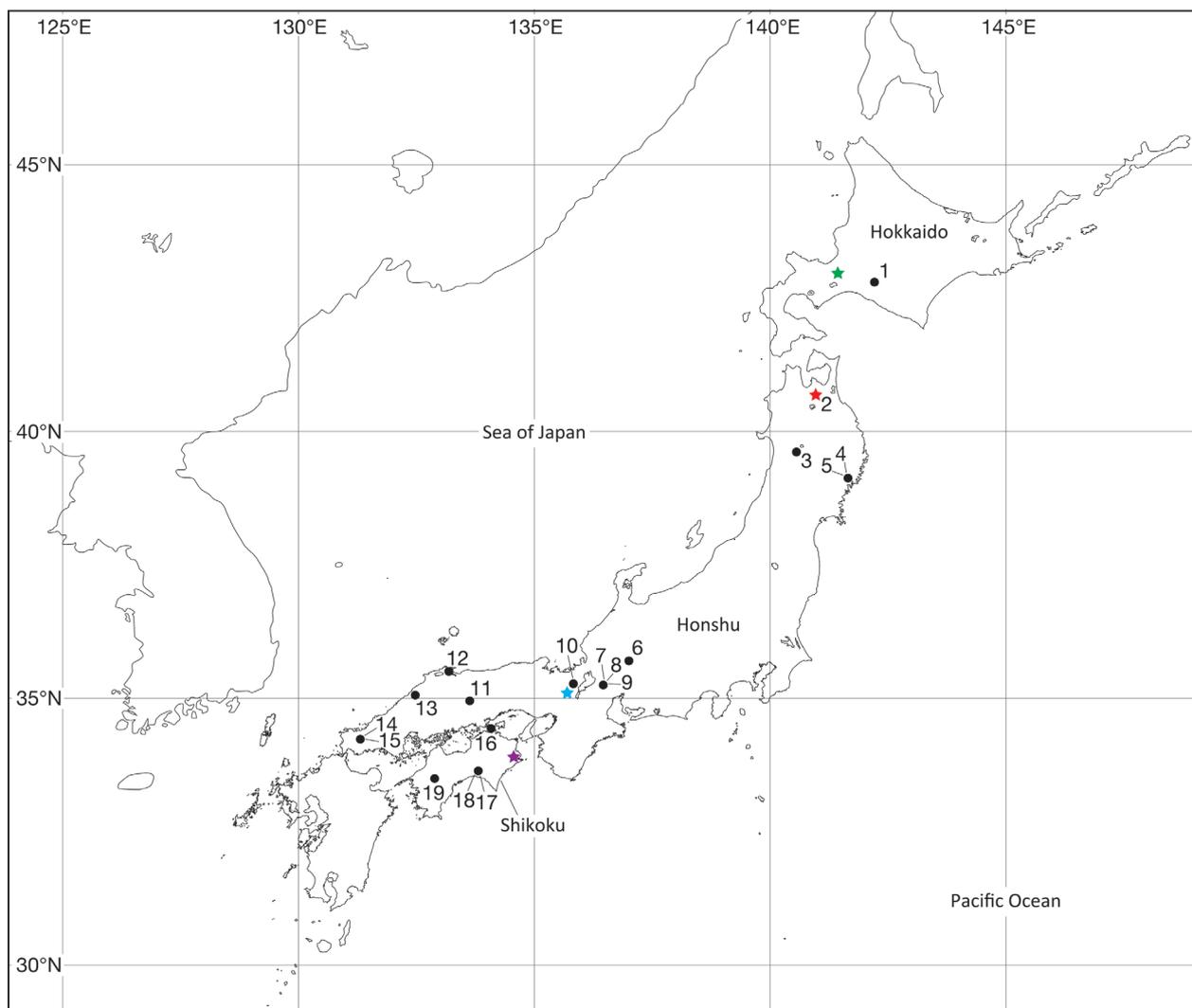
A subterranean species of pseudocrangonyctid amphipod, *Pseudocrangonyx gudariensis* Tomikawa & Sato, **sp. n.**, is described from the spring-fed stream Gudari-numa in Hakoda Mountains, Aomori Prefecture, northern Japan. *Pseudocrangonyx gudariensis* is morphologically similar to *P. coreanus* Uéno, 1966 and *P. febras* Sidorov, 2009 based on its relatively small body size, small number of articles of rami of pleopods, and urosomite 1 without basal setae. However, *P. gudariensis* is distinguished from those species based on the following characteristics: from *P. coreanus*, antenna 2 of female without calceoli, palmar margins of gnathopods 1 and 2 with distally notched robust setae, inner margin of inner ramus of uropod 2 with 4 robust setae, and basal part of inner ramus of uropod 2 without slender seta; and from *P. febras*, carpus of gnathopod 2 without serrate robust setae on posterodistal corners, peduncle of pleopods 1 and 2 with setae, and longer article 2 of uropod 3. Phylogenetic analyses using nuclear 28S rRNA and histone H3, and mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA markers showed that *P. gudariensis* is placed among known *Pseudocrangonyx* Akatsuka and Komai, 1922 species. However, its exact phylogenetic position within the genus could not be determined. The polyphyly of the Japanese *Pseudocrangonyx* species indicates that multiple colonization events of *Pseudocrangonyx* ancestors to the Japanese Archipelago could have occurred. The reliability of the past *Pseudocrangonyx* records from Japan is briefly discussed.

## Introduction

Amphipods that belong to the genus *Pseudocrangonyx* Akatsuka & Komai, 1922 inhabit subterranean waters of Japan, the Korean Peninsula, eastern China, and the Far East of Russia; this genus currently includes 20 species (Sidorov and Gontcharov 2013).

*Pseudocrangonyx* was originally established for the three Japanese subterranean species (Akatsuka and Komai 1922): *P. shikokunis* Akatsuka & Komai, 1922; *P. yezonis* Akatsuka & Komai, 1922; and *P. kyotonis* Akatsuka & Komai, 1922. The first species, *P. shikokunis*, which was subsequently designated as the type species by Barnard and Barnard (1983) by position precedence,

was reported from Tokushima Prefecture in Shikoku, and Hyogo, Okayama, and Yamaguchi Prefectures in the western area of Honshu, Japan (Akatsuka and Komai 1922, Uéno 1927, 1933a, c, Tomikawa et al. 2008); *P. yezonis* was collected in Hokkaido and Akita Prefecture in the northern area of Honshu (Akatsuka and Komai 1922, Uéno 1933b, Matsuda 1954); and *P. kyotonis* has been recorded from Kyoto, Gifu, Shizuoka, and Shimane Prefectures in Honshu (Akatsuka and Komai 1922, Uéno 1927, 1971a, c, Nunomura 1975). Additionally, two continental *Pseudocrangonyx* species were reported from Japanese waters: *P. asiaticus* Uéno, 1934 from Tsushima Island (Uéno 1971b); and *P. coreanus* Uéno, 1966 from Tsushima Island and Shimane Prefec-



**Figure 1.** Map showing the collection localities of the specimens examined in this study and type localities of the known Japanese *Pseudocrangonyx* species. The closed circles indicate the localities of the referred materials used for the phylogenetic analyses. The star in red denotes the type locality of the new species; in purple, *P. shikokunis*; in blue, *P. kyotonis*; and in green, *P. yezonis*. Names of localities are shown in Table 1.

ture in the western area of Honshu (Uéno 1971b, Narahara et al. 2009).

Over recent decades, additional species have been described from the Far East of Russia (e.g., Sidorov and Gontcharov 2013). These results indicate that *Pseudocrangonyx* is highly diversified, and several species await description (Sidorov and Gontcharov 2013). Therefore, it is highly possible that there are also many undescribed species in the Japanese Archipelago.

During field surveys of the benthic invertebrate fauna in the spring-fed stream Gudari-numa in Hakkoda Mountains, Aomori Prefecture in the northern Honshu, two of the authors (AS and AO) and their colleagues collected several *Pseudocrangonyx* specimens. After careful examination of the materials, it was revealed that the collected *Pseudocrangonyx* amphipods represent an undescribed species. Thus, this new species is described herein. In addition, the phylogenetic position of the new species within *Pseudocrangonyx* was estimated using nuclear 28S

rRNA and histone H3, and mitochondrial cytochrome *c* oxidase subunit I and 16S rRNA sequence data.

The taxonomic description was prepared by the first and third authors (KT and AS). The second author (TN) conducted the molecular analyses, the fourth author (YO) assisted in manuscript preparation, and the last author (AO) provided the material of the new species and conducted this study.

## Material and methods

### Sample

Specimens of *Pseudocrangonyx* species were collected from 14 localities in Hokkaido, Honshu, and Shikoku, Japan (Fig. 1). Most of specimens were collected by scooping various groundwater environments in caves with a fine-mesh hand-net. The Gudari-numa specimens were pumped up with 10–55 L of interstitial water at 21–57 cm

beneath the surface of gravelly bottom using a handmade core sampler, and then collected, or directly collected by scooping together with bottom gravels of the stream. Most of the specimens were fixed in approximately 10% formaldehyde solution but a few were in 99% ethanol.

### Morphological observation

All appendages of the examined specimens of the undescribed species were dissected in 70% ethanol and mounted in gum-chloral medium on glass slides under a stereomicroscope (Olympus SZX7). Specimens were examined using a light microscope (Nikon Eclipse Ni) and illustrated with the aid of a camera lucida. The body length from the tip of the rostrum to the base of the telson was measured along the dorsal curvature to the nearest 0.1 mm. The nomenclature of the setal patterns on the mandibular palp follows Stock (1974). The specimens are deposited in the Tsukuba Collection Center of the National Museum of Nature and Science, Tokyo (NSMT) and the Zoological Collection of Kyoto University (KUZ).

### PCR and DNA sequencing

The extraction of genomic DNA from appendage muscles of the *Pseudocrangonyx* materials preserved in 99% ethanol followed Tomikawa et al. (2014). Primer sets for the PCR and cycle sequencing (CS) reactions used in this study were as follows: for 28S rRNA (28S), 28F and 28R (PCR and CS) (Hou et al. 2007) with 28SF and 28SR (CS) (Tomikawa et al. 2012) as internal primers; for histone H3 (H3), H3aF and H3bR (PCR and CS) (Colgan et al. 1998); for cytochrome *c* oxidase subunit I (COI), LCO1490 and HCO2198 (PCR and CS) (Folmer et al. 1994), or jgLCO1490 and jgHCO2198 (Geller et al. 2013), respectively, with M13F and M13R tails (Messing 1983), used for PCR, and then M13F and M13R used as primers for CS, followed Raupach et al. (2015); for 16S rRNA (16S), 16STf (Macdonald III et al. 2005) and 16Sbr (Palumbi 1996; modified to correspond with “Fruit Fly”) (PCR and CS).

The PCR reaction and DNA sequencing for a part of COI and 16S sequences followed Tomikawa et al. (2014); the PCR and CS reactions were performed using a PC-320 Thermal Cycler (ASTEC). Those for the other sequences were performed using the modified method outlined by Nakano (2012) and Tomikawa et al. (2016); reactions were performed using a T-100 Thermal Cycler (Bio-Rad). When using a PC-320 Thermal Cycler, the PCR mixtures were heated to 94°C for 7 min, followed by 35 cycles at 94°C (45 s), 42°C (1 min) and 72°C (1 min), and a final extension at 72°C (7 min). In the other reactions using a T-100, the PCR mixtures were heated to 94°C for 6 min, followed by 35 cycles at 94°C (10 s), 50°C for 28S and H3 or 48°C for COI and 16S (20 s each), and 72°C (1 min 24 s for 28S, 24 s for H3, and 42 s for COI and 16S), and a final extension at 72°C for 6 min. When using a PC-320, the CS conditions were 25 cycles at 96°C (10 s), 50°C (5 s) and 60°C (4 min). The sequencing mixtures for the other reactions were heated to 96°C for 2 min, followed by 40 cycles at 96°C (10 s), 50°C (5

s) and 60°C (36 s). The obtained sequences of a portion of COI and 16S were edited using MEGA6.03 (Tamura et al. 2013), and the reminders were assembled using DNA BASER (Heracle Biosoft S.R.L.). These DNA sequences were deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (DDBJ) (Table 1).

### Molecular phylogenetic analyses

Twenty-one published sequences were obtained from the INSDC for use in molecular phylogenetic analyses (Table 1). Eleven OTUs of the seven *Pseudocrangonyx* species, *P. febras* Sidorov, 2009, *P. holsingeri* Sidorov & Gontcharov, 2013, *P. korkishkoorum* Sidorov, 2006, *P. kseniae* Sidorov, 2012, *P. susanaensis* Labay, 1999, *P. sympatricus* Sidorov & Gontcharov, 2013, and *P. tinovi* Sidorov & Gontcharov, 2013, distributed in the Russian Far East were included in the analyses along with the following three crangonyctoid amphipods as outgroup taxa: *Crangonyx floridanus* Bousfield, 1963, *Crymostygius thingvallensis* Kristjánsson & Svavarsson, 2004, and *Eocrangonyx primoryensis* Stock & Jo, 1990.

The phylogenetic position of the *Pseudocrangonyx* amphipod from the Gudari-numa Stream within the genus was estimated based on the gene fragments of 28S, H3, COI, and 16S sequences. The alignments of H3 and COI was trivial, as no indels were observed. The 28S, and 16S sequences were aligned using MAFFT v. 7.299b L-INS-i (Katoh and Standley 2013). The lengths of 28S, H3, COI and 16S sequence lengths were 1,480, 328, 658, and 431 bp, respectively.

Prior to construction a phylogenetic tree based on the concatenated sequences, maximum likelihood (ML) trees were constructed based on each of the 28S, COI, and 16S markers using RAxML v. 8.2.8 (Stamatakis 2014) with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping (Felsenstein 1985) conducted with 1,000 replicates. Based on the three obtained ML trees (not shown), a 28S (HQ286019) and a COI (HQ286032) sequences of *C. thingvallensis* were removed from the dataset to prevent long branch attraction. Then, 28S sequences were re-aligned using MAFFT L-INS-i and refined with Gblocks Server v. 0.91b (Castresana 2000) with a default setting, and thus their final length was 980 bp. The concatenated sequences yielded 2,397 bp of alignment positions. One of the completely identical sequences (G1297 and G1298) was removed from the dataset using the “pgelimdupseq” command implemented in Phylogears v. 2.0.2014.03.08 (Tanabe 2008).

ML phylogenies were conducted using RAxML v. 8.2.8 with GTRCAT, immediately after nonparametric bootstrapping (BS) conducted with 1,000 replicates. The best fit-partitioning scheme for the ML analysis was identified with the Akaike information criterion (Akaike 1974) using PartitionFinder v. 1.1.1 (Lanfear et al. 2012) with the “all” algorithm: 28S/1st and 2nd positions of H3/3rd position of H3/1st position of COI/2nd position of COI/3rd position of COI/16S. BI and Bayesian poste-

**Table 1.** Samples used for the phylogenetic analyses. The information on the vouchers is accompanied by the collection localities and the INSDC accession numbers. Sequences marked with an asterisk were obtained for the first time in the present study. Acronym: NSMT, the Tsukuba Collection Center of the National Museum of Nature and Science, Tokyo. Identification sources: a, by the first author KT; b, Narahara et al. (2009); c, Nunomura (1975); d, Uéno (1927); e, Uéno (1971a).

| #                      | Species                            | Voucher or isolate # | Locality          | INSDC #   |            |           |           |
|------------------------|------------------------------------|----------------------|-------------------|-----------|------------|-----------|-----------|
|                        |                                    |                      |                   | 28S       | Histone H3 | COI       | 16S       |
| <i>Pseudocrangonyx</i> |                                    |                      |                   |           |            |           |           |
| 1                      | <i>P. yezonis</i> <sup>a</sup>     | G1280                | Mukawa, Hokkaido  | LC171518* | LC171520*  | LC171519* | LC171517* |
| 2                      | <i>Pseudocrangonyx</i> sp.         | NSMT-Cr 24605        | Aomori, Aomori    | LC171498* | LC171500*  | LC171499* | LC171497* |
| 3                      | <i>P. yezonis</i> <sup>a</sup>     | G1279                | Daisen, Akita     | LC171514* | LC171516*  | LC171515* | LC171513* |
| 4                      | <i>Pseudocrangonyx</i> sp.         | G400                 | Ofunato, Iwate    |           |            |           | LC171479* |
| 5                      | <i>Pseudocrangonyx</i> sp.         | G1281                | Ofunato, Iwate    |           |            |           | LC171521* |
| 6                      | <i>P. kyotonis</i> <sup>c</sup>    | G1297                | Gujo, Gifu        | LC171541* | LC171543*  | LC171542* | LC171540* |
| 6                      | <i>P. kyotonis</i> <sup>c</sup>    | G1298                | Gujo, Gifu        | LC171545* | LC171547*  | LC171546* | LC171544* |
| 7                      | <i>Pseudocrangonyx</i> sp.         | G404                 | Taga, Shiga       | LC171488* | LC171489*  |           |           |
| 8                      | <i>Pseudocrangonyx</i> sp.         | G405                 | Taga, Shiga       | LC171491* | LC171493*  | LC171492* | LC171490* |
| 9                      | <i>Pseudocrangonyx</i> sp.         | G406                 | Taga, Shiga       | LC171495* | LC171496*  |           | LC171494* |
| 10                     | <i>Pseudocrangonyx</i> sp.         | G1282                | Otsu, Shiga       |           | LC171523*  |           | LC171522* |
| 11                     | <i>Pseudocrangonyx</i> sp.         | G1283                | Niimi, Okayama    | LC171525* | LC171527*  | LC171526* | LC171524* |
| 12                     | <i>P. kyotonis</i> <sup>c</sup>    | G402                 | Matsue, Shimane   | LC171485* | LC171487*  | LC171486* | LC171484* |
| 13                     | <i>P. coreanus</i> <sup>b</sup>    | G401                 | Ota, Shimane      | LC171481* | LC171483*  | LC171482* | LC171480* |
| 14                     | <i>P. shikokunis</i> <sup>d</sup>  | G1277                | Mine, Yamaguchi   | LC171506* | LC171508*  | LC171507* | LC171505* |
| 15                     | <i>Pseudocrangonyx</i> sp.         | G1278                | Mine, Yamaguchi   | LC171510* | LC171512*  | LC171511* | LC171509* |
| 16                     | <i>Pseudocrangonyx</i> sp.         | G1271                | Takamatsu, Kagawa | LC171502* | LC171504*  | LC171503* | LC171501* |
| 17                     | <i>Pseudocrangonyx</i> sp.         | G1295                | Kami, Kochi       | LC171533* | LC171535*  | LC171534* | LC171532* |
| 18                     | <i>Pseudocrangonyx</i> sp.         | G1296                | Kami, Kochi       | LC171537* | LC171539*  | LC171538* | LC171536* |
| 19                     | <i>Pseudocrangonyx</i> sp.         | G1294                | Seiyo, Ehime      | LC171529* | LC171531*  | LC171530* | LC171528* |
|                        | <i>P. febras</i>                   |                      |                   |           |            | KF153114  |           |
|                        | <i>P. holsingeri</i>               |                      |                   | KJ871679  |            | KF153111  |           |
|                        | <i>P. korkishkorum</i>             | B1                   |                   | KJ871678  |            | KF153107  |           |
|                        | <i>P. korkishkorum</i>             | B2                   |                   |           |            | KF153108  |           |
|                        | <i>P. korkishkorum</i>             | B3                   |                   |           |            | KF153109  |           |
|                        | <i>P. korkishkorum</i>             | N1                   |                   | KJ871676  |            | KF153105  |           |
|                        | <i>P. korkishkorum</i>             | N2                   |                   | KJ871677  |            | KF153106  |           |
|                        | <i>P. kseniae</i>                  |                      |                   | KJ871675  |            | KF153115  |           |
|                        | <i>P. susanaensis</i>              |                      |                   |           |            | KF153113  |           |
|                        | <i>P. sympatricus</i>              |                      |                   |           |            | KF153112  |           |
|                        | <i>P. tiunovi</i>                  |                      |                   | KJ871674  |            | KF153110  |           |
| Outgroup               |                                    |                      |                   |           |            |           |           |
|                        | <i>Crymostygius thingvallensis</i> |                      |                   | HQ286019  |            | HQ286032  | HQ286009  |
|                        | <i>Eocrangonyx primoryensis</i>    |                      |                   |           |            |           | HQ286011  |
|                        | <i>Crangonyx floridanus</i>        | G1322                | Chiba, Chiba      | LC171549* |            | LC171550* | LC171548* |

rior probabilities (PPs) were estimated using MrBayes v. 3.2.6 (Ronquist et al. 2012). The best-fit partition scheme as well as models for each partition were selected based on the Bayesian information criterion (Schwarz 1978) using PartitionFinder with the “all” algorithm: for 28S, GTR+G; for the 1st and 2nd positions of H3, JC69; for the 3rd position of H3, K80+G; for the 1st position of COI, SYM+I; for the 2nd position of COI, HKY85+I+G; for the 3rd position of COI, GTR+I+G; and for 16S, GTR+I+G. Two independent runs of four Markov chains were conducted for 20 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut and Drummond 2013) and the first 50,001 trees were discarded based on these results.

## Results

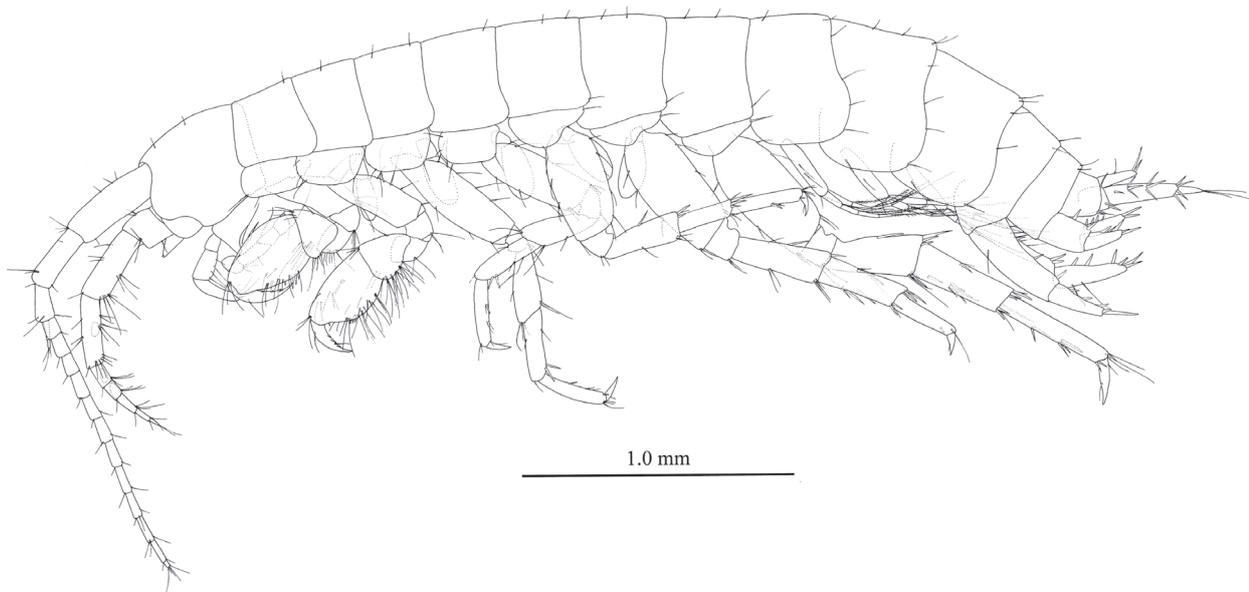
### Taxonomy

#### *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n.

<http://zoobank.org/A821F75A-4FCB-40F7-B8AE-48289A696725>  
New Japanese name: Gudarimekura-yokoebi

Figs 2–9

**Type materials.** Holotype: Male (3.9 mm), NSMT-Cr 24603, Gudari-numa Stream (40°40'21.2"N, 140°55'54.6"E, elev. 589 m), Komagome, Aomori, Aomori Prefecture, Japan, 4 June 2014, collected by A. Ohtaka. Paratypes: 1 female (3.1 mm), NSMT-Cr 24604, locality same as for holotype, 21 June 2015, collected by



**Figure 2.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., holotype, male (3.9 mm), NSMT-Cr 24603. Habitus, lateral view.

A. Ohtaka; 1 female (4.4 mm), NSMT-Cr 24605, locality same as for holotype, 14 March 2015, collected by A. Ohtaka; 1 male (2.5 mm) and 3 females (1.4–2.8 mm), NSMT-Cr 24606, locality same as for holotype, 23 May 2015, collected by A. Ohtaka; 4 females (2.0–2.9 mm) KUZ Z1746, data same as for holotype, collected by A. Ohtaka.

**Type locality.** Japan, Aomori Prefecture: Aomori, Gudari-numa Stream (northern Honshu).

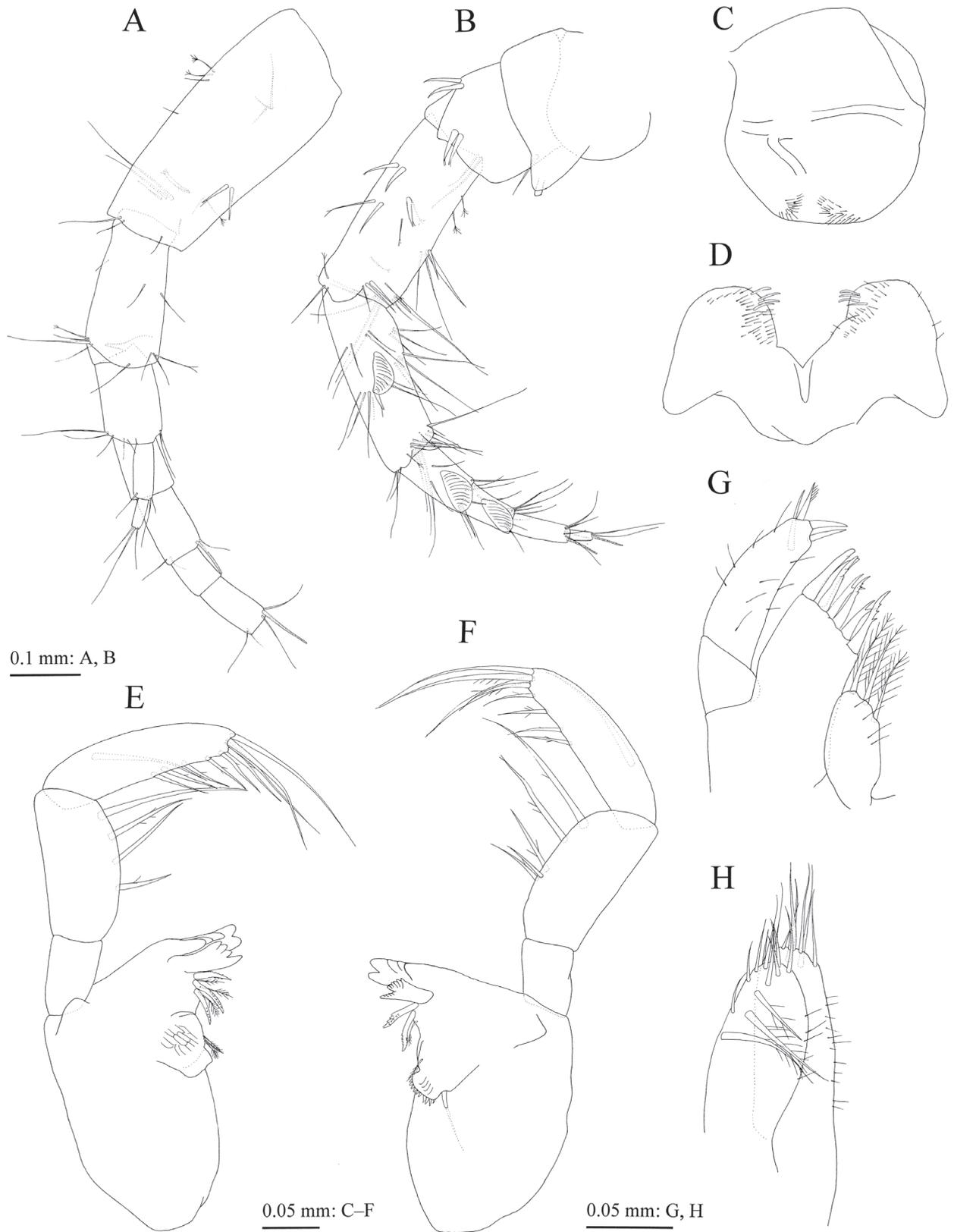
**Description.** *Male* [NSMT-Cr 24603, 3.9 mm]. Head (Fig. 2) with short dorsal setae; rostrum reduced; lateral cephalic lobe rounded; antennal sinus shallow with rounded angle; eyes absent. Pereonites 1–7 with short dorsal setae (Fig. 2); posterolateral margin of pereonites 5–7 with seta (Fig. 2). Dorsal margin of pleonites 1–3 with 5, 6, and 8 setae, respectively (Fig. 7I–K). Dorsal margin of urosomites 1 and 2 each with 4 setae (Fig. 7L, M), dorsal margin of urosomite 3 lacking setae (Fig. 7N). Posterior margin of epimeral plate 1 with 2 setae, posteroventral corner rounded with 1 seta (Fig. 7P); ventral and posterior margins of plate 2 with 1 and 2 setae, respectively, posteroventral corner rounded, with 1 seta (Fig. 7Q); ventral and posterior margins of plate 3 each with 1 seta, posteroventral corner rounded, with 1 seta (Fig. 7R).

Antenna 1 (Fig. 3A) 0.47 times as long as body length, peduncular articles 1 to 3 in length ratio of 1.0 : 0.6 : 0.4; accessory flagellum 2-articulate, terminal article with 3 setae; primary flagellum 12-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 3B) 0.60 times as long as antenna 1; peduncular article 5 with calceolus; flagellum 0.55 times as long as peduncular articles 4 and 5 combined, consisting of 4 articles, first 2 of which with calceolus.

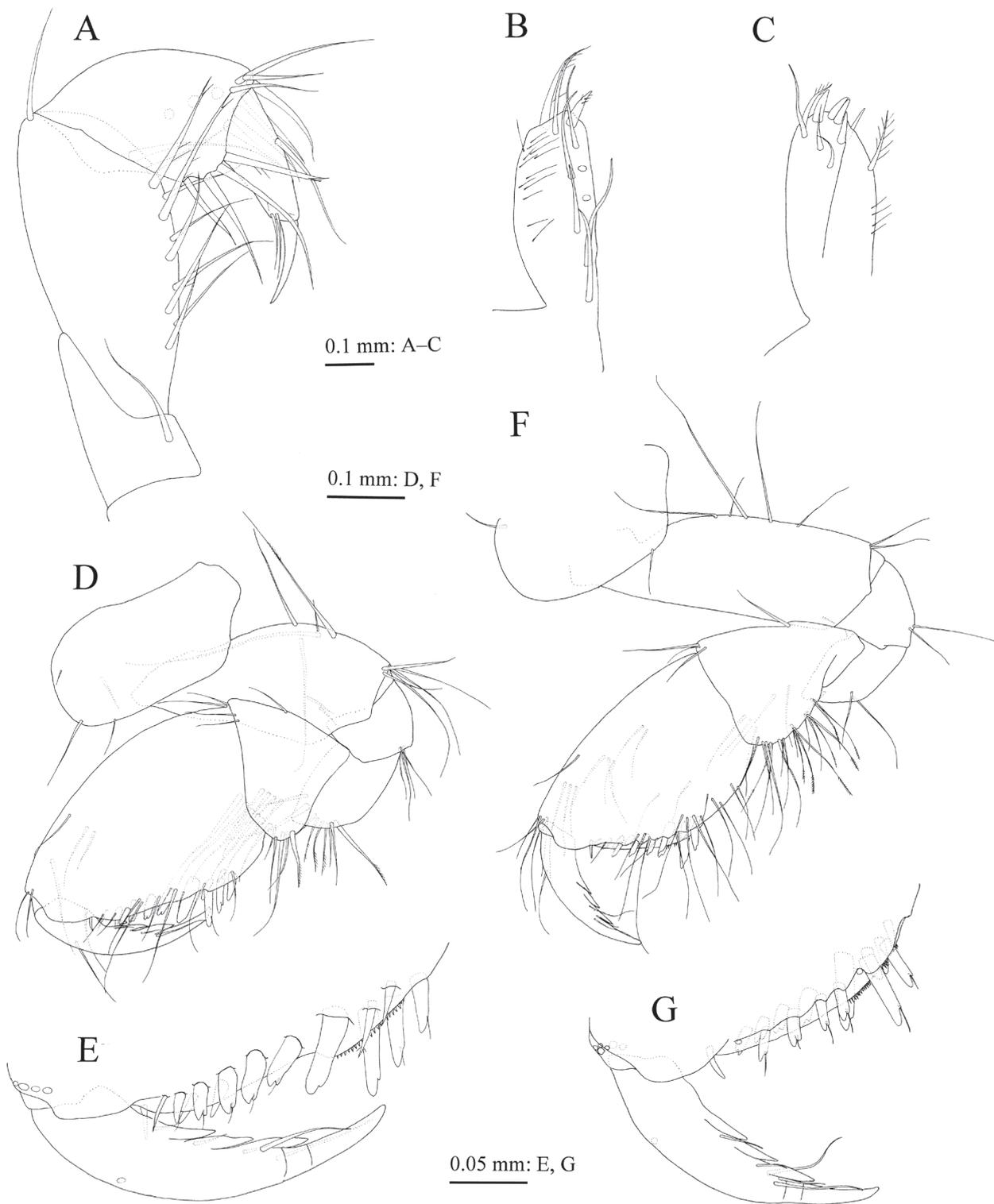
Upper lip (= labrum) (Fig. 3C) with rounded anterior margin, bearing fine setae. Mandibles (Fig. 3E, F) with

left and right incisors both 5-dentate; left lacinia mobilis 4-dentate, right lacinia bifid, bearing many teeth; molar process triturative, molar of right mandible with accessory seta; accessory setal rows of left and right mandibles with 3 and 2 weakly pectinate setae, respectively; palp 3-articulate, article 3 with 1 A-, 4–5 D-, and 4 E-setae. Lower lip (Fig. 3D) with broad outer lobes, mandibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 3G) with inner and outer plates, and palp; inner plate subovate, its medial margin with 3 plumose setae; outer plate subrectangular with 7 serrate teeth apically; palp 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 3 apical and 1 subapical robust setae. Maxilla 2 (Fig. 3H) with oblique inner row of 3 plumose setae on inner plate. Maxilliped (Fig. 4A–C) with inner and outer plates, and palp; inner plate (Fig. 4C) with 3 apical and 2 subapical robust setae; outer plate (Fig. 4B) with 1 apical plumose seta and 1 apical robust seta and 5 medial slender setae; palp (Fig. 4A) 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

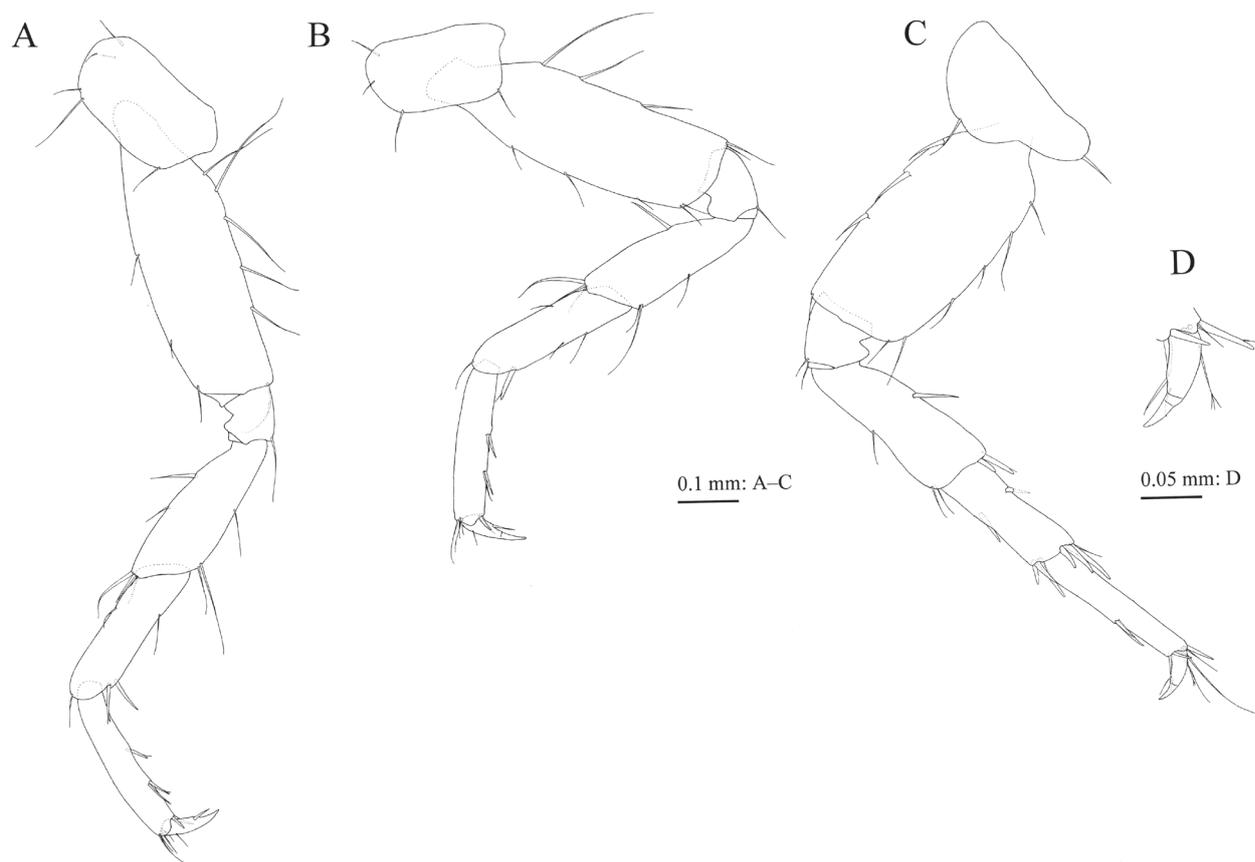
Gnathopod 1 (= pereopod 1) (Fig. 4D, E) with subquadrate coxa, bearing setae on anterodistal corner of coxa, width 2.0 times as long as depth; anterior margin of basis bare, posterior margin of basis with 5 setae; posterodistal corner of carpus with slender setae, some weakly pectinate; propodus stout, subchelate, palmar margin with 6 medial and 5 lateral robust setae, some distally notched (Fig. 4E); posterior margin of dactylus dentate (Fig. 4E). Gnathopod 2 (= pereopod 2) (Fig. 4F, G) with subquadrate coxa bearing setae on its anterior margin and posterodistal corner, width 1.9 times as long as depth; anterior and posterior margins of basis with 0 and 5 setae, respectively; posterodistal corner of carpus with slender seta, some weakly pectinate; propodus stout, subchelate with 6 medial and 8 lateral robust setae along palmar margin, some



**Figure 3.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., holotype, male (3.9 mm), NSMT-Cr 24603. **A** antenna 1, medial view; **B** antenna 2, medial view; **C** upper lip, anterior view; **D** lower lip, ventral view; **E** left mandible, medial view; **F** right mandible, medial view; **G** maxilla 1, dorsal view; **H** maxilla 2, dorsal view.



**Figure 4.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., holotype, male (3.9 mm), NSMT-Cr 24603. **A** maxilliped, dorsal view; **B** inner plate of maxilliped, dorsal view; **C** outer plate of maxilliped, dorsal view; **D** gnathopod 1, lateral view; **E** palmar margin of propodus and dactylus of gnathopod 1, medial view; **F** gnathopod 2, lateral view; **G** palmar margin of propodus and dactylus of gnathopod 2, medial view.



**Figure 5.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., holotype, male (3.9 mm), NSMT-Cr 24603. **A** pereopod 3, lateral view; **B** pereopod 4, lateral view; **C** pereopod 5, lateral view; **D** dactylus of pereopod 5, lateral view.

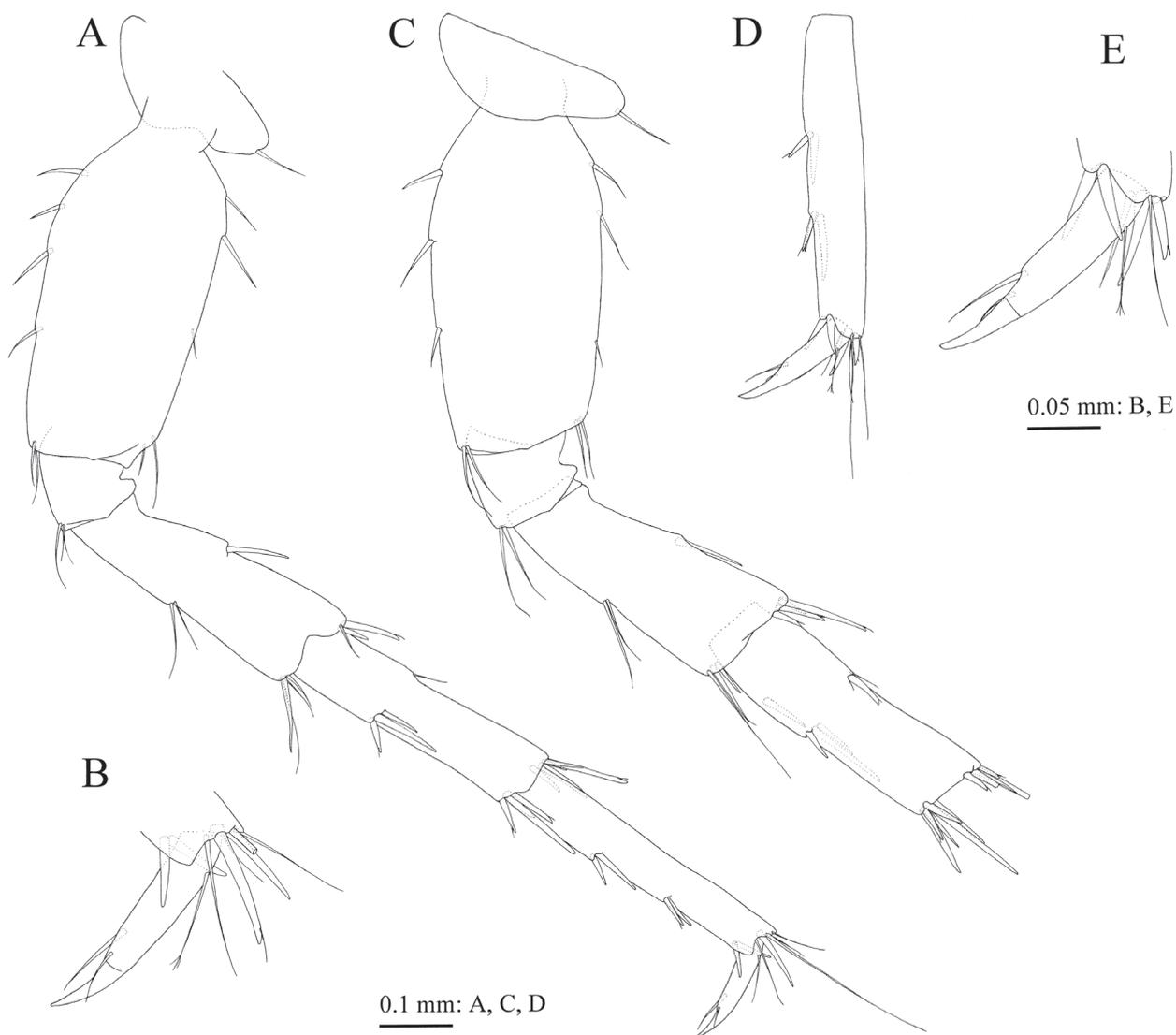
distally notched (Fig. 4G); posterior margin of dactylus dentate (Fig. 4G). Pereopod 3 (Fig. 5A) with subquadrate coxa bearing setae on its anterodistal and posterovertral corners, width 1.8 times as long as depth; anterior and posterior margins of basis with 2 and 5 setae, respectively; merus, carpus, and propodus in length ratio of 1.0 : 1.0 : 0.9; posterior margin of dactylus with 1 seta. Pereopod 4 (Fig. 5B) with subquadrate coxa bearing setae on its anterior margin, anterodistal and posterovertral corners, width 1.8 times as long as depth; anterior and posterior margins of basis each with 3 setae; merus, carpus, and propodus in length ratio of 1.0 : 0.8 : 0.8; posterior margin of dactylus with 1 seta. Pereopod 5 (Fig. 5C, D) with weakly bilobed coxa bearing setae on anterior and posterior lobes; anterior and posterior margins of basis with 3 and 4 setae, respectively; merus, carpus, and propodus in length ratio of 1.0 : 0.8 : 0.9; posterior margin of dactylus with 2 setae (Fig. 5D). Pereopod 6 (Fig. 6A, B) with coxa bearing concave lower margin, posteroproximal corner with 1 seta; anterior and posterior margins of basis with 4 and 3 setae, respectively; merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 1.1; posterior margin of dactylus with 2 setae (Fig. 6B). Pereopod 7 (Fig. 6C–E) with subtriangular coxa, bearing 1 seta on posteroproximal corner; anterior and posterior margins of basis each with 3 setae; merus,

carpus, and propodus in length ratio of 1.0 : 1.1 : 1.2; posterior margin of dactylus with 2 setae (Fig. 6E).

Coxal gills (Fig. 2) on gnathopod 2 and pereopods 3–6; sternal gills absent.

Peduncles of pleopods 1 and 2 (Fig. 7A, C) with 2 and 1 setae on outer margins, respectively; peduncle of pleopod 3 (Fig. 7D) lacking marginal setae. Pleopods 1–3 each with paired retinacula (Fig. 7B), and lacking bifid setae (clothes-pin setae) on inner basal margin of inner ramus.

Uropod 1 (Fig. 7E) with basofacial slender seta on peduncle; inner ramus 0.70 times as long as peduncle, inner margin of former with 2 robust setae, outer margin bare, basal part with 3 slender setae; outer ramus 0.76 times as long as inner, its inner and outer margins with 0 and 2 robust setae, respectively. Uropod 2 (Fig. 7F, G) with inner and outer rami; inner ramus 0.90 times as long as peduncle, its inner margin with 4 robust setae, outer margin bare, distal part with 2 serrate and 4 simple robust setae and 1 slender seta (Fig. 7G); outer ramus 0.89 times as long as inner ramus, its outer margin with 1 robust seta. Uropod 3 (Fig. 7H) with peduncle 0.33 times as long as outer ramus, with 1 dorsal and 3 ventral robust setae; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.36 times as long as proximal article, with 3 distal setae.



**Figure 6.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., holotype, male (3.9 mm), NSMT-Cr 24603. **A** pereopod 6, lateral view; **B** dactylus of pereopod 6, lateral view; **C** coxa–carpus of pereopod 6, lateral view; **D** propodus–dactylus of pereopod 6, lateral view; **E** dactylus of pereopod 7, lateral view.

Telson (Fig. 70) length 1.2 times as long as wide, cleft for 0.08 times of length, each telson lobe with 2 lateral long penicillate setae, 2 apical robust and 1 apical short penicillate setae.

*Female* [NSMT-Cr 24604, 3.1 mm]. Antenna 1 (Fig. 8A) 0.58 times as long as body length, primary flagellum 12-articulate. Antenna 2 (Fig. 8B) 0.64 times as long as antenna 1, calceoli absent; flagellum 0.64 times as long as peduncular articles 4 and 5 combined, 5-articulate, first 2 of which with 1 robust seta, lacking calceoli.

Lacinia mobilis of left mandible 5-dentate.

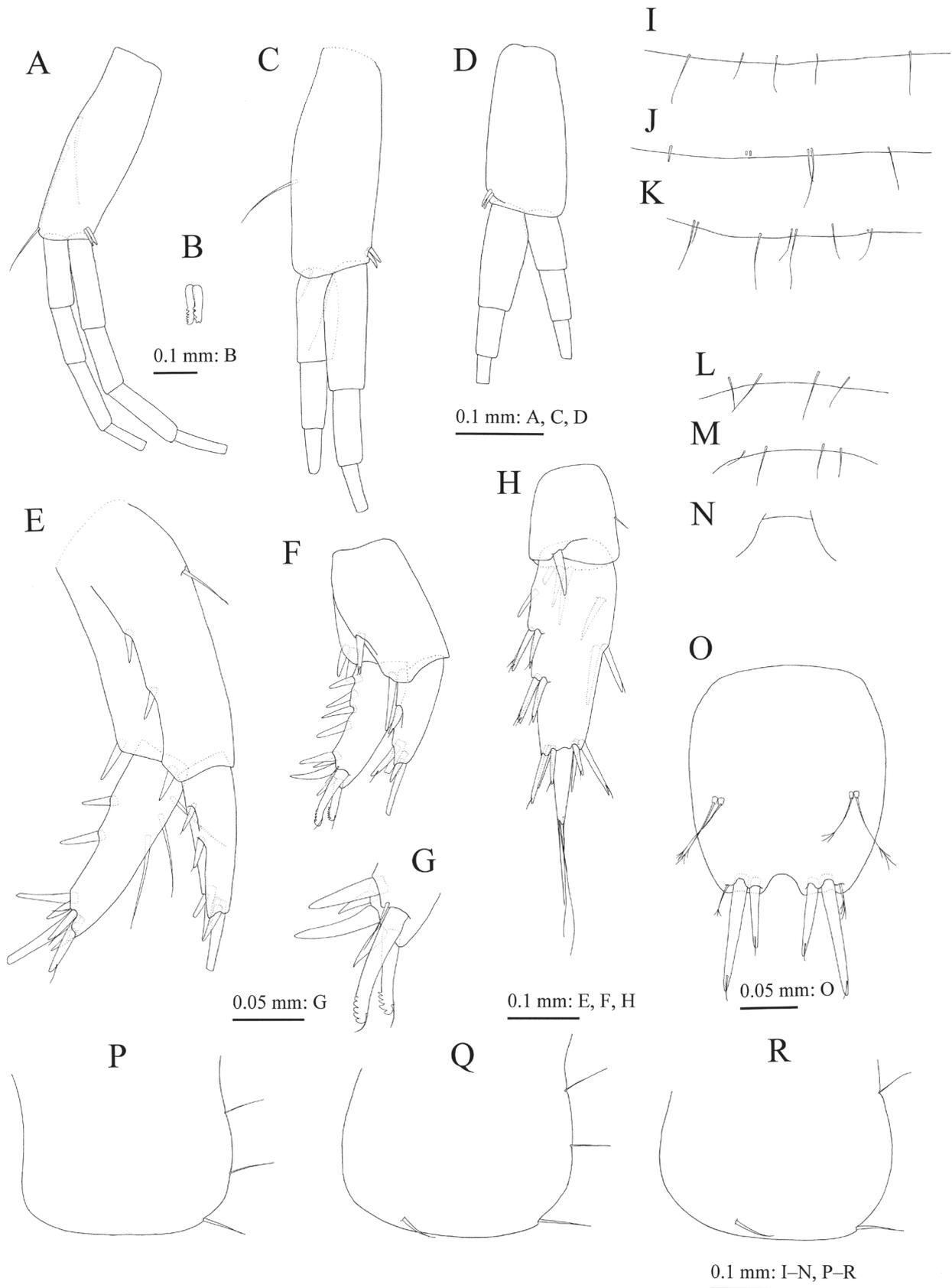
Gnathopod 1 (Fig. 8C, D) with coxa width 1.9 times as long as depth; palmar margin (Fig. 8D) with 4 medial and 4 lateral distally notched robust setae. Gnathopod 2 (Fig. 8E, F) with coxa width 1.8 times as long as depth; palmar margin (Fig. 8F) with 9 medial and 5 lateral robust setae, some distally notched.

Brood plates (Fig. 8G) slender, with numerous setae, on gnathopod 2 and pereopods 3–5.

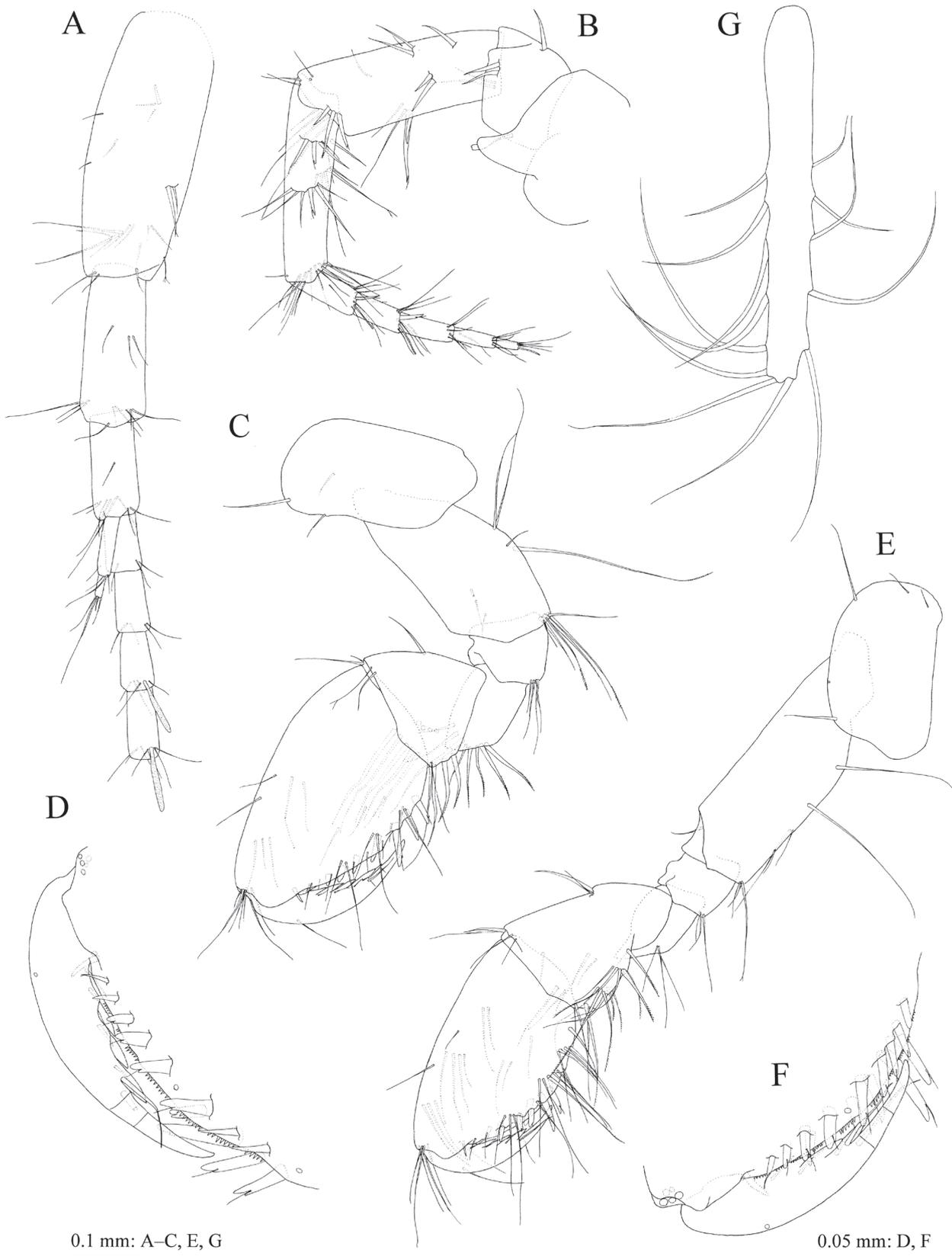
Uropod 1 (Fig. 9A) with 3 robust setae on inner margin of inner ramus, basal part with 2 slender setae; outer ramus 0.80 times as long as inner. Uropod 2 (Fig. 9B) with 6 simple robust setae and 1 slender seta on distal part of inner ramus. Uropod 3 (Fig. 9C) with peduncle 0.32 times as long as outer ramus; terminal article of outer ramus 0.35 times as long as proximal article.

**Etymology.** The specific name is an adjective derived from Gudari-numa, the type locality of the new species.

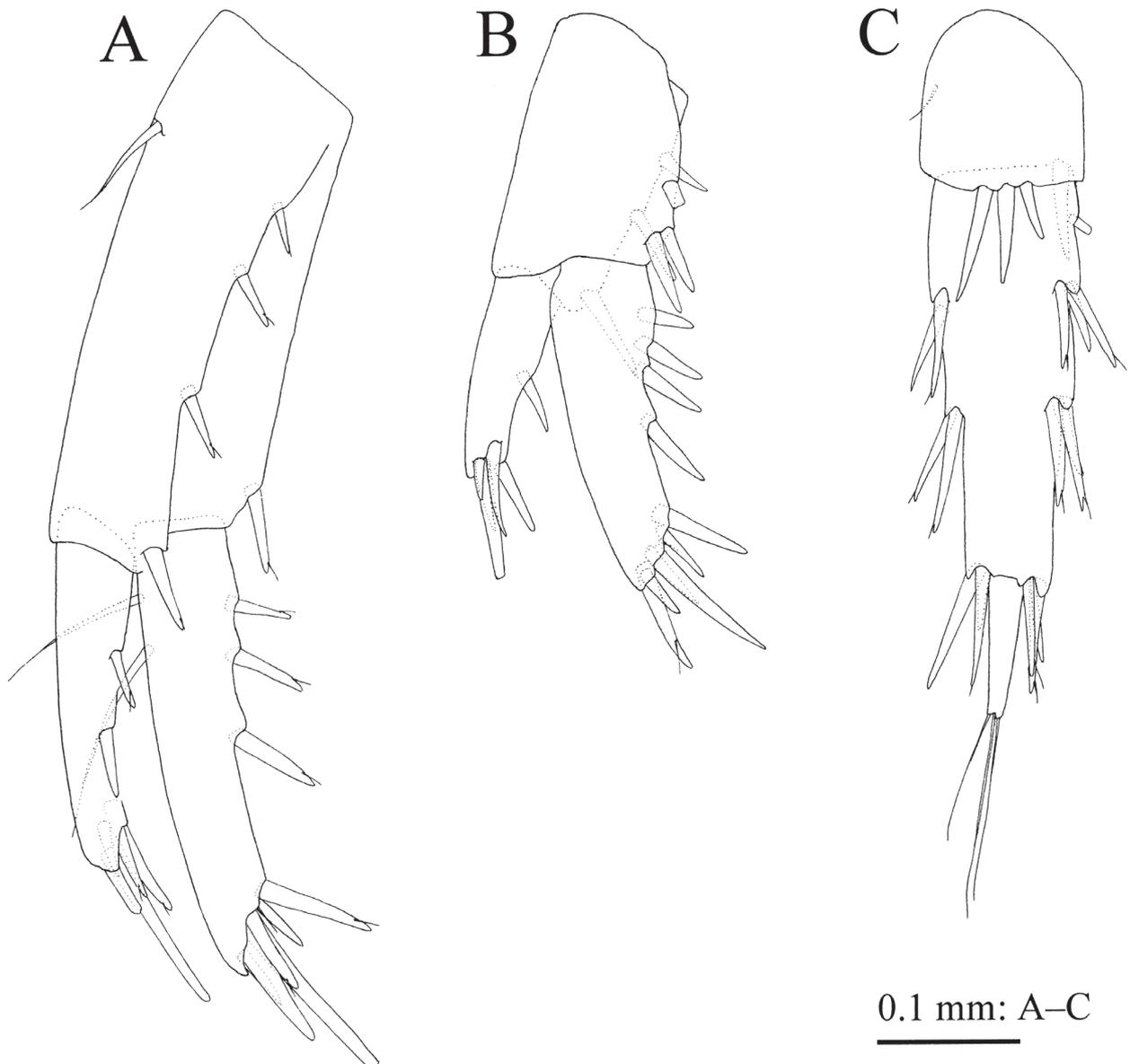
**Distribution and habitat.** This species is known only from the type locality. The specimens were collected from interstitial water in the gravelly bottom. Water temperature of the habitat was stable and around 7°C throughout the year (Baba and Ohtaka unpublished).



**Figure 7.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., holotype, male (3.9 mm), NSMT-Cr 24603. **A** pleopod 1, anterior view; **B** retinacula on peduncle of pleopod 1, anterior view; **C** pleopod 2, anterior view; **D** pleopod 3, anterior view; **E** uropod 1, dorsal view; **F** uropod 2, dorsal view; **G** distal part of inner ramus of uropod 2, dorsal view; **H** uropod 3, dorsal view; **I–K** dorsal margins of pleonites 1–3, respectively, dorsal views; **L–N** dorsal margins of urosomites 1–3, respectively, dorsal views; **O** telson, dorsal view; **P–R** epimeral plates 1–3, respectively, lateral views.



**Figure 8.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., paratype, female (3.1 mm), NSMT-Cr 24604. **A** antenna 1, medial view; **B** antenna 2, medial view; **C** gnathopod 1, lateral view; **D** palmar margin of propodus and dactylus of gnathopod 1, medial view; **E** gnathopod 2, lateral view; **F** palmar margin of propodus and dactylus of gnathopod 2, medial view.

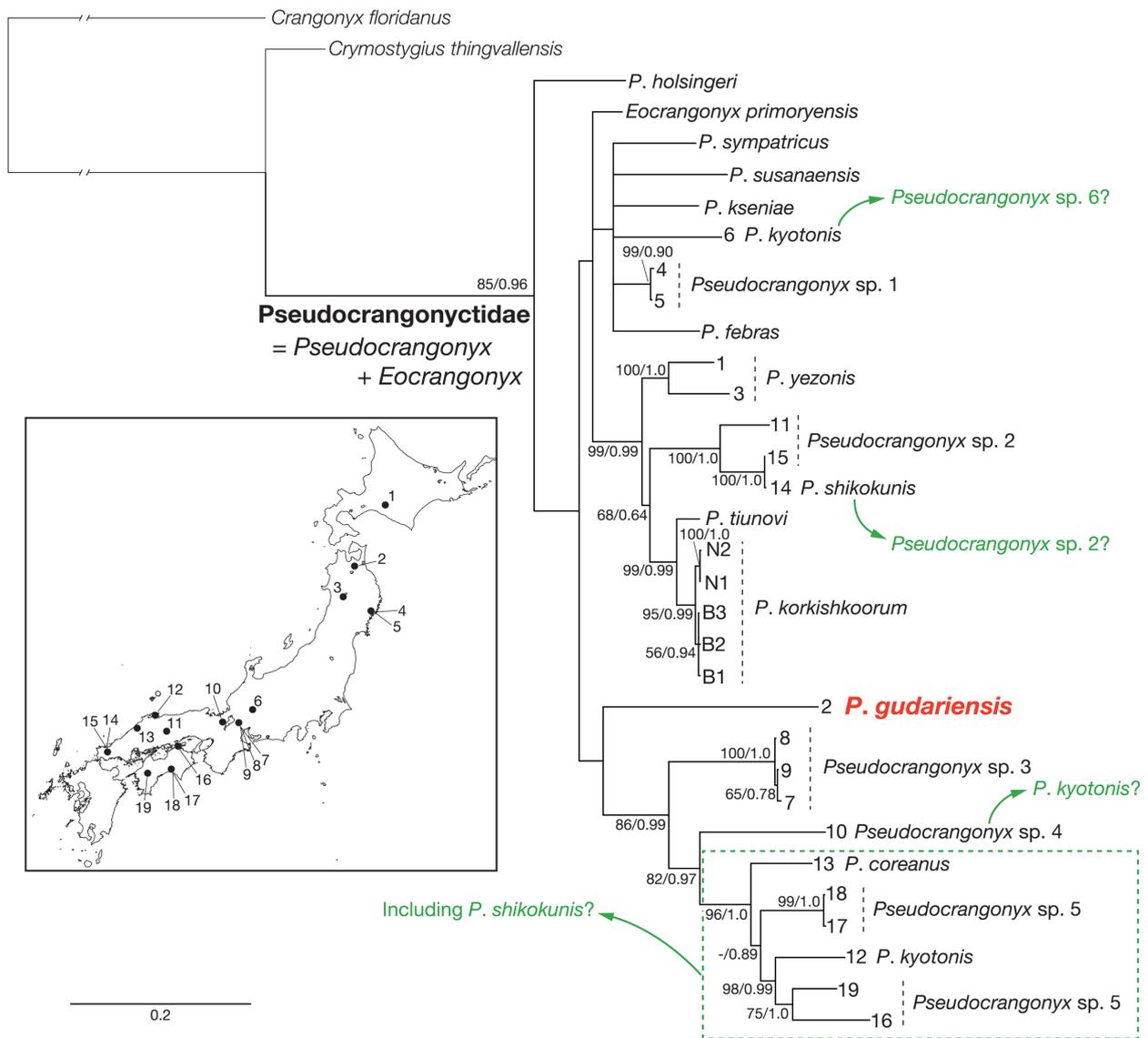


**Figure 9.** *Pseudocrangonyx gudariensis* Tomikawa & Sato, sp. n., paratype, female (3.1 mm), NSMT-Cr 24604. A–C uropods 1–3, respectively, dorsal views.

**Remarks.** *Pseudocrangonyx gudariensis* is morphologically similar to *P. coreanus* described from the Korean Peninsula. The deposited female paratypes of the latter species have calceoli on antenna 2 and pleopods without bifid setae on inner basal margin of inner ramus, which are features that were not mentioned in the original description (NSMT-Cr 13521–13522; Tomikawa and Onodera, personal observation). These two species share the following features: 1) relatively small body size (smaller than 6 mm), 2) eye completely absent, 3) carpus of gnathopod 2 without serrate robust setae on posterodistal corners, 4) outer margin or outer distal corner of pleopods 1 and 2 with setae, 5) inner basal margin of inner ramus of pleopods without bifid setae, and 6) small number of articles (less than 5) of rami of pleopods. However, *P. gudariensis* is distinguished from *P. coreanus* by the fol-

lowing features (features of *P. coreanus* in parentheses): 1) antenna 2 of female without calceoli (present); 2) palmar margins of gnathopods 1 and 2 with distally notched robust setae (absent); 3) inner margin of inner ramus of uropod 2 with 4 (0 or 1) robust setae; and 4) basal part of inner ramus of uropod 2 without slender seta (present).

*Pseudocrangonyx gudariensis* is also similar to *P. febras* from river basin of Primorye, Russia in having 1) relatively small body size (smaller than 6.5 mm), 2) eye completely absent, 3) palmar margins of gnathopods 1 and 2 with distally notched robust setae, 4) small number of articles (less than 6) of rami of pleopods, and 5) urosomite 1 without basal setae. However, *P. gudariensis* is distinguished from the latter by the following features (features of *P. febras* in parentheses): 1) carpus of gnathopod 2 without serrate robust setae on posterodistal cor-



**Figure 10.** Bayesian inference tree for 2,397 bp of nuclear 28S rRNA plus histone H3 and mitochondrial COI and 16S rRNA markers, with the map modified from Fig. 1. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities. Specimen numbers are also shown in Fig. 1 and Table 1.

ners (present), 2) peduncle of pleopods 1 and 2 with setae (absent), and 3) article 2 of uropod 3 longer (shorter) than setae on distal part of article 1.

### Molecular phylogenies

The obtained BI tree (Fig. 10) had an almost identical topology to that of the ML tree ( $\ln L = -13551.11$ ; not shown). The monophyly of the family Pseudocrangonyctidae (*Pseudocrangonyx* + *Eocrangonyx*) was well recovered (BS = 88%, PP = 0.96). However, our analyses failed to obtain robust phylogenetic relationships of the known *Pseudocrangonyx* species, and to determine the precise phylogenetic positions of each of the new species *P. gudariensis* (sample #2), *P. kyotonis* sensu Nunomura (1975) from Gifu (#6), and unidentified *Pseudocrangonyx* amphipods from Iwate (#4, 5). Two monophyletic lineages

received high support values. The one lineage (BS = 99%, PP = 0.99) contained *P. yezonis* clade (BS = 99%, PP = 1.0; #1, 3), the clade (BS = 100%, PP = 1.0) of the *Pseudocrangonyx* species from Okayama (11) and Yamaguchi (#14, 15) including *P. shikokunis* sensu Uéno (1927) (#14), and the clade (BS = 100%, PP = 1.0) comprised two Russian species, *P. korkishkoorum* and *P. tiunovi*. However, the relationships among these three clades were unresolved.

The other monophyletic lineage (BS = 93%, PP = 0.99) consisted of unidentified *Pseudocrangonyx* species from Shiga Prefecture (#7–10), and from Shikoku (#16–19), plus *P. coreanus* sensu Narahara et al. (2009) from Shimane (#10), and *P. kyotonis* sensu Uéno (1971a) from Shimane as well (#12). Three specimens from Shiga (#7–9) formed a well supported clade (BS = 100%, PP = 1.0). This subclade was a sister lineage to the clade

(BS = 88%, PP = 0.97) comprised reminders including a specimen from Shiga (#10). Specimens from Shimane (#12, 13) and Shikoku region (#16–19) formed a monophyletic lineage (BS = 96%, PP = 1.0). Monophyly of the *Pseudocrangonyx kyotonis* sensu Uéno (#12) and two specimens from Kagawa (#16) and Ehime (#19) was well supported (BS = 98%, PP = 0.99); the latter two (#16, 19) formed a clade, but this relationship was not strongly supported in the ML analysis (BS = 69%, PP = 1.0).

## Discussion

As mentioned in the Remarks, *P. gudariensis* is morphologically similar to *P. coreanus* and *P. febras*. These three species share the following characteristics: relatively small body size, absence of basal setae on urosomite 1, and small number of articles of rami of pleopods. However, our phylogenetic analyses failed to recover monophyly of *P. gudariensis* + *P. febras*. In addition, *P. coreanus* sensu Narahara et al. (2009) is genetically distant from *P. gudariensis* and *P. febras*. Therefore, the aforementioned shared characteristics do not reflect phylogenetic relationships of *Pseudocrangonyx* species. Whatever the case, the results of our morphological examination and molecular phylogenetic analyses fully support the distinct taxonomic status of the present new species in this genus.

Sidorov and Holsinger (2007) suggested that the colonization events of the ancestral *Pseudocrangonyx* species to the Japanese Archipelago could have taken place twice: in the Middle–Late Miocene and the Early Pleistocene through land bridges between the continental China and the Japanese Archipelago. The obtained phylogeny also indicated that multiple colonization events of *Pseudocrangonyx* amphipods to the Japanese Archipelago occurred. The present Japanese specimens were split into two clades, and three distinctive lineages, of which phylogenetic positions still remain uncertain. Moreover, the obtained tree showed that the phylogenetic positions of the several Japanese individuals did not reflect their geographical distributions. The specimens distributed in the Chugoku region (#11, 14, 15) did not form a monophyletic group with geographically close samples (e.g., #12, 13). These three specimens formed a clade with the northern Japanese *P. yezonis* and two Russian species. These results indicate that Japanese *Pseudocrangonyx* species experienced a complicated biogeographical history. To clarify the origin and dispersal routes of *Pseudocrangonyx* amphipods, comprehensive taxa sampling and more detailed genetic data are needed.

Our phylogenetic results also revealed that the species diversity of Japanese *Pseudocrangonyx* is quite high, and they should be classified into the known three species and additional undescribed species. Therefore, taxonomic studies should be conducted to determine the systematic accounts of these undescribed amphipods. First, however, the taxonomic status of the three known species described by Akatsuka and Komai (1922) should be revisited. In the

obtained tree, *P. kyotonis* sensu Nunomura (1975) (#6) and sensu Uéno (1971a) (#12) were not monophyletic. The latter was genetically more closely related to the *Pseudocrangonyx* species distributed in Shikoku (e.g., #16, 17), where the type locality of *P. shikokunis* is located. Alternatively, *P. shikokunis* sensu Uéno (1927) (#14) was distantly related to the *Pseudocrangonyx* specimens collected in Shikoku. These results highlighted that the previous studies of *Pseudocrangonyx* in Japan contained misidentified or taxonomically uncertain records.

In addition to *P. gudariensis*, *P. kyotonis* sensu Uéno (1971a), *P. kyotonis* sensu Nunomura (1975), and *P. coreanus* sensu Narahara et al. (2009), five unidentified Japanese *Pseudocrangonyx* species were identified based on molecular phylogenetic analyses (Fig. 10). Among these five species, it is highly possible that *Pseudocrangonyx* sp. 5 and *Pseudocrangonyx* sp. 4 represent the true *P. shikokunis* and *P. kyotonis*, respectively, because their collection localities are close to each of the type localities of these species. Accordingly, *P. kyotonis* sensu Nunomura (1975) and *P. shikokunis* sensu Uéno (1927) could be considered unidentified *Pseudocrangonyx* sp. 6 and *Pseudocrangonyx* sp. 2, respectively. To confirm whether these taxonomic treatments are adequate, molecular phylogenetic analyses including topotypic sequences of described species and detailed morphological analyses are needed.

Our phylogenetic analyses also shed light onto the taxonomic account of *Eocrangonyx* Schellenberg, 1936. This genus have been placed under the family Pseudocrangonyctidae along with *Pseudocrangonyx* (Holsinger 1989). These two genera bear a close resemblance to each other in general morphology. *Eocrangonyx* is distinguished from *Pseudocrangonyx* by the absence of article 2 of uropod 3 (Holsinger 1989). However, Sidorov and Holsinger (2007) revealed the presence of extremely reduced article 2 of uropod 3 in the Russian *E. stygoedincus* (Sidorov and Holsinger, 2007). Subsequently, Tomikawa and Shinoda (2016) also revealed the same characteristics in *E. japonicus* (Uéno, 1930), which is the type species of *Eocrangonyx*. Our phylogenetic tree showed that *Pseudocrangonyx* and *Eocrangonyx* are phylogenetically closely related (Fig. 10). However, the phylogenetic position of *E. primoryensis* remains unresolved. In addition, *E. japonicus* genetic data have been never assessed. To evaluate the independence of *Pseudocrangonyx* and *Eocrangonyx*, and the validity of article 2 of uropod 3 as a generic diagnostic character, it is necessary to clarify the phylogenetic relationships between *Pseudocrangonyx* and *Eocrangonyx* by including additional species and genetic markers. Consequently, there will be an enhanced understanding of species diversity and evolutionary history of the Far Eastern subterranean Crangonyctoidea species.

## Acknowledgements

We thank Dr Yuji Abe (Taga Town Museum), Kokichi Aoya (Daisen City), Dr Mark J. Grygier (Lake Biwa

Museum), Naoyuki Nakahama (Kyoto University), Ryo-suke Okano (Ehime University), Dr Tomislav Karanovic (Sungkyunkwan University), and Naoshi Sato (Ofunato City) for providing specimens of *Pseudocrangonyx*. KT thanks Satoko Tashiro (Hiroshima University), Yukiko Narahara-Nakano (Hiroshima University), and Daisuke Saiga (Hiroshima University) for supporting field work. Thanks are also due to Dr Ronald Vonk (Naturalis Biodiversity Center), Professor Boris Sket (University of Ljubljana), and Dr Michael Ohl (Museum für Naturkunde) for their critical reading and valuable comments on this manuscript. This work was partly supported by JSPS KAKENHI Grant Numbers JP25242015, JP25840140, JP15J00720. The open access publication of this manuscript was supported by the Museum für Naturkunde.

## References

- Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723. doi: 10.1109/TAC.1974.1100705
- Akatsuka K, Komai T (1922) *Pseudocrangonyx*, a new genus of subterranean amphipods from Japan. *Annotationes Zoologicae Japonenses* 10: 119–126.
- Barnard JL, Barnard KH (1983) *Freshwater Amphipoda of the World, I. Evolutionary Patterns and II. Handbook and Bibliography*. Hayfield Associates, Virginia, 830 pp.
- Bousfield EL (1963) New fresh-water amphipod crustaceans from Florida. *Natural History Papers, National Museum of Canada* 18: 1–9.
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552. doi: 10.1093/oxfordjournals.molbev.a026334
- Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* 46: 419–437. doi: 10.1071/ZO98048
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. doi: 10.2307/2408678
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome *c* oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources* 13: 851–861. doi: 10.1111/1755-0998.12138
- Holsinger JR (1989) Allocrangonyctidae and Pseudocrangonyctidae, two new families of Holarctic subterranean amphipod crustaceans (Gammaridea), with comments on their phylogenetic and zoogeographic relationships. *Proceedings of the Biological Society of Washington* 102: 947–959.
- Hou Z, Fu J, Li S (2007) A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution* 45: 596–611. doi: 10.1016/j.ympev.2007.06.006
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. doi: 10.1093/molbev/mst010
- Kristjánsson BK, Svavarsson J (2004) Crymostygidae, a new family of subterranean freshwater gammaridean amphipods (Crustacea) recorded from subarctic Europe. *Journal of Natural History* 38: 1881–1894. doi: 10.1080/00222930310001597295
- Labay VS (1999) Atlas and key to the Malacostraca of fresh- and brackish waters of the Island of Sakhalin. In: *Fisheries Research in Sakhalin-Kuril Region and Adjacent Waters. Volume 2. Sakhalin Book Publishing House, Yuzhno-Sakhalinsk*, 59–73.
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. doi: 10.1093/molbev/mss020
- Macdonald III KS, Yampolsky L, Duffy JE (2005) Molecular and morphological evolution of the amphipod radiation of Lake Baikal. *Molecular Phylogenetics and Evolution* 35: 323–343. doi: 10.1016/j.ympev.2005.01.013
- Matsuda M (1954) *Pseudocrangonyx yezonis* found collected from several places in Akita Prefecture. *Collecting and Breeding* 16: 12–13.
- Messing J (1983) New M13 vectors for cloning. In: Wu R, Grossman L, Moldave K (Eds) *Methods in Enzymology. Volume 101. Recombinant DNA, Part C*. Academic Press, New York, 20–78. doi: 10.1016/0076-6879(83)01005-8
- Nakano T (2012) A new sexannulate species of *Orobodella* (Hirudinida, Arhynchobdellida, Orobodellidae) from Yakushima Island, Japan. *ZooKeys* 181: 79–93. doi: 10.3897/zookeys.181.2932
- Narahara Y, Tomikawa K, Torigoe K (2009) *Pseudocrangonyx coreanus* (Amphipoda, Pseudocrangonyctidae) from a World Heritage Iwami-ginzan silver mine and its cultural landscape, Shimane Prefecture. *Bulletin of the Biogeographical Society of Japan* 64: 171–176.
- Numomura N (1975) *Pseudocrangonyx kyotonis* collected from Kumai-shi-do Cave (Gifu Prefecture). *Nature Study* 21: 11.
- Palumbi SR (1996) *Nucleic acids II: The polymerase chain reaction*. In: Hillis DM, Moritz C, Mable BK (Eds) *Molecular Systematics. Second Edition*. Sinauer Associates, Inc., Sunderland, 205–247.
- Rambaut A, Drummond AJ (2013) Tracer v. 1.6. <http://tree.bio.ed.ac.uk/software/tracer/>
- Raupach MJ, Barco A, Steinke D, Beermann J, Laakmann S, Mohrbeck I, Neumann H, Kihara TC, Pointner K, Radulovici A, Segelken-Voigt A, Wesse C, Knebelberger T (2015) The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. *PLoS ONE* 10: e0139421. doi: 10.1371/journal.pone.0139421
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. doi: 10.1093/sysbio/sys029
- Schwarz G (1978) Estimating the dimension of a model. *The Annals of Statistics* 6: 461–464. doi: 10.1214/aos/1176344136
- Schellenberg A (1936) Die Amphipodengattungen um *Crangonyx*, ihre Verbreitung und ihre Arten. *Mitteilungen aus dem Zoologischen Museum in Berlin* 22: 31–44. doi: 10.1002/mmnz.19370220103
- Sidorov DA (2006) A new species of the genus *Pseudocrangonyx* (Crustacea, Amphipoda, Pseudocrangonyctidae) from Primorye region (Russia). *Zoologicheskii Zhurnal* 85: 1486–1494.
- Sidorov DA (2009) New species of stygobiont amphipod (Crustacea: Amphipoda: Pseudocrangonyctidae) from Primorye, with descrip-

- tion of female of *Pseudocrangonyx levanidovi* Birstein from the Khor River springs. Amurian Zoological Journal 1: 92–105.
- Sidorov DA (2012) *Pseudocrangonyx kseniae*, a new species of Amphipoda (Crustacea, Pseudocrangonyctidae) from subterranean waters of Southern Primorye. Zoologicheskii Zhurnal 91: 30–37.
- Sidorov DA, Gontcharov AA (2013) Studies on subterranean amphipod crustaceans of Primorye, Russia. Part 1. Three new species of the genus *Pseudocrangonyx* from springs and other groundwater habitats in far eastern Russia. Zootaxa 3693: 547–567. doi: 10.11646/zootaxa.3693.4.8
- Sidorov D, Holsinger JR (2007) *Procrangonyx stygoedincus*, a new species of subterranean amphipod (Pseudocrangonyctidae) from the Far East of Russia, with remarks on biogeographic relationships. Crustaceana 80: 417–430. doi: 10.1163/156854007780440984
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. doi: 10.1093/bioinformatics/btu033
- Stock JH (1974) The systematics of certain Ponto-Caspian Gammaridae (Crustacea, Amphipoda). Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 70: 75–95.
- Stock JH, Jo YW (1990) Two Crangonyctidae (Crustacea, Amphipoda) from subterranean waters of the Far East. Stygologia 5: 119–127.
- Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197
- Tanabe AS (2008) Phylogears v. 2.0.2014.03.08. <http://www.fifthdimension.jp/>
- Tomikawa K, Shinoda S (2016) Redescription of a subterranean amphipod, *Eocrangonyx japonicus* (Crustacea, Amphipoda, Pseudocrangonyctidae) from Japan. Crustaceana 89: 583–594. doi: 10.1163/15685403-00003544
- Tomikawa K, Morino H, Ohtsuka S (2008) Redescription of a subterranean amphipod, *Pseudocrangonyx shikokunis* (Crustacea: Amphipoda: Pseudocrangonyctidae) from Japan. Species Diversity 13: 275–286.
- Tomikawa K, Kobayashi N, Kyono M, Ishimaru S-i, Grygier MJ (2014) Description of a new species of *Sternomoera* (Crustacea: Amphipoda: Pontogeneiidae) from Japan, with an analysis of the phylogenetic relationships among the Japanese species based on the 28S rRNA gene. Zoological Science 31: 475–490. doi: 10.2108/zs140026
- Tomikawa K, Tanaka H, Nakano T (2016) A new species of the rare genus *Priscomilitaris* from the Seto Inland Sea, Japan (Crustacea, Amphipoda, Priscomilitaridae). ZooKeys 607: 25–35. doi: 10.3897/zookeys.607.9379
- Tomikawa K, Tashiro S, Kobayashi N (2012) First record of *Gammarus koreanus* (Crustacea, Amphipoda, Gammaroidea) from Japan, based on morphology and 28S rRNA gene sequences. Species Diversity 17: 39–48. doi: 10.12782/sd.17.1.039
- Uéno M (1927) Notes on some subterranean isopods and amphipods of Japan. Memoirs of the College of Science, Kyoto Imperial University, Series B 3: 355–368.
- Uéno M (1930) A new subterranean amphipod from Japan. Annotationes Zoologicae Japonenses 13: 21–23.
- Uéno M (1933a) Crustacea recorded from the subterranean waters of Japan. Shokubutsu oyobi Doubutsu 1: 483–489.
- Uéno M (1933b) Three noticeable freshwater Crustacea of Hokkaido. Annotationes Zoologicae Japonenses 14: 115–122.
- Uéno M (1933c) Subterranean waters and animals of Akiyoshi-dai. Japanese Journal of Limnology 2: 91–95. doi: 10.3739/rikusui.2.91
- Uéno M (1934) Subterranean Crustacea from Kwantung. Annotationes Zoologicae Japonenses 14: 445–450.
- Uéno M (1966) Results of the speleological survey in South Korea 1966 II. Gammarid Amphipoda found in subterranean waters of South Korea. Bulletin of the National Science Museum, Tokyo 9: 501–535.
- Uéno M (1971a) The fauna of the insular lava caves in West Japan. VIII. Subterranean Amphipoda. Bulletin of the National Science Museum, Tokyo 14: 161–170.
- Uéno M (1971b) Subterranean Amphipoda from the Islands of Tsushima. Bulletin of the National Science Museum, Tokyo 14: 161–170.
- Uéno M (1971c) The fauna of the lava caves around Mt. Fuji-san. II. Amphipoda and Cladocera (Crustacea). Bulletin of the National Science Museum, Tokyo 14: 219–220.

# A new species of *Moenkhausia* (Characiformes, Characidae) from the Içá River, Amazon Basin, northern Brazil

Valter M. Azevedo-Santos<sup>1</sup>, Ricardo C. Benine<sup>1</sup>

<sup>1</sup> Laboratório de Ictiologia, Departamento de Zoologia, IBB-UNESP Campus de Botucatu, Botucatu, SP, 18618-970, Brazil

<http://zoobank.org/7578441A-4AF2-4318-8C38-23B1D45E9861>

Corresponding author: Valter M. Azevedo-Santos (valter.ecologia@gmail.com)

## Abstract

Received 30 July 2016  
Accepted 5 October 2016  
Published 18 October 2016

Academic editor:  
Peter Bartsch

## Key Words

Humeral spot  
Putumayo River  
Scale *radii*  
Tooth cusps  
*Moenkhausia tergimacula*

A new species of the genus *Moenkhausia* is described from the Içá River, Amazon Basin. *Moenkhausia britskii* sp. n. is most similar to *M. grandisquamis* and *M. xinguensis*. From these two species it is readily distinguished by the pigmentation pattern of the humeral spot. *Moenkhausia britskii* further differs from *M. xinguensis* by the pattern of *radii* on the scales, which curve upward and downward (*vs.* scales with straight *radii*). Discussions on the putative relationship of the new species with *M. grandisquamis* and *M. xinguensis*, and on the peculiar upward and downward arched scale *radii* are provided.

## Introduction

Eigenmann (1903) proposed the genus *Moenkhausia* to accommodate *Tetragonopterus xinguensis* Steindachner, 1882. In his description Eigenmann (1903: 145) defined the genus simply as “similar to *Markiana*” with “anal naked” and “caudal scaled”. Subsequently, some species originally described in other genera were transferred into *Moenkhausia* (Eigenmann 1910, Eigenmann 1917), and since then, many new species have been described (e.g., Travassos 1964, Fink 1979, Lucena and Lucena 1999, Benine 2002, Benine et al. 2007, Benine et al. 2009, Marinho 2010, Petrolli et al. 2016). At this time, more than 80 valid species (Eschmeyer et al. 2016) are recognized. The genus is widespread in the Neotropical river systems, with its greatest diversity occurring in the Amazon Basin (Eigenmann 1917, Lima et al. 2003).

During a taxonomic study of *M. grandisquamis* (Müller & Troschel, 1845) and *M. xinguensis* (Steindachner, 1882), we verified the existence of an undescribed species, very similar morphologically to the

formers, which is formally described herein. Given such similarity, the lack of phylogenetic definition for the genus, and the fact that the new species bears all the still used traditional generic diagnostic features (see Eigenmann 1903, Eigenmann 1917), we conservatively allocate our new species in *Moenkhausia*.

## Material and methods

Measurements were taken following Fink and Weitzman (1974) and using a digital caliper (0.1 mm of precision). Counts were also performed according to Fink and Weitzman (1974) and taken at the left side of the specimens, whenever possible. Counts are followed by their frequency in parentheses; asterisks indicate values for the holotype. Detailed analysis of teeth, fin rays, and vertebrae were made from cleared and stained (c&s) specimens prepared according to Taylor and Van Dyke (1985), which was dissected following Weitzman (1974). Vertebral counts were taken from x-ray images and

cleared and counterstained (c&s) specimen. Weberian apparatus was counted as four vertebrae; and the fused preural and ural centrum PU1+U1, located in the caudal region, as one element.

Institutional acronyms cited in this work are explicated in Sabaj-Pérez (2016).

## Results

### *Moenkhausia britskii* sp. n.

<http://zoobank.org/ED47F6E9-E4E8-4753-B342-CB879F2A30BC>

Fig. 1

**Holotype.** MZUSP 120691, 52.9 mm SL, Brazil, Amazonas State, Santo Antônio do Içá Municipality, Rio Içá, approximately 60 km above its mouth, 3°0'7.68" S, 69°2'49.52" W, collected by H. A. Britski, N. A. Menezes, J. C. Garavello, T. R. Roberts, S. Gerken, 18 Oct 1968.

**Paratypes.** MZUSP 17537, 16 (1 c&s), 41.3–58.3 mm SL, same data as the holotype.

**Diagnosis.** *Moenkhausia britskii* sp. n. differs from all congeners, except *M. grandisquamis* (Müller & Troschel, 1845), *M. tergimacula* Lucena & Lucena, 1999 and *M. xinguensis* (Steindachner, 1882), by the presence of a single humeral spot, presence of a silvery lateral stripe, absence of a dark line at the base of the anal-fin rays, absence of a caudal lobule mark, dorsal fin hyaline, five series of scales above lateral line and four series of scales below lateral line. *Moenkhausia britskii* differs from *M. tergimacula* by the absence of a dark spot located anteriorly to the dorsal-fin origin; and from *M. xinguensis* by the scales showing upward and downward bent *radii* (vs. scales with straight *radii*) (Fig. 2). Finally, it differs from *M. grandisquamis* by having a larger humeral spot, located over the third to fifth or fourth to sixth lateral-line scales and extending vertically over three or four horizontal scale rows above the lateral line (vs. smaller humeral spot, located over second to third or third to fourth scale of the series immediately above the lateral line) (Fig. 3). The new species also differs by having four or five cusps on the second tooth of the inner row of the premaxilla (Fig. 4) (vs. eight, seven, occasionally six cusps on the



**Figure 1.** *Moenkhausia britskii*, MZUSP 120691, holotype, 52.9 mm SL, from Içá River, Amazon basin.

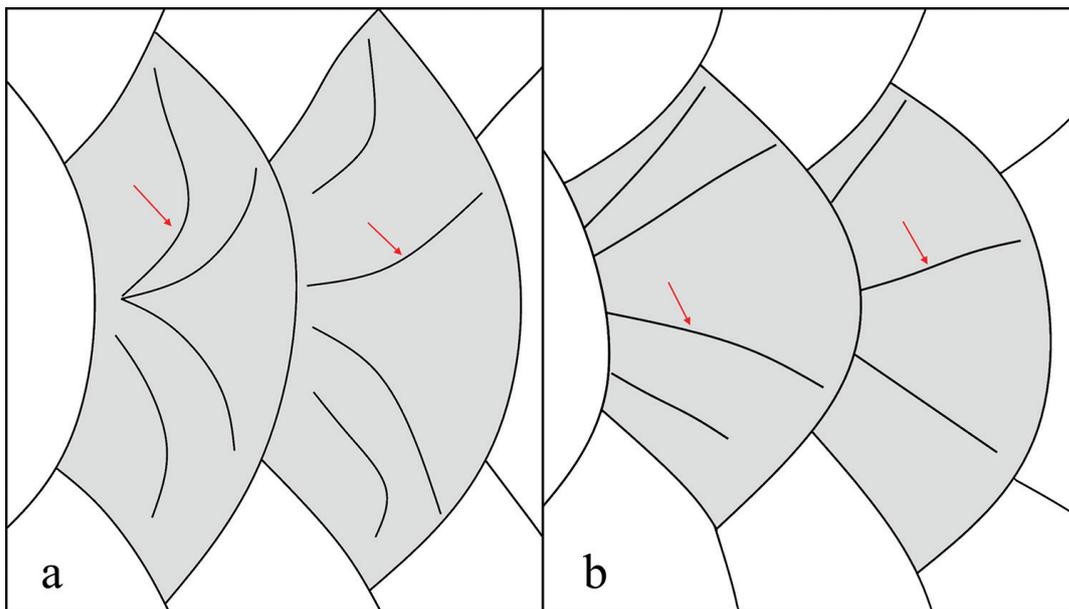
second tooth of the inner row of the premaxilla in *M. grandisquamis*).

**Description.** Morphometric data are given in Table 1. Greatest body depth at dorsal-fin origin. Dorsal profile of body slightly convex from tip of supraoccipital spine to dorsal-fin origin. Dorsal-fin base posteroventrally inclined. Profile straight or slightly convex from end of dorsal fin to adipose-fin origin; adipose-fin base posteroventrally inclined; caudal peduncle concave both dorsally and ventrally; ventral profile of body convex from tip of lower jaw to anal-fin origin; anal-fin base posterodorsally inclined. Prepelvic region transversally flattened, mainly at pelvic-fin insertion.

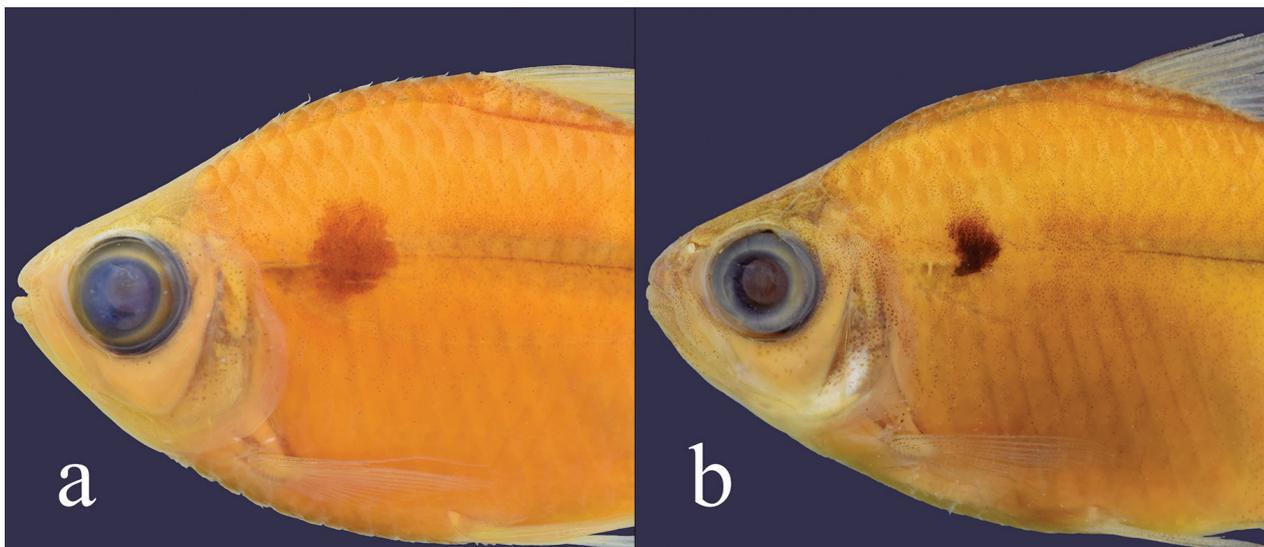
Mouth terminal. Posterior tip of maxilla extending beyond vertical through anterior margin of orbit. Premaxillary teeth arranged in two rows; outer row with 4(1), 5\*(15) or 6 (1) tricuspidate teeth with central cusp longer; inner row with 5\* (17) tetra- to pentacuspitate teeth (Fig. 5). Maxilla with 1\* (14) or 2(3) tricuspid teeth. Dentary with 4\* (17) tetra- to pentacuspitate teeth; central cusp usually longer, followed by five to ten small teeth decreasing in size posteriorly, with one to three cusps.

Dorsal-fin rays ii, 9. Pectoral-fin rays i, 10 (1), i, 11 (10), or i, 12\* (6). Pelvic-fin rays i, 7. Anal-fin rays iii, 23 (4), iii, 24 (4), iii, 25\* (8), or iii, 26 (1). Adipose fin present.

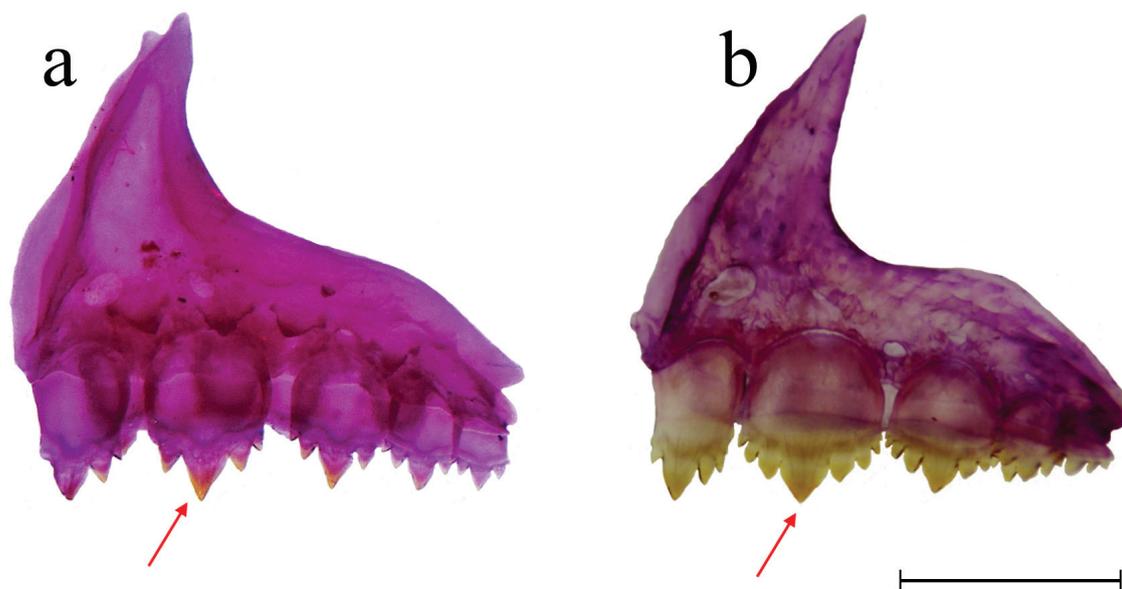
Scales cycloid with striae curved upward and downward. Lateral line slightly curved, with 30 (3), 31 (6), 32\*



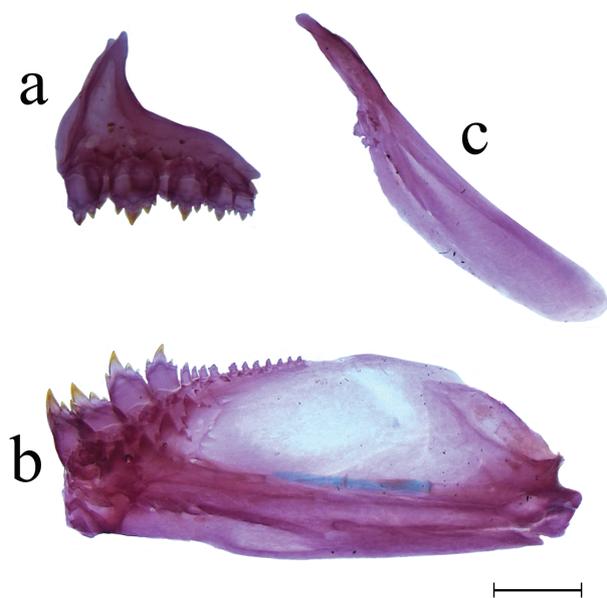
**Figure 2.** Illustration of two scales (gray color) with arrows indicating the radii of the scales: a) *Moenkhausia britskii*, holotype, MZUSP 120691, 52.9 mm SL; b) *Moenkhausia xinguensis*, INPA 39991, 42.3 mm SL.



**Figure 3.** Humeral spot: a) *Moenkhausia britskii*, 50.5 mm SL, paratype, MZUSP 17537; b) *Moenkhausia grandisquamis*, 48.2 mm SL, MZUSP 38244.



**Figure 4.** Arrows (red color) indicating the second tooth of the inner row of the premaxilla: **a)** *Moenkhausia britskii*, paratype, MZUSP 17537; **b)** *Moenkhausia grandisquamis*, MZUSP 38244. Medial view. Scale bar = 1 mm.



**Figure 5.** *Moenkhausia britskii*, MZUSP 17537, paratype: **(a)** premaxilla; **(b)** dentary; **(c)** maxilla. Medial view. Scale bar = 1 mm.

(6) or 33(2) pored scales. Five transversal scales above lateral line and four transversal scales below lateral line. A single series of scales covering base of second to eleventh branched anal-fin rays. Small scales covering the proximal two-third of caudal-fin lobes.

Total number of vertebrae 32 (2). Gill rakers on lower limb 8 (1); 11\* (15); 12 (1); gill rakers on upper limb 7(1), 8(9) or 9\*(7). Supraneurals 4 (2).

**Color in alcohol.** Overall coloration yellow tan. Concentration of few dark chromatophores on upper lip.

Infraorbital and opercular series light silvery due to the scarce remaining guanine pigmentation. Dark chromatophores more densely concentrated along dorsal midline. Sparsely distributed dark chromatophores dorsal of horizontal septum. A dark line marks the horizontal septum. A silvery-gray midlateral stripe extending from posterior margin of humeral spot to caudal peduncle. Irregularly shaped, humeral spot located over third to fifth or fourth to sixth lateral-line scales and extending vertically over three or four horizontal scale rows above lateral line. Paired fins and anal fin hyaline. Adipose with very few dark chromatophores.

**Distribution.** Known from the Igarapé da Cachoeira, Içá River, Amazon basin, Santo Antônio do Içá, Amazonas, Brazil (Fig. 6).

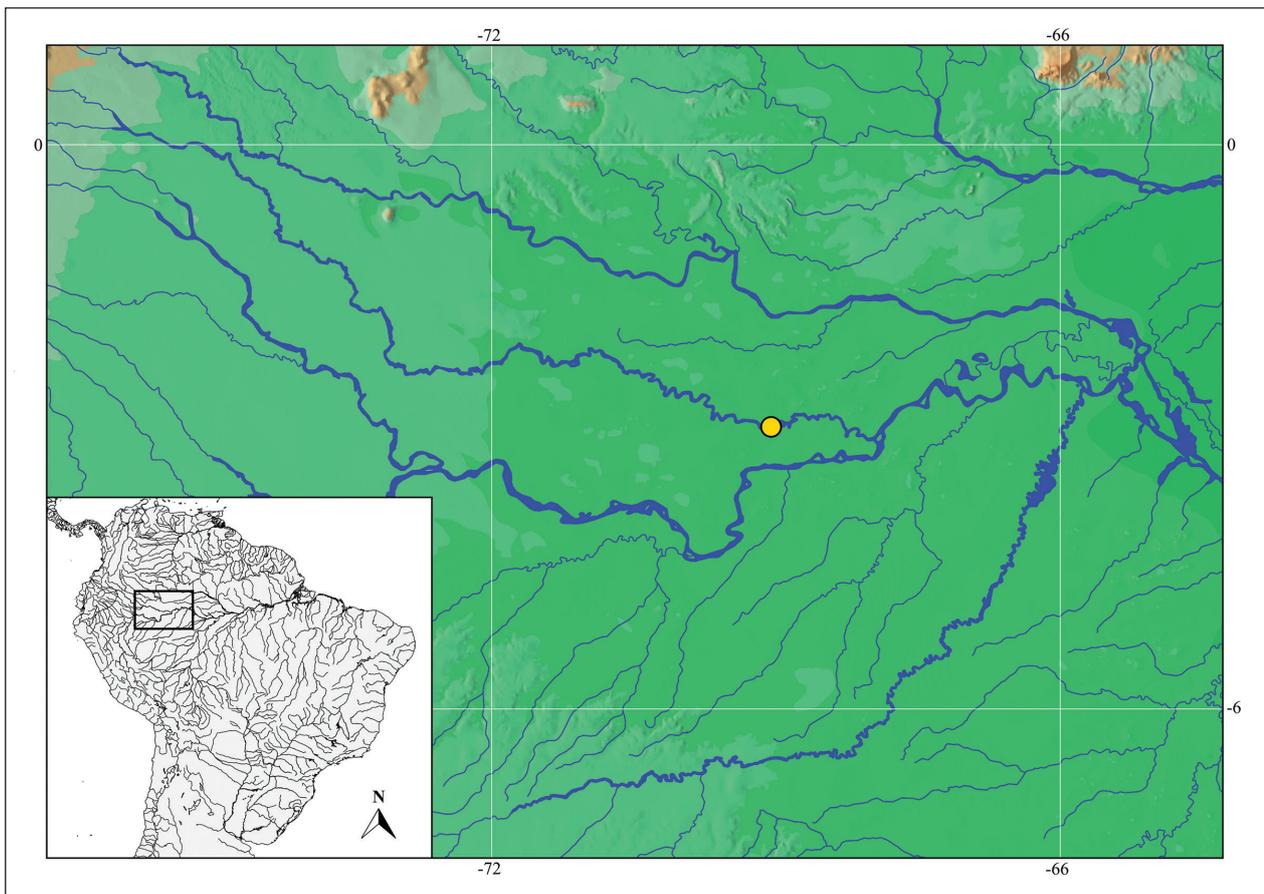
**Etymology.** The specific epithet *britskii* is in honor of Dr. Heraldo Antônio Britski, collector of this new species and for his enormous and valuable contributions to our knowledge of the Neotropical Ichthyology.

## Discussion

Aside from an overall similarity in proportions and morphometric and meristic data, *Moenkhausia britskii* sp. n. and *M. grandisquamis* share with *M. xinguensis* the unique combination of a single humeral spot, presence of a silvery lateral stripe, absence of a dark line at the base of the anal-fin rays, absence of a caudal lobule mark, dorsal fin hyaline, and five series of scales above and four series of scales below lateral line. *Moenkhausia tergimaculata* also fits in this combination except for the humeral spot, which is represented by a field of sparse dark chromato-

**Table 1.** Morphometric data of holotype and paratypes of *Moenkhausia britskii* from Içá River, Amazon basin (n = 17). Range includes the holotype.

| Measurements                           | Holotype | Range     | Mean | SD  |
|--|----------|-----------|------|-----|
| Standard length (mm)                   | 52.9     | 41.3–58.3 | 50.2 | -   |
| <b>Percents of standard length</b>     |          |           |      |     |
| Greatest depth                         | 47.6     | 41.2–47.7 | 45.1 | 1.6 |
| Snout to dorsal-fin origin             | 52.8     | 49.6–53.9 | 52.1 | 1.2 |
| Snout to pectoral-fin origin           | 29.2     | 28.7–31.7 | 30.5 | 0.9 |
| Snout to pelvic-fin origin             | 53.4     | 50.5–55.1 | 52.5 | 1.3 |
| Snout to anal-fin origin               | 70.8     | 67.7–71.7 | 70.3 | 1.1 |
| Caudal peduncle depth                  | 11.5     | 10.5–12.3 | 11.4 | 0.5 |
| Caudal peduncle length                 | 8.9      | 8.5–11.2  | 9.3  | 0.7 |
| Pectoral-fin length                    | 23.2     | 21.5–25.6 | 23.5 | 0.9 |
| Pelvic-fin length                      | 20.3     | 17.9–21.9 | 19.8 | 1.2 |
| Dorsal-fin length                      | 33.7     | 31.2–34.4 | 32.8 | 1.1 |
| Dorsal-fin base                        | 16.8     | 14.6–18.7 | 16.0 | 0.9 |
| Anal-fin length                        | 18.7     | 18.6–22.7 | 20.3 | 1.4 |
| Anal-fin base                          | 30.9     | 28.8–31.4 | 30.1 | 0.7 |
| Eye to dorsal-fin origin               | 36.4     | 34.3–38.8 | 36.7 | 1.1 |
| Dorsal-fin origin to caudal-fin origin | 58.4     | 55.3–58.5 | 56.9 | 1.0 |
| Head length                            | 28.1     | 26.3–30.3 | 28.3 | 1.1 |
| Head depth                             | 31.9     | 29.5–33.1 | 31.5 | 1.2 |
| <b>Percents of head length</b>         |          |           |      |     |
| Snout length                           | 25.8     | 23.2–28.3 | 25.8 | 1.5 |
| Upper jaw length                       | 43.7     | 40.1–47.9 | 44.3 | 2.2 |
| Horizontal orbital diameter            | 44.9     | 41.4–50.6 | 45.3 | 2.8 |
| Least interorbital width               | 53.7     | 46.7–57.9 | 52.1 | 3.0 |



**Figure 6.** Partial map of South America with yellow circle indicating the distribution of *Moenkhausia britskii*.

phores at the humeral area, and an unusual conspicuous dark blotch located just ahead of the origin of the dorsal fin (see Lucena and Lucena 1999 for more details). Thus, these probably closely related species should be necessarily considered in a phylogenetic analysis of the genus, since the group encompasses its type species, *M. xinguensis*.

The peculiar upward and downward arched scale *radii* observed in *Moenkhausia britskii* sp. n., is also present in *M. grandisquamis*. Indeed, this feature was first described by Eigenmann (1917: 67) as “some striae of the scales diverging from the middle line of each scale in nearly opposite directions, up and down” to directly differentiate *M. grandisquamis* from *M. ovalis* (Günther, 1868). Moreover, Eigenmann (1917: 85) emphatically argued that this feature actually would distinguish *M. grandisquamis* “from all others of the genus”, evidencing the uniqueness of such character. Thus, considering the coloration pattern, and overall morphometric and meristic similarities between *M. grandisquamis* and *M. britskii*, it is plausible to consider such a condition synapomorphic and indicative of close relationship between these two species. Nonetheless, our comparative study also demonstrates that *Stichonodon insignis* (Steindachner, 1876) has scales with upward and downward curved *radii*. However, this is a highly modified deep-bodied species currently allocated at the Stethaprioninae (Nelson et al. 2016). Accordingly, occurrence of this trait in distantly related groups must be considered convergent/homoplastic for the time being. Distribution and significance of this rare character for systematics of Characidae shall be evaluated by more detailed comparison and a phylogenetic analysis encompassing more taxa.

**Comparative material.** *Brachychalcinus* sp.: LBP 10653, 1, 58.8 mm SL, **Brazil**. *Moenkhausia agnase*: LBP 14869, 3, 62.1–65.5 mm SL, **Peru**. *Moenkhausia aurantia*: LBP 18999, 4, 20.7–40.9 mm SL, **Brazil**. *Moenkhausia australe*: LBP 4655, 10 of 65, 29.5–36.7 mm SL, **Brazil**. *Moenkhausia bonita*: LBP 7592, 12, 31.1–34.5 mm SL, **Brazil**. *Moenkhausia celibela*: LBP 14250, 7 of 23, 20.6–34.2 mm SL, **Brazil**. *Moenkhausia ceros*: LBP 4504, 12 of 25, 20.7–30.5 mm SL, **Brazil**. *Moenkhausia chrysargyrea*: USNM 226155, 37, 42.4–53.3 mm SL, **Suriname**. *Moenkhausia comma*: LBP 16714, 12, 46.28–63.22 mm SL, **Brazil**. *Moenkhausia copei*: LBP 2300, 1, 36.7 mm SL, **Venezuela**. *Moenkhausia cosmops*: LBP 8164, 6, 43.6–65.2 mm SL, **Brazil**. *Moenkhausia costae*: LBP 10298, 3, 33.7–48.9 mm SL, **Brazil**; LBP 10298, 3, 33.7–48.9 mm SL, **Brazil**. *Moenkhausia cotinho*: MZUSP 29827, 12 of 45, 33.91–49.88 mm SL, **Brazil**. *Moenkhausia dasalmas*: LBP 17135, 8 of 38, 24.5–41.3 mm SL, **Brazil**. *Moenkhausia dichroura*: LBP 3760, 10, 70.23–44.52 mm SL, **Brazil**. *Moenkhausia* aff. *grandisquamis*: MZUSP 099992, 50, 11.3–55.1 mm SL, **Brazil**. *Moenkhausia grandisquamis*: MZUSP 101448, 9, 52.4–96.6 mm SL, **Brazil**; ANSP 175609, 15, 49.5–66.7 mm SL, **Guyana**; MZUSP 38244, 30, topotypes, 25.0–44.1 mm SL, **Suriname**. *Moenkhausia hemigrammoides*: LBP 13243, 3, 31.2–34.6 mm SL,

**Brazil**. *Moenkhausia intermedia*: LBP 17196, 4, 52.74–68.54 mm SL, **Brazil**. *Moenkhausia jamesi*: MZUSP 17583, 2, 54.40–50.35 mm SL, **Brazil**. *Moenkhausia justae*: MZUSP 55752, 12, 51.19–42.03 mm SL, **Brazil**. *Moenkhausia lata*: LBP 19969, 6 of 11, 41.0–61.7 mm SL, **Brazil**. *Moenkhausia levidorsa*: INPA 11611, 5, 40.06–60.46 mm SL, **Brazil**. *Moenkhausia lopesi*: LBP 16063, 8, 40.98–43.52 mm SL, **Brazil**. *Moenkhausia megalops*: INPA 48026, 2, 51.3–65.9 mm SL, **Brazil**. *Moenkhausia oligolepis*: LBP 10807, 5, 31.64–46.4 mm SL, **Brazil**. *Moenkhausia phaenota*: LBP 8029, 9, 45.8–32.7 mm SL, **Brazil**. *Moenkhausia pirauba*: **LBP16148**, 11, 85.2–69.4 mm SL, **Brazil**. *Moenkhausia sanctaefilomenae*: LBP 1474, 7, 25.72–55.18 mm SL, **Brazil**. *Moenkhausia sthenosthoma*: MZUSP 117143, 1, paratype, **Brazil**. *Moenkhausia surinamensis*: LBP 21009, 4, 43.4–63.8 mm SL, **Brazil**. *Moenkhausia tergimacula*: MCP 202287, paratype, **Brazil**. *Moenkhausia veneirei*: MZUSP 119006, holotype, **Brazil**. *Moenkhausia xinguensis*: LBP 16745, 23, 53.6–33.9 mm SL, **Brazil**; INPA 39991, 20, 50.4–34.5 mm SL, **Brazil**; INPA 47044, 38, 51.6–32.8 mm SL, **Brazil**; INPA 40483, 2, 41.8–60.3 mm SL, **Brazil**; INPA 47140, 20.7–42.2 mm SL, **Brazil**. *Poptella* sp. 1: LBP 7792, 10, 31.0–35.3 mm SL, **Brazil**. *Poptella* sp. 2: LBP 4012, 3, 48.5–51.5 mm SL, **Brazil**. *Poptella* sp. 3: MZUSP 117635, 6 of 53, 27.6–36.5 mm SL, **Brazil**. *Stichonodon insignis*: INPA 042300, 1, 68.3 mm SL, **Brazil**. *Tetragonopterus argenteus*: LBP 185, 02, 70.4–71.6 mm SL, **Brazil**. *Tetragonopterus chalceus*: LBP 16663, 5, 40.5–64.8 mm SL, **Brazil**.

## Acknowledgments

We are grateful to Aléssio Datovo and Michel D. Gianeti (MZUSP), Lúcia Py-Daniel and Renildo Ribeiro (INPA), and Claudio de Oliveira (LBP) for curatorial assistance and the loan of material in their care. Radiographs were taken at the facilities of the Laboratório de Ictiologia de Ribeirão Preto (LIRP-USP) under the technical assistance of Hertz F. dos Santos and André L. H. Esguicero. We are also grateful to Fernando R. Carvalho, Maxwell J. Bernt, Donald C. Taphorn, and Peter Bartsch, for suggestions. Marina G. Petrolli assisted with Figure 5 and Bruno F. Melo with Figure 1 and Figure 3. The first author was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

## References

- Benine RC (2002) *Moenkhausia levidorsa*, a new species from Rio Aripuanã, Amazon Basin, Brazil (Characiformes: Characidae). *Ichthyological Exploration of Freshwaters* 13(4): 289–294.
- Benine RC, Castro RMC, Santos ACA (2007) A new *Moenkhausia* Eigenmann, 1903 (Ostariophysi: Characiformes) from Chapada Diamantina, rio Paraguaçu Basin, Bahia, Northeastern Brazil.

- Neotropical Ichthyology 5(3): 259–262. doi: 10.1590/s1679-62252007000300004
- Benine RC, Mariguela TC, Oliveira C (2009) New species of *Moenkhausia* Eigenmann, 1903 (Characiformes: Characidae) with comments on the *Moenkhausia oligolepis* species complex. *Neotropical Ichthyology* 7: 161–168. doi: 10.1590/S1679-62252009000200005
- Eigenmann CH (1903) New genera of South America fresh-water fishes, and new names for some old genera. *Smithsonian Miscellaneous Collection* 45: 144–148.
- Eigenmann CH (1910) Catalogue of the freshwater fishes of the tropical and south temperate America. *Reports of the Princeton University Expeditions to Patagonia* 3(2): 375–511.
- Eigenmann CH (1917) The American Characidae. Part I. *Memoirs of the Museum of Comparative Zoology* 43: 1–102.
- Eschmeyer WN, Fricke R, van der Laan R (2016) *Catalog of Fishes: Genera, Species, References*. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> [accessed 21 April 2016]
- Fink WL, Weitzman SH (1974) The so-called cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). *Smithsonian Contributions to Zoology* 172: 1–46. doi: 10.5479/si.00810282.172
- Fink WL (1979) A new species of *Moenkhausia* from the Mato Grosso region of Brazil (Pisces: Characidae). *Breviora* 450: 1–12.
- Günther A (1868) Diagnoses of some new freshwater fishes from Surinam and Brazil, in the collection of the British Museum. *Annals Magazine Natural History* 1(6): 475–481. doi: 10.1080/00222936808695733
- Lima FCT, Malabarba LR, Buckup PA, Silva JFP, Vari RP, Harold A, Benine R, Oyakawa OT, Pavanelli CS, Menezes NA, Lucena CAS, Malabarba MCSL, Lucena ZMS, Reis RE, Langeani F, Cassati L, Bertaco VA, Moreira C, Lucinda PHF (2003) Genera incertae sedis in Characidae. In: Reis RE, Kullander SO, Ferraris Jr CJ (Eds) *Check List of the Freshwater Fishes of South and Central America*. EDIPUCRS, Porto Alegre, 106–169.
- Lucena ZMS, Lucena CAS (1999) *Moenkhausia tergimacula*, a new species from the upper rio Tocantins, Brazil (Osteichthyes: Characidae). *Ichthyological Exploration of Freshwaters* 10(3): 231–236.
- Marinho MMF (2010) A new species of *Moenkhausia* Eigenmann (Characiformes: Characidae) from the rio Xingu basin, Brazil. *Neotropical Ichthyology* 8(3): 655–659. doi: 10.1590/S1679-62252010000300011
- Müller J, Troschel FH (1845) *Horae ichthyologicae, Beschreibung und Abbildung neuer Fische – Die Familie der Characinen*. Berlin, 40 pp. doi: 10.5962/bhl.title.6935
- Nelson JS, Grande TC, Wilson MVH (2016) *Fishes of the World* (5<sup>th</sup> edn). John Wiley & Sons, 752 pp. doi: 10.1002/9781119174844
- Petrolli MG, Azevedo-Santos VM, Benine RC (2016) *Moenkhausia veneri* (Characiformes: Characidae), a new species from the rio Araguaia, Central Brazil. *Zootaxa* 4105(2): 159–170. doi: 10.11646/zootaxa.4105.2.4
- Sabaj-Pérez MH (Ed.) (2016) *Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference*. Version 4.0. American Society of Ichthyologists and Herpetologists, Washington, D.C. <http://www.asih.org/resources/> [16 August 2016]
- Steindachner F (1876) *Ichthyologische Beiträge* (V). [Subtitles i-v.]. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe* 74(1. Abth.): 49–240.
- Steindachner F (1882) *Beiträge zur Kenntniss der Flussfische Südamerikas* (IV). *Anzeiger der Akademie der Wissenschaften in Wien* 19: 175–180.
- Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage. *Cybium* 9: 107–119.
- Travassos H (1964) Sobre alguns peixes do Estado do Pará, Brasil. *Anais da Academia Brasileira de Ciências* 36: 539–548.
- Weitzman SH (1974) Osteology and evolutionary relationships of the Sternopychidae, with a new classification of stomiatooid families. *Bulletin of the American Museum of Natural History* 153(3): 327–478.



# The collection of Nematomorpha in the Zoological Museum Hamburg, including description of a new species, *Chordodes jelkae* sp. n.

Andreas Schmidt-Rhaesa<sup>1</sup>

<sup>1</sup> Centrum für Naturkunde, University of Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

<http://zoobank.org/FAB1AD5B-087A-45AB-AFA3-8CF3513411F8>

Corresponding author: *Andreas Schmidt-Rhaesa* ([andreas.schmidt-rhaesa@uni-hamburg.de](mailto:andreas.schmidt-rhaesa@uni-hamburg.de))

## Abstract

Received 31 August 2016  
Accepted 31 October 2016  
Published 29 November 2016

Academic editor:  
*Michael Ohl*

## Key Words

Nematomorpha  
*Chordodes jelkae* sp. n.  
horsehair worms  
collection  
revision  
SEM

The collection of horsehair worms in the Zoological Museum of the Centrum für Naturkunde (CeNak) of the University of Hamburg has been revised. All specimens have been investigated by Scanning Electron Microscopy (SEM). A total of 173 specimens from 135 catalogue entries is present in the collection, these represent 39 species from 10 genera (*Gordius*, *Chordodes*, *Paragordius*, *Parachordodes*, *Gordionus*, *Acutogordius*, *Beatogordius*, *Paragordionus*, *Pseudochordodes*, *Nectonema*). Previous revisions of the Nematomorpha material have been done in 1893 by Römer and in 1935 by Heinze. A number of species could not be determined to species level, either because characters were not preserved well enough or because observed characters did not fit available species descriptions. This does account in particular for the genus *Gordius*, where diagnostic characters are few and great uncertainties exist concerning the status of certain species. Therefore, within this genus, many records were only determined as *Gordius* sp., including some specimens that had previously been determined to species level. One new species is described from the material of the collection. This species is named *Chordodes jelkae* sp. n. and it is characterized by the presence of an apical tuft of bristles on simple areoles in combination with the absence of thorn areoles. The holotype of *Chordodes pilosus* Möbius, 1855 was reinvestigated, and its species identity cannot be determined unambiguously. Therefore the name *Chordodes pilosus* is considered a nomen dubium.

## Introduction

The Zoological Museum Hamburg has a collection of 173 specimens from 135 catalogue entries of horsehair worms (Nematomorpha) representing 39 species from 10 genera. A total of about 455 species has been described from all over the world. Important for collection and determination are adult worms in their free-living phase (see Hanelt et al. 2005 and Schmidt-Rhaesa 2013 for recent summaries on the life cycle and other topics), because these are encountered most often and because the cuticle has structures that are relevant for determination. The parasitic juveniles are almost impossible to determine, because they show a different cuticle that does not contain differential structures (see, e.g., Schmidt-Rhaesa 2005).

The only published review of the collection of the Zoological Museum Hamburg is from Römer (1895). He investigated material that was included in the registry catalogue as numbers V2206 to V2234 (without giving these accession numbers in the publication). These are 29 catalogue numbers from 11 species of *Gordius* and *Chordodes*, the only genera available by that time. According to recent systematics, Römer's 10 species belong to four genera: *Gordius*, *Gordionus*, *Parachordodes* and *Chordodes*. The material of three numbers (V2206, 2210, 2219) was lost (noticed by a revision in 1954). Six numbers (V2209–V2212, 2232, V2234) turned out to be mermithid nematodes, among these are two species that were newly described (as nematomorphs) by Römer (1895): *Chordodes liguligerus* Römer, 1895 (see Heinze 1935a) and *C. hamatus*. The latter species was later renamed *Gordiomermis hamatus* (Heinze 1934).

In 1935, Kurt Heinze from Berlin worked on the Hamburg collection and determined a number of specimens, a few of these results were published in Heinze (1935a). The collecting localities of several Hamburg specimens occurred in Heinze's summaries of German Nematomorpha from 1937 and 1941.

Since then, nobody has reviewed the material or deposited new type material until from 2008 on the author deposited several types in the collection.

It is the aim of this summary on the one hand to present an overview of the Nematomorpha material in the Hamburg collection and on the other hand to determine all material according to current standards. This includes investigation by Scanning Electron Microscopy (SEM). The SEM reinvestigation led to several new determinations, in particular a number of determinations at the species level could not be confirmed and hence are treated here as "sp."

## Material and methods

Specimens are conserved in ethanol with a desired concentration of 70%. For the reinvestigation, entire pieces from the midbody region and in some cases the posterior end (about 1–2 mm from the terminal tip) were prepared for Scanning Electron Microscopy (SEM). Pieces were dehydrated in an increasing ethanol series, critically point dried and coated with gold in a sputter coater. Observation took place using a LEO SEM 1524 at 10 kV. Digital images were taken.

## Results and discussion

In the Zoological Museum Hamburg, entries are recorded in a catalogue book, printed as a record card and recorded digitally in a Filemaker file. Entries in the catalogue are hand-written and could not always be read reliably. It was attempted to verify the collection localities, but this was not possible in all cases. Writing of names is given here as good as possible. Citations from the catalogue are given in quotation marks. The main focus is on the older records, which are reinvestigated. Younger records, which are well documented in the literature, are presented only briefly, with reference to the respective publications.

### Genus *Acutogordius* Heinze, 1952

Males in species of *Acutogordius* have a postcloacal crescent, like in *Gordius*, but the tail lobes are pointed and the entire posterior end tapers towards the pointed tip of the tail lobes (Fig. 7D, G, J). Nine species have been described. The distribution of fine bristles in the male anterior end appears to be important for species delimitation, but such fine bristles were not always reported in older descriptions. See Schmidt-Rhaesa and Geraci (2006) for a summary.

### *Acutogordius* cf. *acuminatus* De Miralles & De Villalobos, 1998

**Material examined.** V 2213 (1 male). Fig. 7C–E.

**Location.** Brazil, Joinville, Santa Catarina.

**Remarks from the catalogue.** Collected by "Killmann" (no date given). Received by the museum on June 19, 1890. Determined by Römer in 1893 as *Gordius aquaticus* and by Heinze in 1935 as *Gordius* sp.

**Occurs in publications.** Römer (1895, page 791), briefly in Römer (1896, page 260).

**Remarks.** Römer (1895) describes this specimen as corresponding to *Gordius aquaticus* in almost all respects, except for the absence of lightly coloured spots and the pointed shape of the posterior end. This last character was regarded diagnostic for a new genus *Acutogordius*, introduced by Heinze (1952). As no bristles were observed on the cuticle (Fig. 7C), the specimen is closest to *Acutogordius acuminatus* De Miralles & De Villalobos, 1998, which was described from Brazil, too (De Miralles and De Villalobos 1998, see also Schmidt-Rhaesa and Geraci 2006).

**Material examined.** V7351(b) (1 male). Fig. 7F, G.

**Location.** Brazil, presumably Itapoá, Santa Catarina (see remarks from the catalogue).

**Remarks from the catalogue.** Collected by W. Ehrhardt on January 17, 1910. Received by the museum on November 12, 1910. As location, the catalogue says "Humboldt-Geb. am Itapoéú", some letters are not explicit. This location could not be retrieved, but Itapoá is close. Additionally, there are other catalogue entries with the same date and same collector having as location Joinville, which is close to Itapoá in the province Santa Catarina. Original determination as "Gordiide".

**Occurs in publications.** none

**Remarks.** The catalogue notes "dv", meaning "diverse" as number of specimens. 13 specimens were found, 12 of them are determined as *Chordodes brasiliensis* (see there) and specimen V7351(b) belongs to the genus *Acutogordius*. No bristles were observed on the body cuticle (Fig. 7F) or the posterior end (Fig. 7G), therefore the specimen is closest to the description of *A. acuminatus* De Miralles & De Villalobos, 1998, which was described from Brazil, too (De Miralles and De Villalobos 1998, see also Schmidt-Rhaesa and Geraci 2006).

**Material examined.** V11465 (1 male). Fig. 7H–J.

**Location.** Brazil, Boiteuxburgo, Santa Catarina.

**Remarks from the catalogue.** Collected by Paul Missfeldt (date unknown). Received by the museum on December 5, 1930. Originally determined as "*Gordius*".

**Occurs in publications.** unknown

**Remarks.** The specimen has clearly pointed tail lobes, which carry two longitudinal crests or keels (Fig. 7J). These may be artificial structures, but as they occur on both tail lobes, some doubts remain. There are some scattered bristle-like structures on the cuticle, but it could

not be determined with certainty if these are really cuticular structures (Fig. 7I). Otherwise the cuticle is smooth (Fig. 7H). As bristles are absent on the posterior end, the specimen is closest to the description of *A. acuminatus* De Miralles & De Villalobos, 1998 (see above and De Miralles and De Villalobos 1998, Schmidt-Rhaesa and Geraci 2006).

### Genus *Beatogordius* Heinze, 1934

Characteristic for this genus, which currently includes 21 species (Schmidt-Rhaesa 2013), is that areoles are arranged in longitudinal rows. Two specimens of the collection belong to *Beatogordius*, among them the holotype of the most recently described species in this genus.

#### *Beatogordius* sp.

**Material examined.** V8939 (1 male). Fig. 9A–C.

**Location.** Chile, Coronel, in drinking water.

**Remarks from the catalogue.** collected by R. Paeseler on October 5, 1914. Received by the museum on January 15, 1920. Originally determined as “Gordiide”.

**Occurs in publications.** unknown

**Remarks.** The cuticle is not very well preserved and strongly covered with dirt. Paired elongate areoles arranged in longitudinal rows can be well observed (Fig. 9A), but interareolar structures were not seen. This is characteristic for the genus *Beatogordius* and resembles patterns found especially in *B. latastei* (De Villalobos et al. 2003). The posterior end has short, probably broken, spines around the cloacal opening (Fig. 9B) and postcloacal spines, which extend from posterior of the cloacal opening onto about half of the ventral side of the tail lobes (Fig. 9C). In the anterior region they are stout and gradually become more slender in the posterior region (Fig. 9D). Precloacal bristles were not observed (Fig. 9C), but it cannot be excluded that they were artificially eroded or covered by dirt. Males of all South American species have clear precloacal rows of bristles (De Villalobos et al. 2003).

**Material examined.** V9575 (1 male). Fig. 8I–K.

**Location.** Chile, Limache (writing is not explicit), in river.

**Remarks from the catalogue.** collected by D.C. Bock (date not given). Received by the museum on July 9, 1923. Originally determined as “Gordiide”

**Occurs in publications.** unknown

**Remarks.** The cuticle of this specimen shows longitudinal rows of paired, elongate areoles, which is characteristic for the genus *Beatogordius* (Fig. 8I, J). There is closest resemblance to *B. latastei* (Camerano, 1895) (compare De Villalobos et al. 2003), but for a proper determination the cuticular structures in the interareolar region should be checked, which are not seen clearly due to some covering of dirt between areoles in specimen V9575.

### *Beatogordius chinensis* Schmidt-Rhaesa, 2011

**Material examined.** V13294 (1 female)

**Location.** China, Yunnan province, 2 km south of Haba, Haba Xueshan Mountains (27°21.9'N, 100°08.3'E), 2870m. Collected in a small pond in a dry valley of a brook.

**Remarks from the catalogue.** Collected by J. Hájek and J. Růžička on June 17–20, 2007.

**Occurs in publications.** Schmidt-Rhaesa (2011)

**Remarks.** Holotype and only known specimen as described in Schmidt-Rhaesa (2011).

### Genus *Chordodes* Creplin, 1847

This is a genus with almost 100 described species, most of which occur in the tropics and subtropics and many of which have mantids as host (see Schmidt-Rhaesa et al. 2008, Schmidt-Rhaesa 2013). Characteristic for the genus is a particular type of areoles, crowned areoles that has a stem with an apical “crown” of longer or shorter filaments. Areoles can be very diverse, and several types are present.

#### *Chordodes* sp.

**Material examined.** V2230 (1 female). Fig. 12C–G.

**Location.** Venezuela, Ciudad Bolivar.

**Remarks from the catalogue.** Collected by D. Siegert (no collection date or date when received by the museum given). Determined by Möbius in 1855 as *Chordodes pilosus*, confirmed by Römer in 1893 and Heinze in 1935. Host: *Blabera gigantea*.

**Occurs in publications.** Möbius (1855), Janda (1894, page 604), Römer (1895, page 797), Camerano (1897, page 416), Römer (1896, page 280), Heinze (1935a, page 24).

**Remarks.** This specimen is the holotype for the species *Chordodes pilosus*, described by Möbius in 1855. The genus *Chordodes* had been introduced briefly before (Creplin 1847), and the main reason to distinguish this genus from *Gordius* was the abundant presence of marked areoles. Römer (1895, 1896) reinvestigated the specimen, but without going much further than Möbius' original description. The descriptions were not detailed enough to allow a distinction from the several new species that were described towards the end of the 19<sup>th</sup> century, and Camerano (1897) listed *C. pilosus* under species inquirendae. Later, Heinze (1935a) reinvestigated the specimen again and added some information on the cuticle. The current reinvestigation by SEM shows that crowned areoles, the diagnostic character of the genus *Chordodes*, are present on the cuticle (Fig. 12C–G). Crowned areoles are arranged in clusters, surrounded by circumcluster areoles (Fig. 12F). Simple and tubercle areoles are present. All areoles are very bad in shape and appear worn or eroded, especially the filaments of the crowned areoles (Fig. 12E–G). Nevertheless it can be seen that crowned areoles

with longer apical filaments are present along a longitudinal stretch, probably the ventral midline (Fig. 12C). All observed characters are present in many species of *Chordodes* and their preservation in specimen V2230 is not good enough to characterise a distinct species. Therefore, the species identity of *Chordodes pilosus* cannot be unambiguously determined from the type specimen, and, thus, the name must be regarded a nomen dubium.

**Material examined.** V5320 (1 male, 1 female). Fig. 12H–O.

**Location.** South Africa, Bothaville.

**Remarks from the catalogue.** Collected by Brauns (no date given). Received by the museum on November 13, 1899. Determined as “*Gordius*”. From “*Mantis*”.

**Occurs in publications.** unknown

**Remarks.** The cuticle of the female is in comparably bad shape. Crowned areoles with short and long apical filaments are present (Fig. 12H–K). Tubercle, thorn or bulging areoles could not be observed. The cuticle of the male is only slightly better in preservation (Fig. 12L–O). Crowned areoles with short and longer apical filaments were observed, but the clusters of crowned areoles appear to be heterogeneous. In some clusters a central pair of crowned areoles is surrounded by circumcluster areoles (Fig. 12J), in other clusters the distinction between crowned areoles and circumcluster areoles is not easy to make and there appears to be a more gradual transition between these two types (Fig. 12K). Simple areoles appear to be covered with tiny knobs or spines (Fig. 12M, N). Further areolar types such as tubercle, thorn or bulging areoles were not observed. Therefore the determination of both specimens cannot go beyond the genus *Chordodes*.

### *Chordodes brasiliensis* Janda, 1893

**Material examined.** V7351 (a) and (c-m) (10 males and 2 females). Fig. 12P–S, 13 A–F.

**Location.** Brazil, presumably Itapoá, Santa Catarina (see remarks from the catalogue).

**Remarks from the catalogue.** Collected by W. Ehrhardt on January 17, 1910. As location, the catalogue says “Humboldt-Geb. am Itapoeú”, some letters are not clear. This location could not be retrieved, but Itapoá is close. Additionally, there are other catalogue entries with the same date and same collector having as location Joinville, which is close to Itapoá in the province Santa Catarina.

**Occurs in publications.** None

**Remarks.** The catalogue notes “dv”, meaning “diverse” as number of specimens. Thirteen specimens were found, one of them (b) is determined as *Acutogordius* cf. *acuminatus* (see there), the others are determined as *Chordodes brasiliensis*. The cuticle has numerous isolated, tall and slender crowned areoles (Fig. 12P–S, 13A, B), which is typical for *Chordodes brasiliensis* (see De Villalobos et al. 2004, Schmidt-Rhaesa et al. 2008). One

specimen (e) has areoles with very long apical filaments along a longitudinal line (Fig. 13C, D), presumably on the ventral side. Such crowned areoles with long filaments have been reported only from females (Camerano 1897, De Villalobos et al. 2004).

### *Chordodes jandae* Camerano, 1895 or *C. skorikovi* Camerano, 1903

**Material examined.** V4064 (1 male and 1 female). Fig. 13G–J.

**Location.** Indonesia, Sumatra (as “O. Sumatra, Dali, Indrapura Estab”).

**Remarks from the catalogue.** Collected by “Gebr. Siemssea” (no date given). Received by the museum on March 21, 1896. Determined as “*Gordiiden*”.

**Occurs in publications.** unknown

**Remarks.** The simple areoles have the shape of blackberry areoles (Fig. 13I). Bulging, tubercle and thorn areoles are present (Fig. 13I). The crowned areole clusters are composed of approximately 12 areoles (Fig. 13J) and are therefore intermediate between the numbers given for *C. jandae* (20) and *C. skorikovi* (7–8) (see Schmidt-Rhaesa et al. 2008).

### *Chordodes janovyi* Bolek, Schmidt-Rhaesa, Hanelt & Richardson, 2010

**Material examined.** V13291 (1 male)

**Location.** Cameroon, Menoua river drainage in the village Bawa (5°24'N, 10°03'E).

**Remarks from the catalogue.** Collected in June 2006.

**Occurs in publications.** Bolek et al. (2010)

**Remarks.** **Holotype** of this species. See Bolek et al. (2010) for all information about this specimen.

**Material examined.** V13292

Same data as for V13291, paratype of this species. See Bolek et al. (2010) for all information about this specimen.

### *Chordodes japonensis* Inoue, 1951

**Material examined.** V4599 (sex unknown because of damaged posterior end). Fig. 13K, L.

**Location.** “Japan, Schikoku, Tosa, Mano Bogawa, Kamigori”

**Remarks from the catalogue.** Collected by Lenz on July 27, 1897. Received by the museum on August 11, 1897. Determined as “*Gordiide*”.

**Occurs in publications.** unknown

**Remarks.** The cuticle of the specimen is in most of the investigated part covered by the unstructured larval cuticle (Fig. 13L). In the parts where the adult cuticle can be observed (Fig. 13K, L) structures correspond to the descriptions given for *Chordodes japonensis* (see, e.g., Inoue 1952, Schmidt-Rhaesa 2004).

**Material examined.** V13282 (female).

**Location.** Japan, Campus of Nakamura Gakuen Sanyo High School, Imajukuaoki 1042-33, Nishi-ku, Fukuoka City, Fukuoka Prefecture, Kyushu (33°33'58"N, 130°17'22"E).

**Remarks from the catalogue.** Collected by Yuriko Kuribashi on October 12–18, 2008 from host *Hierodula patellifera*.

**Occurs in publications.** Schmidt-Rhaesa and Urabe (2009).

**Remarks.** This specimen is only briefly mentioned in Schmidt-Rhaesa and Urabe (2009) as a new record of location.

### *Chordodes cf. japonensis*

**Material examined.** V5202 (1 male). Fig. 13M–P.

**Location.** Japan, Tokyo

**Remarks from the catalogue.** Collected by Jourdan (no date given). Received by the museum on February 14, 1899. Found in an undetermined mantid. Determined as “*Gordius*”.

**Occurs in publications.** unknown

**Remarks.** The cuticle is not in very good shape, but it largely corresponds to the cuticular pattern of *C. japonensis* (compare specimen V4599 and Inoue 1952, Schmidt-Rhaesa 2004) (Fig. 13M–O).

### *Chordodes cf. joyeuxi* Dorier, 1935

**Material examined.** V5778 (1 female). Fig. 14A–F.

**Location.** Indonesia, Sumatra, Lomgei Lalah, Indragiri

**Remarks from the catalogue.** Collected by W. Burcharde (no date given). Received by the museum on August 26, 1901. Determined as “*Gordius*”.

**Occurs in publications.** unknown

**Remarks.** The simple areoles have the shape of blackberry areoles (Fig. 14B, F), bulging areoles are absent. Thorn and tubercle areoles are present (Fig. 14B–E), thorn areoles are quite large (Fig. 14B, C). Crowned areoles with short apical filaments and those with long filaments both occur (Fig. 14B, D, F). According to the key from Schmidt-Rhaesa et al. (2008) the specimens closely resembles *C. joyeuxi* from Vietnam.

### *Chordodes parabipilus* Kintsurashvili, Schmidt-Rhaesa & Gorgadze, 2011

**Material examined.** V13269 (1 female, holotype)

**Location.** Georgia, village Tsitamuri, 20 km from Tbilisi.

**Remarks from the catalogue.** Collected by Temur Gvalia on September 30, 2002, given by N. Kintsurashvili. Determined by Schmidt-Rhaesa in 2008.

**Occurs in publications.** Kintsurashvili et al. (2011)

**Remarks.** **Holotype** of this species. See Kintsurashvili et al. (2011) for all information about this specimen.

### *Chordodes polycoronatus* Schmidt-Rhaesa & Brune, 2008

**Material examined.** V11540 (1 male). Fig. 14G–I.

**Location.** Langcat, Sumatra, Indonesia.

**Remarks from the catalogue.** Collected by Ernst Versmann (date unknown). Received by the museum on March 9, 1931. Originally determined as “*Gordius*”.

**Occurs in publications.** unknown

**Remarks.** With the large clusters of crowned areoles (Fig. 14G, I) the specimen closely resembles *C. polycoronatus*, described by Schmidt-Rhaesa and Brune (2008) from Malaysia.

### *Chordodes queenslandi* Schmidt-Rhaesa, 2002

**Material examined.** V2231 (1 female)

**Location.** Australia, Sydney.

**Remarks from the catalogue.** Collected by Dämle (no collection date or date when received by the museum given). Determined by Römer in 1893 as *C. bouvieri*, by Heinze in 1935 as *C. modiglianii* and by Schmidt-Rhaesa in 2002 as *C. queenslandi*.

**Occurs in publications.** Römer (1895, page 797), briefly in Römer (1896, page 283), Heinze (1935a, page 24), Schmidt-Rhaesa (2002b, page 1573).

**Remarks.** As explained in Schmidt-Rhaesa (2002b), this specimen corresponds to the (then) newly described species *C. queenslandi* and does not fit the descriptions of the other two species *C. bouvieri* and *C. modiglianii*.

### *Chordodes jelkae* sp. n.

<http://zoobank.org/7962277E-8D0A-48C6-A164-A3818DB5FD00>

**Material examined.** V10960 (2 females). Fig. 15A–F.

**Location.** Rwanda, Kabgayi, “Poste de Kigali”.

**Holotype.** Specimen 10960 (I).

**Remarks from the catalogue.** Collected by “Freres Rodrigues v.d. Weissen Vätern” (no collection date). Received by the museum on September 20, 1929.

**Etymology.** The species is named after and dedicated to my oldest daughter, Jelka.

**Description.** The two females measure 90 (I) and 85 (II) mm in length and 0.9/1.0 mm in diameter, respectively. The anterior end tapers towards the tip. The body colour is a dark brown general occurrence, with brown basic colour and darker patches (the “leopard pattern”). The anterior tip is white and blends into the brown body colour. Specimen (I) is well preserved, characters of specimen (II) are less well visible (Fig. 15F). The following description is therefore based on specimen (I),

which is designated as the holotype. The cuticle shows the characteristic types of areoles known from other species of *Chordodes*. The simple areoles are roundish, often longer than broad (Fig. 15C, E). Their longer axis is perpendicular to the longitudinal axis of the animal. Simple areoles are closely together (Fig. 15C, E). On the apical side they have a tuft of short (about 3 µm) bristles (Fig. 15C–E), in some cases these fine bristles are dissolved and do not form a clear tuft. Among the simple areoles are occasional tubercle areoles, the tubercle is in some cases pointed (Fig. 15E). Thorn areoles were not observed. Crowned areole clusters are composed of two central crowned areoles with moderately long apical filaments (< 20 µm), which are surrounded by 10–12 circumcluster areoles (Fig. 15C). The apical filaments of the crowned areoles originate laterally around a more or less flat apical surface of the areole, this surface is divided by several grooves into several compartments (Fig. 15C). The apical filaments may divide basally, but more distal divisions were not observed. The circumcluster areoles have a tuft of bristles on top (Fig. 15C). This tuft varies in its form, it either resembles the tuft of simple areoles as all bristles originate in the center of the apical surface or, in some circumcluster areoles, the apical surface is flat and the bristles extend laterally, comparable (though shorter) than in crowned areoles (Fig. 15C). Along one longitudinal line, probably the ventral midline, crowned areoles have very long apical filaments (approximately 170 µm) (Fig. 15B), in contrast to crowned areoles in the remaining parts of the body, which have shorter apical filaments (Fig. 15A).

**Remarks.** Species of *Chordodes* are quite similar in their cuticular structure. Species are distinguished by the presence or absence of particular types of areoles or when cuticular structures exhibit a particular substructure. Characteristic for this new species is the form of the simple areoles. In most species of *Chordodes* the simple areoles have either no bristles or scattered fine bristles on their apical surface. SEM investigations reveal that small bristles may be more abundant than known on the basis of traditional light microscopical investigation and they may even form small tuft-like structures as present, e.g. in *C. parabipilus* Kintsurashvili, Schmidt-Rhaesa & Gorgadze, 2011 (Kintsurashvili et al. 2011), *C. moutoni* Camerano, 1895 (Schmidt-Rhaesa and Yadav 2013) and *C. combi-areolatus* Schmidt-Rhaesa, Limatemjen & Yadav, 2015 (Schmidt-Rhaesa et al. 2015). Compared to these species, the tuft of bristles is larger in *C. jelkae* sp. n. A distinct tuft of bristles is present in *C. villalobi*, a species from Malaysia (Schmidt-Rhaesa and Brune 2008), but this is more pronounced than in *C. jelkae* sp. n. In combination to the shape of the tuft of bristles on the simple areoles, an important difference between all mentioned species and *C. jelkae* sp. n. is that thorn areoles are absent in *C. jelkae* sp. n., but present in all other species. Additionally, simple areoles are closer together in *C. jelkae* sp. n. than in *C. villalobi*. Characters in specimen 10960 (II) are not as

well visible and its assignment to *C. jelkae* sp. n. is likely, but not certain.

### Genus *Gordionus* Müller, 1926

The genus *Gordionus* is characterized by the following character combination: one type of areoles present on the cuticle, male posterior end with bristles anterolateral of the cloacal opening and spines posterior of the cloacal opening and directly around it. Currently, 56 species are included in this genus (Schmidt-Rhaesa 2013), but it is currently not well understood, whether these species are real, because the amount of character variation is not clear. There is a broad range of minute cuticular differences between *Gordionus* specimens and it was suspected that such a range reflects intraspecific variability. This was for example made probable for the species *G. violaceus* and *G. wolterstorffii*, which, although having quite dissimilar cuticular patterns, are connected by a range intermediate cuticular patterns (Schmidt-Rhaesa 2001). Unpublished molecular results (COI-gene) support that there are two species, one (*G. violaceus*) with one typical cuticular pattern and another (*G. wolterstorffii*) with a variable cuticular pattern. This would mean that some species descriptions (see, e.g., Heinze 1941) fall into the range of cuticular variation of *G. wolterstorffii*. This does, for example, account to *G. scaber*, of which some specimens in the collection have been determined. Because the mentioned molecular results have not been published yet, I decide here to retain the determination as *G. scaber*, when specimens correspond to the character description as given by Müller (1926) and Heinze (1937, 1941).

### *Gordionus* sp.

**Material examined.** V2215 (3 females and one specimen without posterior end). Fig. 9E.

**Location.** “East Africa, middle Ussuri” (as “Ostafrika, mittlerer Ussuri”) [a river Ussuri could not be located].

**Remarks from the catalogue.** Collected by “Fischer” (no collection date or date when received by the museum given). *Carabus smaragdinus* is given as host. Determined by Römer in 1895 and Heinze in 1935 as male of *Gordius aquaticus*.

**Occurs in publications.** Römer (1895, page 793), briefly in Römer (1896, page 260).

**Remarks.** Presently, the vial contains four specimens: three females, of which one was investigated here by SEM and one specimen in an extra vial, this specimen is lacking the posterior end. According to the catalogue the number of specimens should be 2 and the determination says “*Gordius aquaticus* ♂”. Römer (1895) mentions 2 males and 4 females from this locality and this host, therefore it remains unknown, how many specimens were included in this vial originally. The potential host is the beetle *Carabus smaragdinus*. It is not mentioned, whether all specimens originate from the one host and the host

is not included in the vial. Additionally, the beetle was probably not determined correctly, as *C. smaragdinus* is not an African species (according to Wikipedia). The cuticle contains polygonal areoles (Fig. 9E) that are clearly separated from each other and therefore it is most likely that this specimen belongs to the genus *Gordionus*.

**Material examined.** V2217 (according to catalogue 3 males, only 1 male found in vial). Fig. 9F–H.

**Location.** Germany (not further specified), from carabid beetle.

**Remarks from the catalogue.** Collected by “v. Siebold” (no collection date or date when received by the museum given). Determined by Von Siebold as *Gordius subbifurcatus*, by Römer in 1893 as *Gordius tolosanus*. Genus corrected to *Parachordodes* by Heinze in 1935 (according to additional label in vial). Additional remark: type material of *Gordius subbifurcatus*.

**Occurs in publications.** Römer (1895, page 794).

**Remarks.** *Gordius subbifurcus* Von Siebold, 1848 (not *subbifurcatus* as in catalogue!) is synonymous to *Parachordodes tolosanus* (Dujardin, 1842) (see, e.g., Heinze 1941). Von Siebold (1848) did not really describe this species, but writes about a horsehair worm emerging from *Molops elatus*: “it possesses a slight longitudinal furrow at the posterior end, therefore I will call it ... *Gordius subbifurcus*”. According to the catalogue, the worm(s) of number V2217 are the type material of *Gordius subbifurcus*, but I regard this as not likely. First, neither in the vial, nor in the catalogue, it is marked as type material. Second, according to the catalogue, Von Siebold collected the material himself from an undetermined carabid beetle. In Von Siebold's 1848 publication he mentions *G. subbifurcus* twice, first from *Molops elatus* sent by Prof. Fuchs and second from *Calathus cisteloides* from the helminthological collection in Freiburg. Both these reports do not correspond to the data given in the Hamburg catalogue.

Finally, the determination as *Parachordodes tolosanus* could not be supported here. The genus *Parachordodes* has two types of areoles, one of which are the so-called superareoles (see, e.g., Schmidt-Rhaesa 2013). The SEM investigation showed only one type of areoles (Fig. 9F), therefore this specimen likely belongs to the genus *Gordionus*. The posterior end shows a row of precloacal bristles that is continuous anterior of the cloacal opening and not divided into two lateral rows. This single character has been taken to name a new genus, *Semigordionus* (Heinze 1952), with one species from Austria. Zanca and Schmidt-Rhaesa (2006) found it more appropriate to regard *Semigordionus* as a member of the genus *Gordionus*, but did not formalize this step, because further investigations of the posterior end of *S. circumannulatus* should be awaited.

**Material examined.** V2236 (II) (1 female) One specimen is indicated in the catalogue, but 2 specimens are present. The second specimen (I) is determined as *Parachordodes tolosanus*. Fig. 9I.

**Location.** Germany, Göttingen.

**Remarks from the catalogue.** Collected by Schlotthauber (no collection date or date when received by the museum given). Determined by Schlotthauber in 1854 as *Gordius aquaticus*, corrected by Heinze in 1935 to *Parachordodes tolosanus*. Host: *Harpalus ruficornis* crossed out and replaced by *Ophonus cephalotes*.

**Occurs in publications** (as *P. tolosanus*). Heinze (1937, page 285), Heinze (1941, page 24).

**Remarks.** Only one type of areoles is present (Fig. 9I), no superareoles could be found. Therefore the specimen is determined as *Gordionus* sp. As the catalogue indicates only one specimen, it is unknown whether this second specimen does belong to the vial originally. If it does, it is unknown whether both specimens emerged from the beetle.

**Material examined.** V9615 (2 females) (3 specimens are indicated in the catalogue). Fig. 9J, K.

**Location.** Macedonia, stream to river Vardar, close to Veles (as “Bach zum Vardoe, N. v. Veles”)

**Remarks from the catalogue.** collected by F. Ahlborn in 1916 (date not given). Received by the museum on November 22, 1923. Originally determined as “*Gordius*”.

**Occurs in publications.** unknown

**Remarks.** The cuticle of both specimens has roundish areoles surrounded by a broad and flat interareolar space, in which interareolar bristles are present (Fig. 9J, K). This somewhat resembles *G. violaceus*, but in that species the interareolar furrows are not so broad. Specimens V9615 cannot be assigned to a species of *Gordionus* with certainty.

**Material examined.** V11117 (1 male). Fig. 9L–O.

**Location.** Germany, ditch in Lokstedt, Hamburg.

**Remarks from the catalogue.** Collected by H. Richter (date unknown). Received by the museum on July 25, 1930. Determined by Heinze in 1935 as *Gordionus scaber*.

**Occurs in publications.** Heinze (1937, page 309), Heinze (1941, page 32)

**Remarks.** The areoles on the cuticle of this specimen are not clearly recognizable and appear to be artificially altered (Fig. 9L). Therefore an exact determination cannot be given. The posterior end (Fig. 9M–O) contains, as far as could be observed, mainly unbranched precloacal (Fig. 9N) and circumcloacal (Fig. 9O) bristles.

**Material examined.** V12470 (1 male). Fig. 10A.

**Location.** Germany, Friedrichstadt an der Eider.

**Remarks from the catalogue.** collected by Niggemeyer (no date given). Received by the museum on May 16, 1936. Determined by Thiel in 1936 as *Gordius aquaticus*.

**Occurs in publications.** unknown

**Remarks.** The posterior end of this specimen is unfortunately lost during preparation for SEM, but notes taken before the loss indicate that a postcloacal crescent is absent. The cuticle has one type of areoles (Fig. 10A), it is possible that this specimen belongs to *G. violaceus*, but preservation is not good enough for a certain determination.

**Material examined.** V13313 (1 male)

**Location.** Japan, Hirakura, Higaashim.

**Remarks from the catalogue.** Collected by Takuya Sato on September 12, 2008. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13316 (1 male)

**Location.** Japan, Hirakura, Nishimata.

**Remarks from the catalogue.** Collected by Takuya Sato on September 29, 2008. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13319 (1 male)

**Location.** Japan, Kumano, Misen.

**Remarks from the catalogue.** Collected by Takuya Sato on September 14, 2009. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13320 (1 male)

**Location.** Japan, Hirakura, Nishimata.

**Remarks from the catalogue.** Collected by Takuya Sato on September 14, 2009. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13361 (1 female; transferred from V2220). Fig. 10B.

**Location.** Germany, Göttingen, Hainholz, in source.

**Remarks from the catalogue.** Collected by Schlotthauber (no collection date or date when received by the museum given). Determined by Römer in 1893 as *Gordius violaceus*, corrected by Heinze in 1935 to *Parachordodes tolosanus*.

**Occurs in publications.** Römer (1895, page 794, as *G. violaceus*), probably Heinze, only as "Göttingen" (1937, page 283; 1941, page 26, as *P. tolosanus*).

**Remarks.** SEM reinvestigation showed that the female does not have superareoles as required for the original determination as *P. tolosanus*. Because it has only one type of areoles (Fig. 10B), it is designed as *Gordionus* sp. The specimen was formerly included in a lot of five worms under the number V2220.

**Material examined.** V13362 (1 male; transferred from V2225). Fig. 10C.

**Location.** Germany, Hamburg, river Bille.

**Remarks from the catalogue.** Collected by Steinblinck (no date given), received by the museum on January

1, 1895. Determined by Römer in 1893 as *Gordius violaceus*, corrected to *Gordionus meissneri* by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 794), briefly in Heinze (1937, page 302), Heinze (1941, page 45).

**Remarks.** SEM reinvestigation shows partly fused areoles (Fig. 10C), consistent with patterns found in the character range of *G. wolterstorffii* (see Schmidt-Rhaesa 2001). *Gordionus meissneri*, to which the specimen was originally assigned, has isolated areoles that are not fused to short rows as in V13362 (see, e.g., Heinze 1941). The specimen was formerly included in a lot with another male worms (determined as *G. violaceus*) under the number V2225.

#### *Gordionus bageli* Schmidt-Rhaesa & Gusich, 2010

**Material examined.** V13278 (1 male)

**Location.** Switzerland, Rösental near Liestal, Tafeljura. In *Thuja* forest.

**Remarks from the catalogue.** Collected by Valeria Viktoria Gusich in 2008.

**Occurs in publications.** Schmidt-Rhaesa and Gusich (2010).

**Remarks.** **Holotype** for this species. See Schmidt-Rhaesa and Gusich (2010) for all information about this specimen.

#### *Gordionus barbatus* Schmidt-Rhaesa & Cieslak, 2008

**Material examined.** V13259 (1 male)

**Location.** Spain, Lleida, Llesp, Barranc Basculina (42°27'24.5"N, 0°44'57"W).

**Remarks from the catalogue.** Collected by J. Fresneda, I. Ribera and A. Cieslak on August 2, 2006. Received by the museum on July 3, 2008.

**Occurs in publications.** Schmidt-Rhaesa and Cieslak (2008).

**Remarks.** **Holotype** of this species. See Schmidt-Rhaesa and Cieslak (2008) for all information about this specimen.

#### *Gordionus chinensis* (Villot, 1874)

**Material examined.** V13250 (1 male)

**Location.** Japan, Yumitohara river, Totsu river system, Kii Peninsula.

**Remarks from the catalogue.** Collected by Takuya Sato in 2007. Received by the museum on May 20, 2008.

**Occurs in publications.** Schmidt-Rhaesa and Sato (2009).

**Remarks.** see Schmidt-Rhaesa and Sato (2009) for all information about this specimen.

**Material examined.** V13251 (1 male)

Same data as V13250, see there.

**Material examined.** V13312 (1 male)

**Location.** Japan, Arida, Ninomata.

**Remarks from the catalogue.** Collected by Takuya Sato on September 13, 2008. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13314 (1 male)

**Location.** Japan, Hirakura, Higaashim.

**Remarks from the catalogue.** Collected by Takuya Sato on September 12, 2008. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13315 (1 male)

**Location.** Japan, Miyagawa, Muzutani.

**Remarks from the catalogue.** Collected by Takuya Sato on September 29, 2008. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13317 (1 male)

**Location.** Japan, Kumano, Sankou.

**Remarks from the catalogue.** Collected by Takuya Sato on October 13, 2010. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13318 (1 male)

**Location.** Japan, Kumano, Sankou.

**Remarks from the catalogue.** Collected by Takuya Sato on October 13, 2010. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13321 (1 male)

**Location.** Japan, Kumano, Sankou.

**Remarks from the catalogue.** Collected by Takuya Sato on October 13, 2010. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

### ***Gordionus kii* Schmidt-Rhaesa & Sato, 2009**

**Material examined.** V13252 (1 male)

**Location.** Japan, Yumitehara river, Totsu river system, Kii Peninsula.

**Remarks from the catalogue.** Collected by Takuya Sato in 2007. Received by the museum on May 20, 2008.

**Occurs in publications.** Schmidt-Rhaesa and Sato (2009).

**Remarks.** **Holotype** for this species. See Schmidt-Rhaesa and Sato (2009) for all information about this specimen.

### ***Gordionus scaber* Müller, 1926**

**Material examined.** V2221 (1 male). Fig. 10D, E.

**Location.** Germany, Hamburg, Bergedorf.

**Remarks from the catalogue.** Collected by Thalenhorst (no collection date or date when received by the museum given). Determined by Römer in 1893 as *Gordius violaceus* and by Heinze in 1935 as *Gordionus scaber* (s. str.).

**Occurs in publications.** Römer (1895, page 794), briefly in Heinze (1937, page 309), Heinze (1941, page 32).

**Remarks.** Although not optimally preserved, it is evident that the areoles are isolated or partly fused in short rows (Fig. 10D), which corresponds to the description of *Gordionus scaber scaber* (see, e.g., Heinze 1941).

**Material examined.** V10924 (1 male). Fig. 10F–H.

**Location.** Germany, Medingen, Bad Bevensen, presumably stream Wohbeck (as “aus dem Wohbach”, “Wohbeck is the current name”).

**Remarks from the catalogue.** Collected by F. Richter (date not given). Received by the museum on June 29, 1929. Determined by Heinze in 1935 as *Gordionus scaber*.

**Occurs in publications.** Heinze (1937, page 309), Heinze (1941, page 32).

**Remarks.** The cuticle shows roundish areoles with an individual character, but which are partly fused with neighbouring areoles in longitudinal direction (not figured). This is consistent with the pattern described for *Gordionus scaber* (e.g., Heinze 1941). The posterior end has, as far as can be observed, unbranched preloocal bristles (Fig. 10F, H), but these are covered with some dirt and a potential fine branching, as is described by, e.g., Heinze (1941) for *G. scaber*, could not be excluded with certainty. Therefore, Heinze's determination as *G. scaber* is confirmed here with the additional remark that this may fall into the range of characters for a polymorphic species *G. wolterstorffii*.

### ***Gordionus turkensis* Schmidt-Rhaesa & Cieslak, 2008**

**Material examined.** V13262 (1 male)

**Location.** Turkey, Karabük Rd. E80, E cross to Gerede, stream in Cayorenguney (40°48'23"N, 32°16'.5"E), 1231 m.

**Remarks from the catalogue.** Collected by I. Ribera on April 28, 2006. Received by the museum on July 3, 2008.

**Occurs in publications.** Schmidt-Rhaesa and Cieslak (2008).

**Remarks.** **Holotype** of this species. See Schmidt-Rhaesa and Cieslak (2008) for all information about this specimen.

***Gordionus violaceus* (Baird, 1853)**

**Material examined.** V2225 (originally 2 males, 1 male is regarded as *Gordionus* sp. and transferred to a new catalogue number 13362). Fig. 10I, J.

**Location.** Germany, Hamburg, river Bille.

**Remarks from the catalogue.** Collected by Steinblinck (no date given). Received by the museum on January 25, 1895. Determined by Römer in 1893 as *Gordius violaceus*, corrected to *Gordionus meissneri* by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 794), briefly in Heinze (1937, page 302), Heinze (1941, page 45).

**Remarks.** SEM reinvestigation shows one type of areoles surrounded by interareolar bristles (Fig. 10I), therefore the determination as *G. violaceus* is supported.

**Material examined.** V11455 (2 females). Fig. 10K, L.

**Location.** Germany, Rügen.

**Remarks from the catalogue.** Collected by A. Thienemann (date unknown). Received by the museum on November 12, 1930. Originally determined by A. Thienemann as *Parachordodes tolosanus*. Corrected by Heinze in 1935 to *Gordionus violaceus*.

**Occurs in publications.** unknown. Heinze (1937, 1941) mentions Rügen as location for *G. violaceus*, but with addition "Stubbnitz", which is not given in the catalogue. Therefore this may be a different specimen.

**Remarks.** In both females the cuticle has round areoles completely surrounded by interareolar bristles (Fig. 10K, L), which is characteristic for *G. violaceus* (compare, e.g., Schmidt-Rhaesa 2001).

***Gordionus wolterstorffii* (Camerano, 1888)**

**Material examined.** V2218 (2 males, 1 female according to catalogue, presently only 1 male in vial). Fig. 11A.

**Location.** Germany, Göttingen, Hainholz, in source.

**Remarks from the catalogue.** Collected by Schlotthauber (no collection date or date when received by the museum given). Determined by Römer in 1893 as *Gordius tolosanus*, corrected to *Gordionus scaber scaber* by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 794), briefly in Römer (1896, page 262), Heinze (1937, page 309), Heinze (1941, page 32).

**Remarks.** Collector and collection dates are similar to V2220 determined as *Parachordodes tolosanus*. The cuticle shows irregular rows of fused areoles (Fig. 11A). According to Heinze (1941) *Gordius scaber scaber* has isolated areoles or areoles are fused to short rows, but the individual character of areoles remains visible. Such description does not correspond to the pattern observed in specimen V2218 by SEM. The long fused rows of areoles correspond to the description given for *Gordionus scaber lineatus* (e.g., in Heinze 1941), but also correspond to the description of *G. wolterstorffii* (Camerano, 1888) (see, e.g., Schmidt-Rhaesa 2001).

**Material examined.** V2222 (in the catalogue 5 males and 2 females are indicated, presently only 1 male is present). Fig. 11B, D.

**Location.** Germany, Kissingen (additional remark "Poppenhausen" in Römer 1895), in a fountain in field.

**Remarks from the catalogue.** Collected by Dömling (no date given). Received by the museum on September 23, 1893. Determined by Römer in 1893 as *Gordius violaceus* and by Heinze in 1935 as *Gordionus scaber*.

**Occurs in publications.** Römer (1895, page 794), briefly in Heinze (1937, page 309), Heinze (1941, page 32).

**Remarks.** The cuticle shows irregular rows of fused areoles (Fig. 11B). As described above (V2218), this is characteristic for *G. wolterstorffii* rather than for *G. scaber*.

**Material examined.** V5255 (1 male). Fig. 11C, E.

**Location.** Germany, Hamburg, Elbe river.

**Remarks from the catalogue.** Collected by Wiebelitz (no date given). Received by the museum on June 14, 1899. Determined by Heinze in 1935 as *Gordionus scaber*.

**Occurs in publications.** unknown

**Remarks.** The cuticle shows irregular rows of fused areoles (Fig. 11C). As described above (V2218), this is characteristic for *G. wolterstorffii* rather than for *G. scaber*.

**Genus *Gordius* Linné, 1758**

Species of the genus *Gordius* are not easy to determine due to their scarceness of diagnostic characters, especially in female worms. Additionally, the range of intraspecific variation for several characters is not known (Schmidt-Rhaesa 2010). Finally, fine structural characters such as the presence and distribution of fine bristles have been observed only with SEM, but are not reported in older species descriptions (see Schmidt-Rhaesa 2010).

Characteristic for the genus is the presence of a semi-circular or parabolic cuticular fold posterior of the ventral cloacal opening, which is called the postcloacal crescent. Posterior of this structure, the body is divided into paired lateral tail lobes. In *Gordius*, the tail lobes are rounded, in contrast to the genus *Acutogordius*, which has pointed tail lobes and is the only other genus with a postcloacal crescent (see below). In females, there is no difference to females of other genera, therefore females are more difficult to assign. The body cuticle in *Gordius* specimens can be either smooth or ornamented with roundish or polygonal structures called areoles. Areoles are present in other genera, too, especially in *Gordionus*. A smooth cuticle is only present in *Gordius*, but it has been described from several species (15 European species according to Schmidt-Rhaesa 2010). Therefore, a smooth cuticle indicates that the specimen belongs to *Gordius*, but it is not indicative for a certain species. Most species can only be determined with the help of certain structures, mostly bristles, on the male posterior end. In worn or dirty specimens such characters may not be visible, therefore determination requires some caution. This is the reason that in this reinvestigation a number of determinations to species level are changed to *Gordius* sp.

**Gordius sp.**

**Material examined.** V 2207 (1 male). Fig. 1A.

**Location.** Germany, Kellersee, Holstein.

**Remarks from the catalogue.** Collected by "v. Pöpinghausen" (no date given), received by the museum on August 10, 1894. Determined as *G. aquaticus* by Römer in 1893 and as *G. mülleri* by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 791), Heinze (1937, page 320), Heinze (1941, page 59) (both Heinze's records with "?").

**Remarks.** In *Gordius muelleri* Heinze, 1933 (writing in catalogue *mülleri*) there is some distance between the postcloacal crescent and the beginning of the bifurcation of the tail lobes (see Heinze 1933, 1937, 1941). As in the present specimen the postcloacal crescent is directly at the point of bifurcation (Fig. 1A), the determination as *G. muelleri* cannot be confirmed.

**Material examined.** V 2208 (3 specimens, 1 male was reinvestigated here). Fig. 1B, C.

**Location.** Germany, Plön, Holstein.

**Remarks from the catalogue.** Collected by "Dr. G. Duncker" (no date given), received by the museum on January 18, 1894. Determined as *G. aquaticus* by Römer in 1893 and as *G. albopunctatus* by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 791), probably Heinze (1937, page 57), Heinze (1941, page 319) [several specific locations around Plön are listed].

**Remarks.** The cuticle is, in the investigated part, covered with material of unknown nature (Fig. 1C). The polygonal areoles, characteristic for *G. albopunctatus* (see, e.g. Schmidt-Rhaesa and Kristensen 2006) could not be observed and, therefore, the specimens are regarded here as *Gordius* sp.

**Material examined.** V2214 (1 female). Fig. 1D.

**Location.** Chile

**Remarks from the catalogue.** No detailed collecting locality in Chile mentioned, collector unknown. No collection date given and no date when received by the museum. Determined by Römer in 1893 as *G. aquaticus*, confirmed by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 792), briefly in Römer (1896, page 260).

**Remarks.** Female worm with smooth cuticle (Fig. 1D), which occurs only in some species of *Gordius*. As *Gordius aquaticus* is restricted to the Palaearctic, hence this is probably another species of *Gordius*, such as *G. robustus*, but this cannot be determined with a female specimen.

**Material examined.** V2216 (1 female). Fig. 1E.

**Location.** Chile, Valdivia river.

**Remarks from the catalogue.** In catalogue: "Coll. Mich. 33", on record card "Michaelsen". No further collection date or date when received by the museum given. Determined by Römer in 1893 as *Gordius aquaticus*, also mentioned "Heinze 1935".

**Occurs in publications.** Römer (1895, page 793), briefly in Römer (1896, page 260).

**Remarks.** Female worm with smooth cuticle (Fig. 1E), see discussion under V2214.

**Material examined.** V2223 (in the catalogue 1 male and 1 female are mentioned, currently two fragments are present, from which at least one is from the male). Fig. 1F, G.

**Location.** Germany, Hamburg (no further details).

**Remarks from the catalogue.** Collector unknown, no date given. Date when received by the museum not given. Determined by Römer in 1893 as *Gordius violaceus* and by Heinze in 1935 as *Gordius mülleri*.

**Occurs in publications.** Römer (1895, page 794), briefly in Heinze (1937, page 320), Heinze (1941, page 59).

**Remarks.** The SEM reinvestigation shows a slight pattern of areoles on the cuticle (Fig. 1F). The cloacal opening, which should be oval in *G. muelleri* (see, e.g., Heinze 1941) is covered and not visible (Fig. 1G). The tail lobes of specimen V2223 are about twice as long as they are broad and not as short as they should be in *G. muelleri* (see, e.g., Heinze 1941). Therefore, the determination as *G. muelleri* is regarded as questionable.

**Material examined.** V2227 (1 female). Fig. 1H.

**Location.** "Viti Islands" (probably Viti Levu, Fiji-Islands).

**Remarks from the catalogue.** Collected or donated by "M.G.". No collection date or date when received by the museum given. Host: *Phybalosoma pythonis* (Phasmatodea). Determined by Römer in 1893 as *Gordius aeneus*, determination checked and confirmed by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 794), Römer (1896, page 268).

**Remarks.** *Gordius aeneus* is regarded an invalid species name (Schmidt-Rhaesa 2013). SEM investigation of specimen V2227 shows a smooth cuticular surface (Fig. 1H), therefore an assignment to the genus *Gordius* is certain, but a concrete species cannot be determined.

**Material examined.** V2228 (1 female). Fig. 2A.

**Location.** unknown ("?" in catalogue).

**Remarks from the catalogue.** Collector unknown. No collection date or date when received by the museum given. Determined by Römer in 1893 as *Gordius fulgur*, determination confirmed by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 795), briefly in Römer (1896, page 267).

**Remarks.** SEM investigation of specimen V2228 shows a smooth cuticular surface (Fig. 2A), therefore an assignment to the genus *Gordius* is certain, but a concrete species cannot be determined. Therefore, the original determination as *G. fulgur* was changed to *Gordius* sp.

**Material examined.** V2238 (1 specimen of unknown sex). Fig. 2B.

**Location.** Tanzania, Bagamoyo.

**Remarks from the catalogue.** Collected by Stuhlmann. No collection date or date when received by the museum given. Determined first as *Gordius aquaticus*,

but "*aquaticus*" was later crossed out. No name of reviser is given. Worm found in an undetermined cricket.

**Occurs in publications.** unknown

**Remarks.** The worm is still inside the cricket, some loops of it are visible through an injury in the anterior part of the abdomen. Body ends were not removed, and a sex determination is therefore not possible. A piece of worm was removed from the outermost loop. The cuticle is smooth (Fig. 2B), therefore it is likely that this is a *Gordius* specimen.

**Material examined.** V2977 (1 female). Fig. 2C.

**Location.** Japan, Lake Candidius.

**Remarks from the catalogue.** Collected by H. Sauter (no date given), received by the museum on April 22, 1908. Originally determined as "*Chordodes*".

**Occurs in publications.** unknown

**Remarks.** SEM investigation reveals a smooth cuticle (Fig. 2C), therefore the specimen belongs to the genus *Gordius*.

**Material examined.** V3314 (1 female). Fig. 2D.

**Location.** "Elsass, Tannenberg bei Saales, 640m, in einer Heuschrecke". Revised location: France, Saales. Tannenberg is probably a sanatorium close to Saales.

**Remarks from the catalogue.** Collected by Dr. Alb. Graser (no date given), received by the museum on July 12, 1909. Host: a cricket.

**Occurs in publications.** unknown.

**Remarks.** The cuticle is smooth, but contains small keel-like structures perpendicular to the longitudinal body axis (Fig. 2D). These structures are unknown from species of *Gordius*.

**Material examined.** V4413 (1 male). Fig. 2E, F.

**Location.** Germany, "Bergedorf" close to Hamburg.

**Remarks from the catalogue.** Collected by "W. Fischer" (date not given). Received by the museum on October 1, 1896, determined as "Gordiide".

**Occurs in publications.** unknown

**Remarks.** The cuticle contains shallow areoles with polygonal or irregular outline (Fig. 2E). The postcloacal crescent borders the point of bifurcation of the two tail lobes, it is short and semicircular and extends slightly onto the tail lobes (Fig. 2F). Most of these characters fit to the description of *G. undulatus* as given by Heinze (1937), with the exception that in this species, there is a distance between the postcloacal crescent and the point of bifurcation of the tail lobes. With the exception of *G. digitatus*, which has strongly interdigitating areoles (see Schmidt-Rhaesa 2010), *G. undulatus* is the only species with areoles of an irregular shape. Therefore a definitive determination can currently not be given.

**Material examined.** V4598 (sex unknown, because posterior end is missing). Fig. 2G.

**Location.** Japan, Schikoku, Mano bogawa, Kamigore, Tosa.

**Remarks from the catalogue.** Collected by Lenz on July 27, 1897. Received by the museum on August 11, 1897. Originally determined as "Gordiide".

**Occurs in publications.** unknown

**Remarks.** The specimen has a smooth cuticle (Fig. 2G), but because the posterior end is missing, no further determination can be given.

**Material examined.** V4611 (1 male, 1 female). Fig. 2H–K.

**Location.** Italy, South Tyrol ("Süd-Tirol").

**Remarks from the catalogue.** Collected by Timm (no date given), received by the museum on August 21, 1897. Determined as "*Gordius*".

**Occurs in publications.** unknown

**Remarks.** Both specimens differ in their cuticular structure, which may indicate that they belong to different species. The female has a smooth cuticle (not figured), while the cuticle of the male has elongate areoles that are partly confluent with neighbouring ones and that are arranged perpendicular to the longitudinal body axis (Fig. 2H, I). This cuticular structure does match best, though not exactly, the description for *G. tirolensis* Heinze, 1937, a species described from Austria (Susalitsch, Kärnten) and also from South Tyrol (Brixen) (Heinze 1937, 1941). The posterior end contains a large postcloacal crescent that has a short distance to the cloacal opening and extends well onto the comparably long tail lobes (length to width slightly > 2:1) (Fig. 2J). The postcloacal crescent is angled. Around the cloacal opening, bordered posteriorly by the postcloacal crescent, there is a depressed area that is bordered by a fringed rim (Fig. 2K). The exact nature of this structure could not be detected, but it does not seem to be a row of bristles as is present in some (non-European) species such as *G. paranensis* (see below and Schmidt-Rhaesa et al. 2000) or *G. attoni* (see, e.g., Schmidt-Rhaesa et al. 2003). The description of the posterior end does not fit *G. tirolensis* (Heinze 1937, 1941) and therefore V4611 is treated here as *Gordius* sp.

**Material examined.** V4981 (1 male). Fig. 3A, B.

**Location.** Germany, Hamburg, Flottbeck, in drainage tubes.

**Remarks from the catalogue.** Collected by Ansoerge (no date given), received by the museum on November 19, 1897. Determined by Heinze in 1935 as *G. aquaticus*.

**Occurs in publications.** Heinze (1937, page 331) and Heinze (1941, page 70). In both cases, Heinze adds the date "19.11.97", the date when received by the museum.

**Remarks.** The SEM reinvestigation shows a smooth cuticle (Fig. 3A) and some short bristles on the inner side of the tail lobes (Fig. 3B, C). As it is not clear whether such bristles are present in *G. aquaticus* (compare Schmidt-Rhaesa 2010), the specimen is referred to here as *Gordius* sp.

**Material examined.** V5046 (1 female). Fig. 3D.

**Location.** Switzerland, "Axenstr. bei Tells Platte" (probably close to Sisikon, Uri, Switzerland).

**Remarks from the catalogue.** Collected by "Michaelsen 20.VI.98", received by the museum on June 30, 1898. Determined as "*Gordius*". Found in man ("im Menschen").

**Occurs in publications.** unknown

**Remarks.** The cuticle is smooth, but has some fungus-like covering (Fig. 3D), the determination is not possible with certainty.

**Material examined.** V5459 (1 male). Fig. 3E, F.

**Location.** Germany, Hamburg, river Elbe (“Elbe bei Hamburg”).

**Remarks from the catalogue.** Collected by A. Westphalen (no date given). Received by the museum on April 26, 1900. Determined by Heinze in 1935 as *Gordius mülleri*.

**Occurs in publications.** Heinze (1937, page 320), Heinze (1941, page 59)

**Remarks.** The cuticle does not contain areoles (Fig. 3E). The posterior end is in bad shape, the postcloacal crescent is directly at the point of bifurcation of the tail lobes (Fig. 3F). As explained for specimen V2207, this argues against a determination as *G. muelleri*.

**Material examined.** V5523 (1 female). Fig. 3G.

**Location.** Germany, Rahlstedt (“Alt-Rahlstedt”), under a flower pot.

**Remarks from the catalogue.** Collected by Ilse Schultze (date not given). Received by the museum on September 15, 1900. Determined by Heinze in 1935/36 as “*Gordius ? aquaticus*”.

**Occurs in publications.** Heinze (1937, page 331), Heinze (1941, page 70).

**Remarks.** The cuticle of this specimen is smooth (Fig. 3G), therefore no further determination can be given.

**Material examined.** V5773 (1 male). Fig. 3H, I.

**Location.** Germany, Eppendorfer Mühlenteich, Hamburg.

**Remarks from the catalogue.** Collected by “Lehrer” (teacher ?) Wagner (date unknown). Received by the museum on July 2, 1901. Determined by Heinze in 1935 as *Gordius mülleri* (= *muelleri*).

**Occurs in publications.** Heinze (1937, page 320), Heinze (1941, page 59).

**Remarks.** The body cuticle is heavily covered with some artificial crust (Fig. 3H), therefore it could not be determined with certainty, whether areoles are present or not. The posterior end is clearer, but does not correspond to the descriptions given for *G. muelleri* (e.g. in Heinze 1941), because the postcloacal crescent is directly on the point of bifurcation of the tail lobes in V5773 (Fig. 3I) and not, as in *G. muelleri*, in some distance from it.

**Material examined.** V6341 (1 female). Fig. 3J.

**Location.** Germany, Hessen, Stream Olbe.

**Remarks from the catalogue.** Collected by G. Ulmer in July 1900. Received November 14, 1903. Determined by Heinze in 1935 as *Gordius stellatus*.

**Occurs in publications.** Heinze (1941, page 71) (not in Heinze 1937)

**Remarks.** The cuticle is smooth with some irregularly placed shallow elevations (Fig. 3J). Star-like structures on the cuticle as described for *G. stellatus* are likely artificial (Heinze 1937) and it is not clear whether this

species is valid or not (e.g. Schmidt-Rhaesa 1997). This reinvestigation cannot assign the specimen to any species of *Gordius* with certainty.

**Material examined.** V6488 (1 male). Fig. 3K–M.

**Location.** Germany, Hannover, River Örtze.

**Remarks from the catalogue.** Collected by Th. Lindemann (no date given). Received by the museum on July 28, 1904. Determined by Michaelsen in 1904 as *Gordius aquaticus* and by Heinze in 1935 as *Gordius setiger*.

**Occurs in publications.** Heinze (1937, page 316), Heinze (1941, page 58)

**Remarks.** The cuticle has very indistinct elevations that might represent areoles (Fig. 3K). The entire cuticle has a fine striation (Fig. 3L). The posterior end has some fine bristles on the inner side of the tail lobes. The postcloacal crescent is narrow and semicircular, it extends onto the tail lobes (Fig. 3M). The current characters do not fit to a certain species of *Gordius*, but are regarded not significant enough to consider this a new species, therefore the specimen is determined as *Gordius* sp. . Heinze's determination as *G. setiger* might be based on the presence of areoles, however, the elevations are so slight that it is not sure whether these can really be called areoles.

**Material examined.** V6826 (2 males and one specimen of unknown sex, posterior end is missing). Fig. 4A, B.

**Location.** China, province Fokien, Futschou.

**Remarks from the catalogue.** Collected by “Cons. G. Siemssen” (no date given). Received by the museum on April 4, 1906.

**Occurs in publications.** unknown

**Remarks.** The specimen has a smooth cuticle (Fig. 4A). The posterior end has a broad postcloacal crescent directly at the point of bifurcation of the two tail lobes (Fig. 4B). Scattered bristles were observed on the posterior end of one male on the entire tail lobes extending to lateral of the cloacal opening. Due to some covering with dirt, such bristles could not be observed in the other male. Details such as bristles are not very well known from Asian species of *Gordius* and the specimen is kept here as *Gordius* sp.

**Material examined.** V7456 (1 female). Fig. 4C.

**Location.** China, province Fujian (as “Prov. Fo-Kien”).

**Remarks from the catalogue.** collected by “Consul G. Siemssen” (no date given), received by the museum on September 8, 1911. Determined as “*Gordius*” by Angener or Augener (as “Ang. det. 1931” or “Aug. det. 1931”).

**Occurs in publications.** unknown

**Remarks.** The specimen has a smooth body cuticle (Fig. 4C), therefore no further determination is possible.

**Material examined.** V7724 (1 male). Fig. 4D, E.

**Location.** China, province Fujian (as “Prov. Fokien”).

**Remarks from the catalogue.** collected by “Consul G. Siemssen” (no date given), received by the museum on September 4, 1912. Determined as “Gordiide”.

**Occurs in publications.** unknown

**Remarks.** The body cuticle is smooth (Fig. 4D). The posterior end has the postcloacal crescent (Fig. 4E), which is characteristic for the genus *Gordius*. Parts of the posterior end are shrunken or covered with dirt, therefore no further structures could be observed and no further determination could be made.

**Material examined.** V8077 (1 male). Fig. 4F, G.

**Location.** Italy, Sorgono, Sardinia.

**Remarks from the catalogue.** collected by A.H. Krause (no date given), received by the museum on March 14, 1913. Determined as "*Gordius*".

**Occurs in publications.** unknown

**Remarks.** The cuticle is not in good shape, but it appears to be smooth (Fig. 4G), except for the posterior end, where some decent structuring into shallow areoles is present. The posterior end has a semicircular postcloacal crescent extending onto the tail lobes (Fig. 4F), some bristles are present in the posterior part of the inner side of the tail lobes. This description does not really fit present descriptions, but differences are not very large, therefore the specimen is kept here as *Gordius* sp. .

**Material examined.** V9075 (2 females). Fig. 4H.

**Location.** France, surroundings of Montpellier.

**Remarks from the catalogue.** collected by K. Schmalzfuss (no date given). Received by the museum on December 23, 1920. Originally determined as "*Gordiide*". Although the catalogue indicates only one specimen, two females were found in the vial, named here (I) and (II).

**Occurs in publications.** unknown

**Remarks.** Both specimens have a smooth cuticle covered by some dirt (Fig. 4H). Therefore they belong to the genus *Gordius*, but a further determination is not possible.

**Material examined.** V9214 (unknown sex, because posterior end is missing). Fig. 4I.

**Location.** Chile, Victoria in province Malleco (swimming in river).

**Remarks from the catalogue.** collected by C. Bock in February 1920. Received by the museum on August 12, 1921.

**Occurs in publications.** unknown

**Remarks.** The cuticle is smooth (Fig. 4I), therefore this specimen must belong to the genus *Gordius*, but a further determination is not possible, because the posterior end is missing.

**Material examined.** V10154 (1 male). Fig. 4J.

**Location.** Germany, Hamburg, Süderelbe (river Elbe).

**Remarks from the catalogue.** Collected by N. Peters (date only given), received by the museum on June 7, 1927. Determined by Heinze in 1935 as *Gordius muelleri* (as *mülleri*).

**Occurs in publications.** unknown

**Remarks.** Unfortunately the posterior end of this specimen was lost during preparation for SEM, but it was

a male posterior end with a postcloacal crescent. The cuticle is smooth (Fig. 4J).

**Material examined.** V10529 (1 male). Fig. 4K, L.

**Location.** Brazil, Itatiaia, state of Rio de Janeiro, 700m, in a puddle.

**Remarks from the catalogue.** Collected by F. Ohaus (date unknown), donated to the museum by Titschak, received by the museum on March 15, 1928.

**Occurs in publications.** unknown

**Remarks.** This specimen clearly belongs to the genus *Gordius* due to the presence of a postcloacal crescent (Fig. 4L), but the cuticle is in bad shape (Fig. 4K) and a further determination cannot be given.

**Material examined.** V10599 (1 male). Fig. 4M–O.

**Location.** probably Croatia, Rijeka. Writing in catalogue: "Umgeg. v. Fiume", Fiume is the Italian and Hungarian name for Rijeka (source: Wikipedia). This is supported by the added word "Jugoslavien" (Yugoslavia) in different handwriting.

**Remarks from the catalogue.** no collector indicated, given to the museum by de Gries. Received by the museum on September 24, 1928. Additional remark: from *Lacerta muralis* (common wall lizard).

**Occurs in publications.** unknown

**Remarks.** This is a *Gordius* specimen with smooth cuticle (Fig. 4M), but with roundish areoles in the posterior end. Some bristles were found on the edge of the tail lobes (Fig. 4O), but a further determination cannot be given. Lizards are not known and extremely unlikely as hosts. As no further specification of where the worm was found in relation to the lizard is given, it cannot be excluded that the worm has been eaten by the lizard and was found in the mouth.

**Material examined.** V10955 (1 male). Fig. 5A.

**Location.** Germany, stream Linau in Witzeeze, Schleswig-Holstein.

**Remarks from the catalogue.** Collected by G. Duncker (date only given on label in vial: August 26, 1929), received by the museum on September 2, 1929. Determined by Heinze in 1935 as *Gordius setiger*. (label in vial says: "*Gordius* spec. *setiger* oder *mülleri*. Vermutl. doch *setiger* Schneider").

**Occurs in publications.** Heinze (1937, page 316), Heinze (1941, page 58).

**Remarks.** Unfortunately the posterior end of this specimen was lost during preparation for SEM, but it was a male posterior end with a postcloacal crescent. There are flat areoles (Fig. 5A), which are present in several species, therefore the determination cannot go beyond *Gordius* sp.

**Material examined.** V11466 (1 male and fragments of probably 2 further specimens; only male was investigated). Fig. 5B, C.

**Location.** Brazil, Boiteuxburgo, Santa Catarina.

**Remarks from the catalogue.** Collected by Paul Missfeldt (no date given). Received by the museum on December 5, 1930. Originally determined as "*Gordius*".

**Occurs in publications.** unknown

**Remarks.** The body cuticle is smooth (Fig. 5B). The postcloacal crescent is semicircular (Fig. 5C). Few bristles are present along the lateral sides, extending onto the tail lobes. The fine structure of species of *Gordius* from South America is not well known and therefore this specimen cannot be determined further than to *Gordius* sp.

**Material examined.** V11943 (1 male). Fig. 5D, E.

**Location.** Hungary. No further specification is given here, but number V11942 is also listed in the catalogue as a gordiid, with the same collector and location “Ungarn, Warmbad Villach, Thermenabfluss”. This means the worm was found in the drainage of a spa. Today, Villach is a village in Austria, until 1918 Austria and Hungary were united, which could explain the catalogue entry. It is assumed that the same locality is valid also for V11943. A vial V11942 is present in the collection, but it contains no specimen.

**Remarks from the catalogue.** Collected by Gebhard (no date given) and received by the museum on September 28, 1933. Original determination as “Gordiiden”.

**Occurs in publications.** unknown

**Remarks.** The specimen has a postcloacal crescent bordering the point of bifurcation of the tail lobes (Fig. 5E) and the cuticle is smooth (Fig. 5D). Some bristles appear to be present in the posterior end on the sides of the tail lobes.

**Material examined.** V12712 (1 female). Fig. 5F.

**Location.** Germany, Schiffbeker Moor, Hamburg.

**Remarks from the catalogue.** Collected by Mrs. Helme (date unknown) and received by the museum on October 12, 1937.

**Occurs in publications.** unknown

**Remarks.** The cuticle is smooth (Fig. 5F), a further determination cannot be given.

**Material examined.** V13285 (1 male)

**Location.** Japan, Katsura river, Arashiyama, Kyoto prefecture (35°00'48"N, 135°40'40"E).

**Remarks from the catalogue.** Collected by Kazuo Isobe on March 13, 2008.

**Occurs in publications.** Schmidt-Rhaesa and Urabe (2009).

**Remarks.** This male *Gordius* specimen could not be determined further, see Schmidt-Rhaesa and Urabe (2009) for further information and discussion.

**Material examined.** V13305 (1 male)

**Location.** Japan, Kumano, Misen.

**Remarks from the catalogue.** Collected by Takuya Sato on September 14, 2009. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13306 (1 male)

**Location.** Japan, Kumano, Nadani.

**Remarks from the catalogue.** Collected by Takuya Sato on October 14, 2007. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13307 (1 male)

**Location.** Japan, Arida, Ninomata.

**Remarks from the catalogue.** Collected by Takuya Sato on September 30, 2009. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13308 (1 male)

**Location.** Japan, Arida, Ninomata.

**Remarks from the catalogue.** Collected by Takuya Sato on September 13, 2009. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13309 (1 male)

**Location.** Japan, Kumano, Nadani.

**Remarks from the catalogue.** Collected by Takuya Sato on November 10, 2008. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13310 (1 male)

**Location.** Japan, Kumano, Nadani.

**Remarks from the catalogue.** Collected by Takuya Sato on October 15, 2007. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

**Material examined.** V13311 (1 male)

**Location.** Japan, Kumano, Nadani.

**Remarks from the catalogue.** Collected by Takuya Sato on November 15, 2006. Received by the museum in 2011.

**Occurs in publications.** Sato et al. (2012)

**Remarks.** Specimen is a voucher for a phylogenetic analysis in Sato et al. (2012).

### *Gordius aquaticus* Linné, 1758

**Material examined.** V12685 (1 male). Fig. 5G, H.

**Location.** Germany, Hamburg.

**Remarks from the catalogue.** Collected by M. Grosse (date not given). Received by the museum on August 5, 1937.

**Occurs in publications.** unknown.

**Remarks.** The cuticle of this specimen is smooth (Fig. 5G) and the posterior end (Fig. 5H) does not include any bristles or further structures, therefore this species is determined as *G. aquaticus*.

#### ***Gordius balcanicus* Schmidt-Rhaesa, 2010**

**Material examined.** V13270 (1 male)

**Location.** Serbia, Kučaj Mountains, Dubašnica, Mikuljska River.

**Remarks from the catalogue.** Collected by Ajtić on October 3, 2006.

**Occurs in publications.** Schmidt-Rhaesa (2010).

**Remarks. Holotype** of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

#### ***Gordius balticus* Schmidt-Rhaesa, 2010**

**Material examined.** V13268 (1 male)

**Location.** Estonia, South Estonia (exact location unknown), in rainwater barrel.

**Remarks from the catalogue.** The collector according to the label is "Elli" in 2008.

**Occurs in publications.** Schmidt-Rhaesa and Prous (2010).

**Remarks. Holotype** of this species. See Schmidt-Rhaesa and Prous (2010) for all information about this specimen.

**Material examined.** V13288 (1 female)

**Location.** Estonia, rainwater container in Suurküla, Häädemeeste.

**Remarks from the catalogue.** Collected by M. Looring on August 23, 2006.

**Occurs in publications.** Schmidt-Rhaesa and Prous (2010).

**Remarks. Paratype** of this species. See Schmidt-Rhaesa and Prous (2010) for all information about this specimen.

#### ***Gordius digitatus* Schmidt-Rhaesa, 2010**

**Material examined.** V13271 (1 male)

**Location.** Croatia, Plitvice Lakes (Plitvička Jezera).

**Remarks from the catalogue.** Collected by M. Teiwes on August 8, 2000.

**Occurs in publications.** Schmidt-Rhaesa (2010).

**Remarks. Holotype** of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

#### ***Gordius helveticus* Schmidt-Rhaesa, 2010**

**Material examined.** V13272 (1 male)

**Location.** Switzerland, Meiringen (Berner Oberland), altitude 793m.

**Remarks from the catalogue.** Collected by Axel Groenvelde on July 18, 2008.

**Occurs in publications.** Schmidt-Rhaesa (2010).

**Remarks. Holotype** of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

**Material examined.** V13277 (1 male)

Same data as for V13272, paratype of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

#### ***Gordius karwendeli* Schmidt-Rhaesa, 2010**

**Material examined.** V13273 (1 male)

**Location.** Germany, Karwendel Mountains, 4–4.5 km NE of Mittenwald. Found in a (not water covered) gravel-bed along stream Seinsbach at roadside during heavy rains.

**Remarks from the catalogue.** Collected by Maaik van Rijn and Axel Groenvelde on June 25, 2002.

**Occurs in publications.** Schmidt-Rhaesa (2010).

**Remarks. Holotype** of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

#### ***Gordius longissimus* Römer, 1895**

**Material examined.** V2229 (1 female). Fig. 5I.

**Location.** "Südsee" (= South Sea), no further specification.

**Remarks from the catalogue.** Collected by "M.G." (writing not explicit). No collection date given and no date when received by the museum. From *Saturnia* (Lepidoptera). Determined by Römer in 1893 as *G. longissimus*, confirmed by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 796), Römer (1896, page 273), Heinze (1933, page 109), Heinze (1934, page 189).

**Remarks. Holotype** of this species. Römer (1895) regards this specimen as a new species due to its extreme length and, in comparison to *G. fulgur*, another species of extreme length, by differences in diameter and colour. Nowadays it is generally assumed that size (length and width) as well as colouration patterns are quite variable, therefore these are no good characters to justify a new species description. One further difference is that *G. longissimus* is lacking an iridescence of the cuticle that *G. fulgur* has. The morphological reason for such iridescence is unknown. The cuticular surface is smooth (Fig. 5I). This is regarded to be a quite weak description for a species, but no changes of the systematical status are proposed here. The length was measured here as 126 cm, which is close to the 132 cm given by Römer. Gordiids are somewhat flexible when preserved and a correct length measurement is almost impossible. The host, a butterfly, is unusual for gordiids (see Schmidt-Rhaesa 2013).

#### ***Gordius paranensis* Camerano, 1892**

**Material examined.** V5035 (1 male, 2 females). Fig. 5J, K.

**Location.** Paraguay, San Bernardino.

**Remarks from the catalogue.** Collected by “Cons. Wiengreen” (no date given). Received by the museum on April 30, 1898. Determined as “*Gordius*”.

**Occurs in publications.** unknown

**Remarks.** The male contains in the posterior end a semicircular row of bristles anterior of the cloacal opening (Fig. 5K), this is characteristic for the species *G. paranensis* from South American and New Zealand (see Schmidt-Rhaesa et al. 2000). The two females have a smooth cuticle (Fig. 5J), although they cannot be assigned to a particular species of *Gordius* they are assumed to be conspecific with the male specimen.

**Material examined.** V11709 (8 males; 1 male investigated by SEM). Fig. 5L.

**Location.** Brazil, Boiteuxburgo, Santa Catarina.

**Remarks from the catalogue.** Collected by Paul Missfeldt (date unknown). Received by the museum on September 8, 1932. Originally determined as “Gordiide”.

**Occurs in publications.** unknown

**Remarks.** The male contains in the posterior end the semicircular row of bristles characteristic for this species (Fig. 5L).

#### *Gordius pesici* Schmidt-Rhaesa, 2010

**Material examined.** V13274 (1 male)

**Location.** Montenegro, Budva.

**Remarks from the catalogue.** Collected by Vladimir Pešić on September 2, 2006.

**Occurs in publications.** Schmidt-Rhaesa (2010).

**Remarks.** **Holotype** of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

#### *Gordius plicatissimus* Heinze, 1952

**Material examined.** V5524 (1 male). Fig. 5M–O.

**Location.** Switzerland, Grindelwald.

**Remarks from the catalogue.** Collected by Michaelsen (no date given). Received by the museum on September 15, 1900. Determined as “*Gordius*”. Host: *Decticus verrucivorum*.

**Occurs in publications.** unknown

**Remarks.** Areoles are large, prominent and slightly oval (Fig. 5N), the arrangement is mostly perpendicular to the longitudinal body axis (Fig. 5M). This arrangement mostly corresponds to the description for *G. plicatissimus* Heinze, 1952, a species reported from Austria (Rosenau am Hengstpaß) (Heinze 1952, see also Schmidt-Rhaesa 2010). The posterior end is strongly shrunken and few details can be observed (Fig. 5O). The postcloacal crescent extends onto the tail lobes, it is broad and parabolic. There are few bristles along the edge of the tail lobes. With exception of the bristles, which were not described by Heinze (1952), the characteristics of the posterior end correspond to *G. plicatissimus*.

**Material examined.** V13332 (1 male). Fig. 6A, B.

**Location.** Austria, Salzburg, St. Gilgen, Falkenstein, 717 m. The specimen was found alive during excavations of a historical source in about 2 m depth.

**Remarks from the catalogue.** Collected by an unknown person on August 30, 2011. Donated to the museum by Robert Lindner, Haus der Natur in Salzburg, Austria in July 2012.

**Occurs in publications.** no

**Remarks.** Areoles are prominent and slightly oval, the arrangement is perpendicular to the longitudinal body axis (Fig. 6A). The broad postcloacal crescent is angled and extends well onto the tail lobes (Fig. 6B). Tiny bristles are present lateral of the postcloacal crescent and on the tail lobes. These characters correspond well to the description for *G. plicatissimus* (Heinze 1952, see also Schmidt-Rhaesa 2010).

#### *Gordius cf. polychaetus* Tang, 1934

**Material examined.** V13283 and V13284 (1 male each)

**Location.** Japan, Sobu Valley, Kaminyu, Yogo Town, Shiga Prefecture (35°34'14"N, 136°12'41"E), on the surface of snow.

**Remarks from the catalogue.** Collected by Daiki Kado on November 22, 2008.

**Occurs in publications.** Schmidt-Rhaesa and Urabe (2009).

**Remarks.** Both specimens have numerous fine bristles on their cuticle, which corresponds to the description of *G. polychaetus* Tang, 1934. See Schmidt-Rhaesa and Urabe (2009) for documentation and discussion.

#### *Gordius serratus* Schmidt-Rhaesa, 2010

**Material examined.** V13280 (1 male)

**Location.** Papua New Guinea, Lahamenegu piggery, Goroka, Eastern Highlands Province, from river water.

**Remarks from the catalogue.** Collected by Ifor Owen, presumably in 1975.

**Occurs in publications.** Schmidt-Rhaesa (2010).

**Remarks.** **Holotype** of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

**Material examined.** V13281 (1 male)

Same data as for V13280, paratype of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

#### *Gordius setiger* Schneider, 1866

**Material examined.** V2224 (1 male). Fig. 6C, D.

**Location.** Germany, Hamburg, Alster.

**Remarks from the catalogue.** Collected by Graeser (no collection date or date when received by the museum given). Determined by Römer in 1893 as *Gordius violaceus* and by Heinze in 1935 as *Gordius setiger*.

**Occurs in publications.** Römer (1895, page 794), briefly in Heinze (1937, page 316), Heinze (1941, page 58).

**Remarks.** The SEM reinvestigation shows inconspicuous areoles (Fig. 6C) and a short, semicircular postcloacal crescent (Fig. 6D). The characters are not very well visible, but correspond to the description for *G. setiger* (see, e.g., Heinze 1941).

**Material examined.** V5940 (1 male). Fig. 6E–G.

**Location.** Germany, river Elbe close to Lauenburg.

**Remarks from the catalogue.** Collected by “Wilh. Meyer” on June 1, 1902. Received by the museum on July 1, 1902. Determined by Heinze in 1935 as *Gordius muelleri*.

**Occurs in publications.** Heinze (1937, page 320), Heinze (1941, page 59)

**Remarks.** Areoles on the cuticle are roughly polygonal in shape and attach closely to each other (Fig. 6E). Few areoles appear a bit stretched, i.e. are broader than long. The narrow, semicircular postcloacal crescent is directly at the point of bifurcation of the tail lobes (Fig. 6F, G). The cloacal opening is covered by dirt, but appears to be slightly oval (Fig. 6G). Heinze's determination as *G. muelleri* cannot be supported here. *Gordius muelleri* has areoles with broad interareolar spaces, a broad postcloacal crescent in distinct distance from the point of bifurcation of the tail lobes and an oval cloacal opening (e.g. Heinze 1941). Shape and position of the postcloacal crescent, in addition to the mostly polygonal areoles correspond with *G. setiger*, although the slightly oval cloacal opening and some “stretched” areoles are not completely corresponding characters.

### *Gordius cf. setiger* Schneider, 1866

**Material examined.** V9000 (1 male). Fig. 6H–J.

**Location.** Germany, lake “Behler See” near Timmen-  
dorf, Holstein.

**Remarks from the catalogue.** collected by “Gast” with unknown date. Received by the museum on June 15, 1920. Determined by Heinze in 1935 as *Gordius setiger*.

**Occurs in publications.** Heinze (1937, page 316), Heinze (1941, page 58)

**Remarks.** The two species *G. setiger* and *G. albopunctatus* are very similar and differ only in few details. According to Heinze (1941), *G. albopunctatus* has polygonal areoles that are slightly stretched and their longer axis is perpendicular to the longitudinal body axis. *Gordius setiger* also has polygonal areoles, but they are irregularly polygonal without being stretched. *Gordius albopunctatus* has a parabolic postcloacal crescent, while this is semicircular in *G. setiger*. The specimen V9000 has clearly visible areoles that are slightly stretched as in *G. albopunctatus* (Fig. 6H). The postcloacal crescent, however, is semicircular as in *G. setiger* (Fig. 6I, J). The position of the cloacal opening in V9000 is directly anterior of the postcloacal crescent (Fig. 6I, J), while in both species, *G. albopunctatus* and *G. setiger*, there

is some distance between these two structures. In summary, V9000 is intermediate between *G. setiger* and *G. albopunctatus*, which could hint to some yet unknown intraspecific variation.

**Material examined.** V9900 (2 males). Fig. 6K–P.

**Location.** Germany, Hamburg, river Elbe, Parkhafen (writing is not explicit).

**Remarks from the catalogue.** collected by H. Meves (date not given). Received by the museum on May 29, 1925. Originally determined as “Gordiiden”. On the vial label, but not in the catalogue is written: determined as *Gordius setiger* by R. Arndt in 1968.

**Occurs in publications.** unknown

**Remarks.** The two males of this lot show characters as described above for *G. setiger* and *G. albopunctatus* (see above under V9000). Male I has polygonal areoles (Fig. 6K), part of which appears to be stretched as in *G. albopunctatus*. The postcloacal crescent is semicircular as in *G. setiger* (Fig. 6L, M). Male II has polygonal, unstretched areoles as in *G. setiger* (Fig. 6N) and a narrow, but parabolic postcloacal crescent as in *G. albopunctatus* (Fig. 6O, P). Therefore, these two males support the discussion under V9000 that there are intermediate stages between the two species *G. setiger* and *G. albopunctatus*.

### *Gordius spiridonovi* Schmidt-Rhaesa, 2010

**Material examined.** V13286 (1 male)

**Location.** Estonia, Lake Peipsi (about 58°40'N, 27°30'E).

**Remarks from the catalogue.** Collected by an unknown person on June 9, 1980.

**Occurs in publications.** Schmidt-Rhaesa and Prous (2010).

**Remarks.** **Holotype** of this species. See Schmidt-Rhaesa and Prous (2010) for all information about this specimen.

**Material examined.** V13287 (1 male)

**Location.** Russia, Udoha River (tributary of the Shelon River), near Borovit, Pskov Region.

**Remarks from the catalogue.** Collected by T. Timm on July 7, 1971.

**Occurs in publications.** Schmidt-Rhaesa and Prous (2010).

**Remarks.** **Paratype** of this species. See Schmidt-Rhaesa and Prous (2010) for all information about this specimen.

### *Gordius terminosetosus* Schmidt-Rhaesa, 2010

**Material examined.** V13279 (1 male)

**Location.** Estonia, Sauga Stream, western Estonia (58°31.54'N, 24°42.16'E).

**Remarks from the catalogue.** Collected by H. Timm on August 16, 1993.

**Occurs in publications.** Schmidt-Rhaesa and Prous (2010).

**Remarks.** Holotype of this species. See Schmidt-Rhaesa and Prous (2010) for all information about this specimen.

### *Gordius undulatus* Heinze, 1937

**Material examined.** V5521 (1 male). Fig. 7A, B.

**Location.** Germany, Hamburg, Winterhude.

**Remarks from the catalogue.** Collected by Braun (writing not explicit, but this name is given by Heinze 1941) on August 10, 1900. Received by the museum on September 1, 1900, determined by “Heinze 1935/6” as *Gordius undulatus*.

**Occurs in publications.** Heinze (1937, page 320), Heinze (1941, page 60).

**Remarks.** This is the **holotype** for this species. (Heinze 1937, see also 1941) describes characteristic features as follows: the posterior end has a small crescent-shaped postcloacal crescent, which is at some distance from the point of bifurcation of the tail lobes. The cloacal opening is round. The cuticle has clearly visible areoles which are irregularly shaped and partly form indentations. These characteristics could be confirmed by the SEM reinvestigation only in part. The shape of the postcloacal crescent is as given by Heinze (1937), but its distance from the point of bifurcation of the tail lobes is difficult to determine (Fig. 7B). The posterior end is slightly curled along the ventral side and the anterior region between the tail lobes is filled with dirt. I have the impression that there is no distance between the postcloacal crescent and the point of bifurcation, but this is not certain. The cuticle was investigated adjacent to the region, where Heinze had removed a piece for his investigation. The cuticle is partly covered by contamination and areoles appear not as clear as stated by Heinze (1937). They are mostly polygonal in shape (Fig. 7A), but some indentations occur.

### *Gordius zwicki* Schmidt-Rhaesa, 2010

**Material examined.** V13275 (1 male)

**Location.** Russia, Russian Far East, small forest tributary in the upper course of Milogradovka River, 25 km NW of Milogradovo.

**Remarks from the catalogue.** Collected by Peter Zwick on June 13, 1998.

**Occurs in publications.** Schmidt-Rhaesa (2010).

**Remarks.** **Holotype** of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

**Material examined.** V13276 (1 male)

Same data as for V 13275, paratype of this species. See Schmidt-Rhaesa (2010) for all information about this specimen.

### Genus *Nectonema* Verrill, 1897

*Nectonema* is the only marine genus of horsehair worms (Nematomorpha) and includes 5 species. Two of them (*N. agile*, *N. munidae*) were repeatedly found, all other species were only caught on single occasions.

### *Nectonema munidae* Brinkmann, 1930

**Material examined.** V13289 (2 females)

**Location.** Norway, Korsfjorden near Bergen, 550–700m.

**Remarks from the catalogue.** Collected by A. Schmidt-Rhaesa, Martin V. Sørensen, Reinhardt Møbjerg Kristensen and Henrik Glenner in August 2009. From host *Munida tenuimana*.

**Occurs in publications.** none.

**Remarks.** none.

**Material examined.** V13290 (2 males)

**Location.** Norway, Korsfjorden near Bergen, 550–700m.

**Remarks from the catalogue.** Collected by A. Schmidt-Rhaesa, Martin V. Sørensen, Reinhardt Møbjerg Kristensen and Henrik Glenner in August 2009. From host *Munida tenuimana*.

**Occurs in publications.** none.

**Remarks.** none.

### Genus *Parachordodes* Camerano, 1897

Species in this genus have two types of areoles, the second, larger type are large areoles that seem to be fusion products of several smaller areoles (Schmidt-Rhaesa 2002a, 2013). They are called superareoles. In total 15 species are known. Several additional specimens without superareoles have been described as *Parachordodes* and not formally removed from this genus, yet (Schmidt-Rhaesa 2013).

### *Parachordodes tolosanus* (Dujardin, 1842)

**Material examined.** V2220 (3 males and 2 females are indicated in the catalog, but actually only 2 males and 1 female are present; female transferred to new catalogue number, see below). Fig. 11K–M.

**Location.** Göttingen, Hainholz, in source (Germany).

**Remarks from the catalogue.** Collected by Schlotthauber (no collection date or date when received by the museum given). Determined by Römer in 1893 as *Gordius violaceus*, corrected by Heinze in 1935 to *Parachordodes tolosanus*.

**Occurs in publications.** Römer (1895, page 794), probably Heinze (1937, page 283) and Heinze (1941, page 26).

**Remarks.** Collector and collection dates are similar to V2218 determined as *Gordionus wolterstorffii*. The fate of two missing specimens is unknown. Two specimens

(1 male and 1 female) were reinvestigated by SEM. The cuticle of the male contains superareoles (Fig. 11K) and corresponds to the description of *P. tolosanus* (see, e.g., Heinze 1941). The female does not have superareoles, but only one type of areoles, it is therefore designed as *Gordionus* sp. under the new catalogue number V13361.

**Material examined.** V2236 (I) (1 male) One specimen is indicated in the catalogue, but 2 specimens are present. The second specimen is determined as *Gordionus* sp.; Fig. 11N, O.

**Location.** Göttingen, Germany

**Remarks from the catalogue.** Collected by Schlotthauber (no collection date or date when received by the museum given). Determined by Schlotthauber in 1854 as *Gordius aquaticus*, corrected by Heinze in 1935 to *Parachordodes tolosanus*. Host: *Harpalus ruficornis* crossed out and replaced by *Ophonus cephalotes*.

**Occurs in publications.** Heinze (1937, page 285), Heinze (1941, page 24).

**Remarks.** Superareoles are present (Fig. 11N, O), therefore the determination as *P. tolosanus* is confirmed. A second specimen was found in the vial, which shows a different cuticular pattern and which is here determined as *Gordionus* sp. (V2236 II). As the catalogue indicates only one specimen, it is unknown whether this second specimen does belong to the vial originally. If it does, it is unknown whether both specimens emerged from the beetle.

**Material examined.** V2237 (1 male). Fig. 11P, Q.

**Location.** Göttingen, Germany

**Remarks from the catalogue.** Collected by Schlotthauber (no collection date or date when received by the museum given). Determined by Schlotthauber in 1854 as *Gordius aquaticus*, corrected by Heinze in 1935 to *Parachordodes speciosus*. Host: *Harpalus distinguendus*.

**Occurs in publications.** Heinze (1937, page 285), Heinze (1941, page 24).

**Remarks.** SEM reinvestigation shows superareoles on the cuticle (Fig. 11P) that correspond to those described from *P. tolosanus* rather than those for *P. speciosus* (see Heinze 1941).

### Genus *Paragordionus* Heinze, 1935

The genus *Paragordionus* resembles *Gordionus* in many respects, but the cuticle contains two types of areoles. The larger areoles are clustered together, such clusters can be composed of two or of several areoles. The large areoles are called megareoles (see Schmidt-Rhaesa 2002a, 2013, Schmidt-Rhaesa and Wagner 2013). Only five species have been described, and species limits require further investigations (Schmidt-Rhaesa and Wagner 2013).

### *Paragordionus dispar* (Müller, 1926)

**Material examined.** V7449 (II) (1 male). Fig. 11I, J.

**Location.** Germany, Berchtesgaden.

**Remarks from the catalogue.** Collected by "Heinr. Baur" (not well readable) (no date given). Received by the museum on August 1, 1911. Determined by Heinze in 1935 as *Paragordionus dispar*. Although the catalogue says that only one specimen belongs to this lot, two specimens are present. These are marked with (I) and (II).

**Occurs in publications.** Heinze (1935b, page 677), Heinze (1937, page 276), Heinze (1941, page 18).

**Remarks.** The two specimens (I) and (II) both belong to the genus *Paragordionus*, because they possess clusters of megareoles (see Schmidt-Rhaesa 2002a). In specimen (II) the clusters of megareoles are smaller and include maximally three megareoles (Fig. 11I, J), while in specimen (I) clusters are larger and include clusters of up to seven megareoles (Fig. 11F, H). These differences correspond to the published descriptions of two different species, *P. dispar* and *P. vej dovskyi* (see, e.g., Heinze 1941). However, according to results of Schmidt-Rhaesa and Wagner (2013) the size and composition of the clusters are quite variable and species limits in this genus should be reinvestigated. As specimen (II) corresponds to the determination given by Heinze, it is assumed that this is the specimen he investigated and reported in the literature. Where the second specimen (I) comes from, whether it was originally present, but overlooked or whether it was added later is unknown and cannot be reconstructed.

### *Paragordionus ibericus* Schmidt-Rhaesa & Cieslak, 2008

**Material examined.** V13260 (1 male)

**Location.** Spain, Puerto de los Cotos, Arroyo de la Laguna Grande de Penalara (40°49'59"N, 3°56'09"W), 1600 m.

**Remarks from the catalogue.** Collected by I. Ribera and A. Cieslak on July 1, 2006. Received by the museum on July 3, 2008.

**Occurs in publications.** Schmidt-Rhaesa and Cieslak (2008).

**Remarks.** Holotype of this species. See Schmidt-Rhaesa and Cieslak (2008) for all information about this specimen.

**Material examined.** V13261 (1 male)

Same data as V13260 (but no type material), see there.

### *Paragordionus vej dovskyi* (Janda, 1894)

**Material examined.** V7449 (I) (1 male). Fig. 11F–H.

**Location.** Germany, Berchtesgaden.

**Remarks.** See V7449 (II) under *Paragordionus dispar* for all remarks and explanations.

**Genus *Paragordius* Camerano, 1897**

Specimens belonging to this genus are easy to recognize, because females have three lobes around the central and terminal cloacal opening and males have very long paired tail lobes (length: width 2:1 or longer). This genus includes the only species among Nematomorpha, where parthenogenetic reproduction was shown (*P. obamai*; Hanelt et al. 2012), the type material is in the collection (see below).

***Paragordius* sp.**

**Material examined.** V3039 (2 males). Fig. 8A–D.

**Location.** Mexico, Hacienda de Ixtapa Tepic.

**Remarks from the catalogue.** Collected by Dr. P. (?) Hacker (no date given). Received by the museum on October 2, 1908. Determined as “Gordiide”. Only 1 male is registered in the catalogue, while 2 males were found in the vial.

**Occurs in publications.** unknown

**Remarks.** The long tail lobes of the male posterior end are characteristic for the genus *Paragordius* (Fig. 8C, D), but no further characters could be identified to determine the exact species.

**Material examined.** V8948 (1 female). Fig. 8E, F.

**Location.** Chile, river Estero Manco (as “Fluss Manco”) near Coronel.

**Remarks from the catalogue.** Collected by R. Paessler on December 16, 1915, received by the museum on January 15, 1920. Originally determined as “Gordiide”.

**Occurs in publications.** unknown

**Remarks.** The posterior end has three lobes, two broader and a slender one, which is typical for females of the genus *Paragordius* (Fig. 8F). The cuticle contains regular depressions and also longitudinal fissures (Fig. 8E). This character does not correspond to previous descriptions of species of *Paragordius*, but because part of the characters might be artificial, more specimens should be investigated before calling this a new species.

**Material examined.** V8962 (1 female). Fig. 8G, H.

**Location.** Chile, river Estero Manco (as “Fluss Manco”) near Coronel.

**Remarks from the catalogue.** Collected by R. Paessler in January 1917, received by the museum on January 15, 1920. Originally determined as “Gordiide”.

**Occurs in publications.** unknown

**Remarks.** The posterior end has three lobes, two broader and a slender one, which is typical for females of the genus *Paragordius* (Fig. 8H). The cuticle appears to be smooth, but has many small cracks (Fig. 8G), which is probably artificial.

***Paragordius obamai* Hanelt, Bolek & Schmidt-Rhaesa, 2012**

**Material examined.** V13265–13267 (1 female each number)

**Location.** Reared from a cyst from a *Biomphalaria pfeifferi* snail collected at Kasabong stream, a small stream in Nyanza province, Kenya (approx. 1170 m altitude), which flows into Winam Bay of Lake Victoria, 2 km to the south.

**Remarks from the catalogue.** Received from Ben Hanelt.

**Occurs in publications.** Hanelt et al. (2012).

**Remarks.** V13265 is the **holotype** for this species, V13266 and V13267 are **paratypes**. See Hanelt et al. (2012) for all information about this specimen. *Paragordius obamai* is the only known nematomorph reproducing by parthenogenesis.

**Genus *Pseudochordodes* Carvalho, 1942**

*Pseudochordodes* is a genus with distribution from the southwestern Nearctic to the Neotropis. Eight species have been described. The males have an unbranched posterior end (= without tail lobes) and the cuticle of both sexes has two types of areoles (see Schmidt-Rhaesa 2013 for summary).

***Pseudochordodes meridionalis* Carvalho & Feio, 1950**

**Material examined.** V2930 (1 female). Fig. 12A, B.

**Location.** Guatemala

**Remarks from the catalogue.** From bequest of “O. Hoffmann der Jul. Groth”. Received by the museum on February 14, 1908. Originally determined as “*Chordodes*”.

**Occurs in publications.** unknown

**Remarks.** SEM reinvestigation reveals larger and smaller areoles (Fig. 12A, B) in an arrangement that corresponds to the description of *Pseudochordodes meridionalis* (see, e.g., Carvalho and Feio 1950, De Miralles and De Villalobos 1996, De Villalobos et al. 2005, 2008 [in part as *Neochordodes meridionalis*]). The cuticle contains areoles with an apical tubercle (Fig. 12B), such areoles appear also to be present in the specimen figured by De Villalobos et al. (2005, her Fig. 6B) and have not been described for this genus before.

**Undetermined**

**Material examined.** V2226 (1 female). Fig. 16A.

**Location.** USA, Arizona, Prescott.

**Remarks from the catalogue.** Collected by Rodenburg (written in Römer 1895 as Bodenburg) (no collection date or date when received by the museum given). Determined by Römer in 1893 as *Gordius violaceus*, determination confirmed by Heinze in 1935.

**Occurs in publications.** Römer (1895, page 794), briefly in Römer (1896, page 267).

**Remarks.** The cuticle of this specimen shows areoles with no clear border and no interareolar structures (Fig. 16A). The surface of the areoles contains grooves, which are oriented parallel to the longitudinal body axis (Fig. 16A). There are regions between the areoles, where grooves are more numerous and create somewhat like a dense cluster of cuticular cords. There is some resemblance to *Pseudochordodes gordioides*, which is present in the Southwestern USA, but here a second type of areoles is more pronounced and has a different surface structure (see, e.g. Schmidt-Rhaesa et al. 2003). Therefore it is not clear to which genus or species this specimen belongs.

**Material examined.** V2233 (1 male). Fig. 16B, C.

**Location.** "Bahia"

**Remarks from the catalogue.** Collected by Bolau (no date given). Received by the museum on September 23, 1893. Determined by Römer in 1893 as *C. variopapillatus*, holotype of this species. Heinze in 1935 writes "specimen in bad condition, not to determine [sehr schlecht erhalten, indeterminable]" and replaces "*C. variopapillatus*" by "*Gordius spec.?*".

**Occurs in publications.** Römer (1895, page 800), briefly in Römer (1896, page 287), Heinze (1935a, page 23).

**Remarks.** The specimen is indeed in bad condition. No areoles were observed on the cuticle (Fig. 16B), therefore, the assignment to *Chordodes* is certainly wrong. As Römer (1895) described different kinds of "papillae" (= areoles) it appears likely that the current specimen is not the specimen investigated by Römer. SEM investigation reveals numerous tube-like structures (Fig. 16B, C), similar structures have been described by Schmidt-Rhaesa et al. (2001) and were assumed not to be cuticular structures. Heinze (1935a) has interpreted these structures as fungal hyphae that break through the cuticle.

**Material examined.** V5522 (1 specimen, sex unknown). Fig. 16D, E.

**Location.** Germany, Ahrensburg.

**Remarks from the catalogue.** Collected by Post (no date given). Received by the museum on September 8, 1900. Determined by Heinze in 1935 as "*Gordius ? undulatus*".

**Occurs in publications.** unknown (not in Heinze 1937 and 1941).

**Remarks.** The posterior end is damaged, the sex cannot be determined. The cuticle is heavily covered with some covering, probably fungus (Fig. 16D, E). In some parts areoles are visible, these are irregularly shaped. The presence of areoles is in agreement with an assignment to the genera *Gordius* or *Gordionus*, but a further determination cannot be given.

**Material examined.** V6622 (sex not determined). Fig. 16F, G.

**Location.** Germany, Hamburg.

**Remarks from the catalogue.** Collected by Rump (no date given). Received by the museum on November 10, 1904. Host: *Vanessa io* (European peacock, currently *Aglais io* or *Inarchis io*, Lepidoptera).

**Occurs in publications.** unknown

**Remarks.** The cuticle is smooth and has some folds (Fig. 16F, G), but no proper determination is possible. Lepidoptera are not or only exceptionally host to nematomorphs (see Schmidt-Rhaesa 2013), therefore this specimen might not be a horsehair worm.

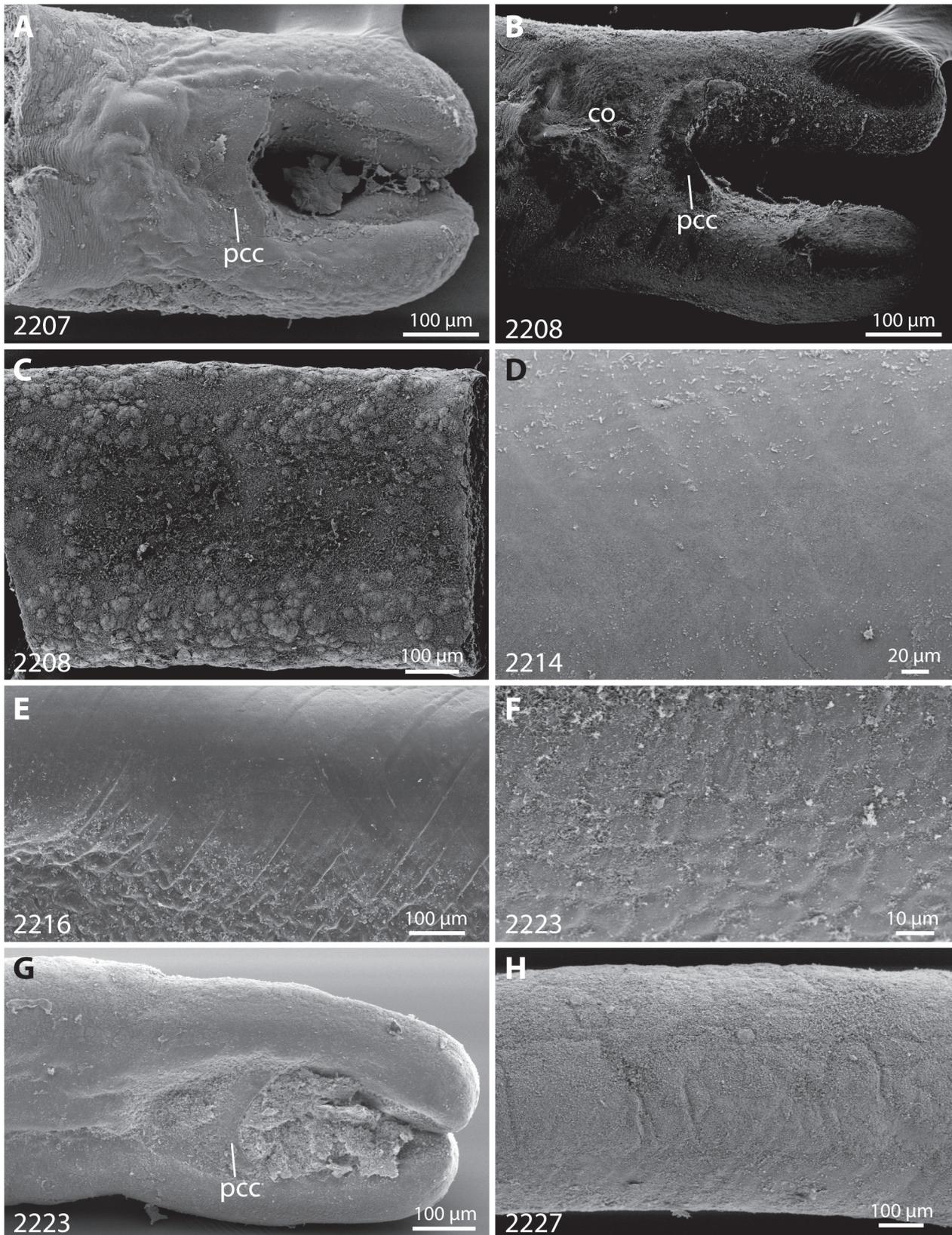
**Material examined.** V7028 (1 specimen). Fig. 16H.

**Location.** Portugal, Leixões

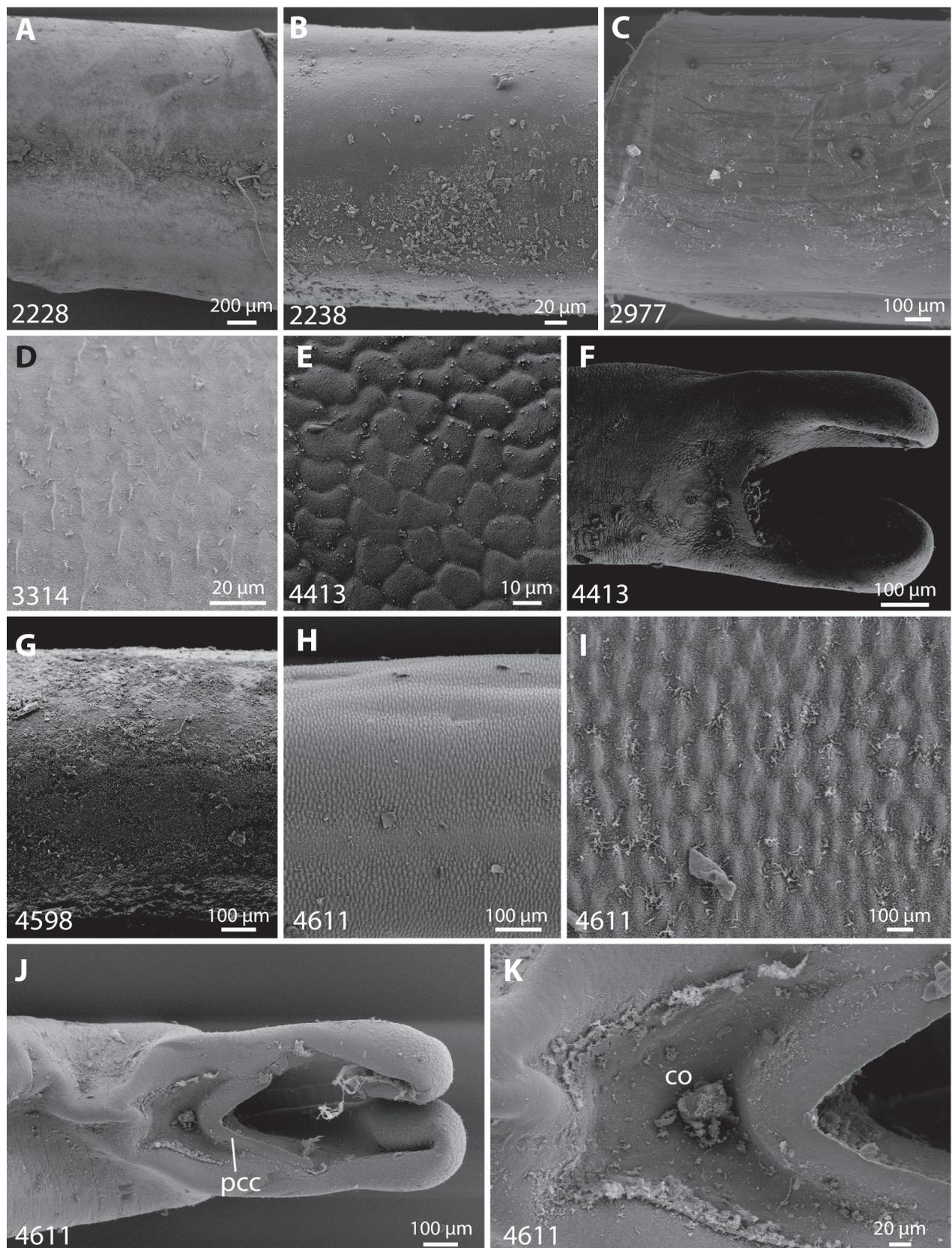
**Remarks from the catalogue.** Collected by F. Ohaus on August 25, 1904. Received by the museum on January 1, 1907. Determined as "Gordiide".

**Occurs in publications.** unknown

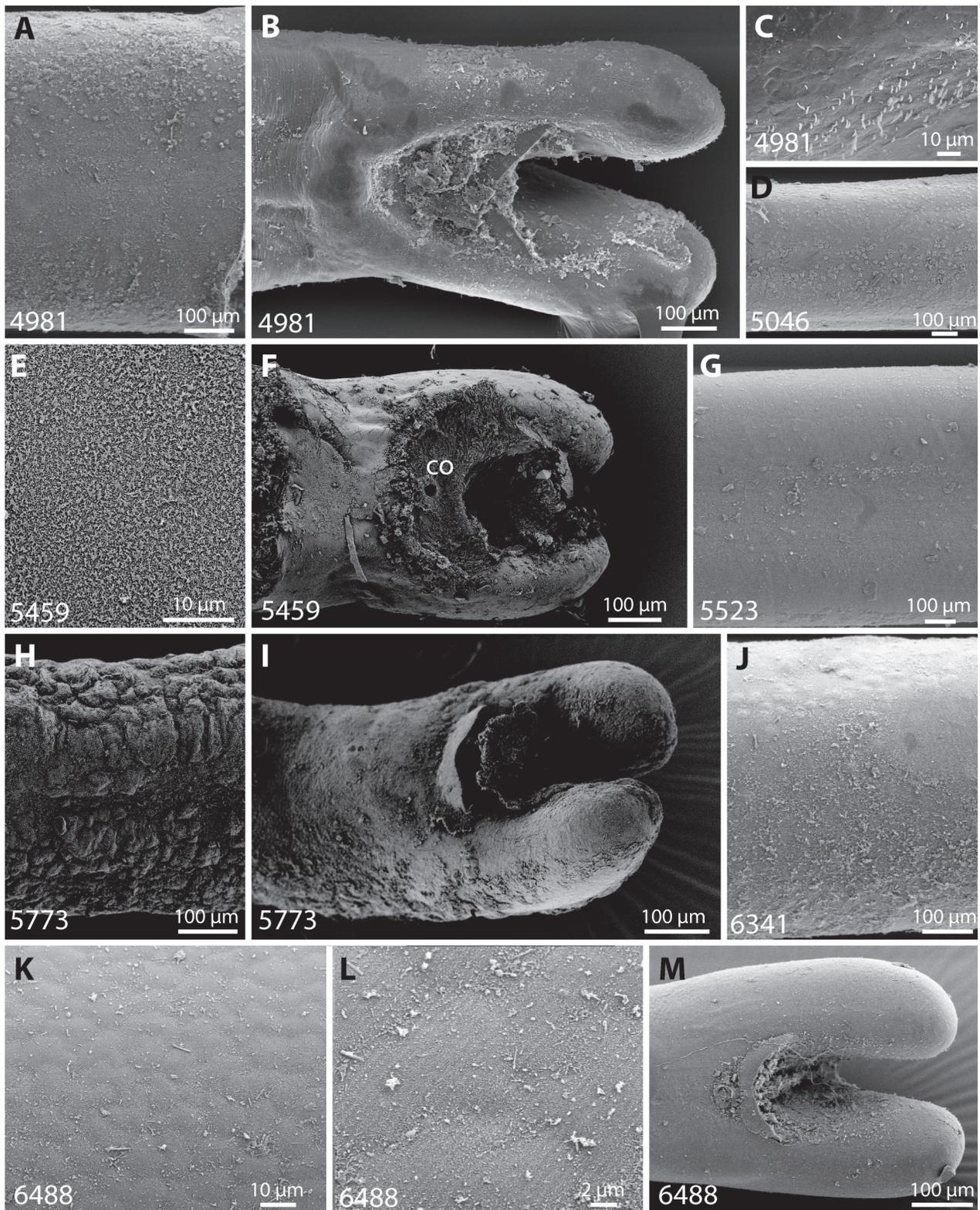
**Remarks.** Cuticle smooth, covered with dirt or fungi (Fig. 16H), no proper determination possible.



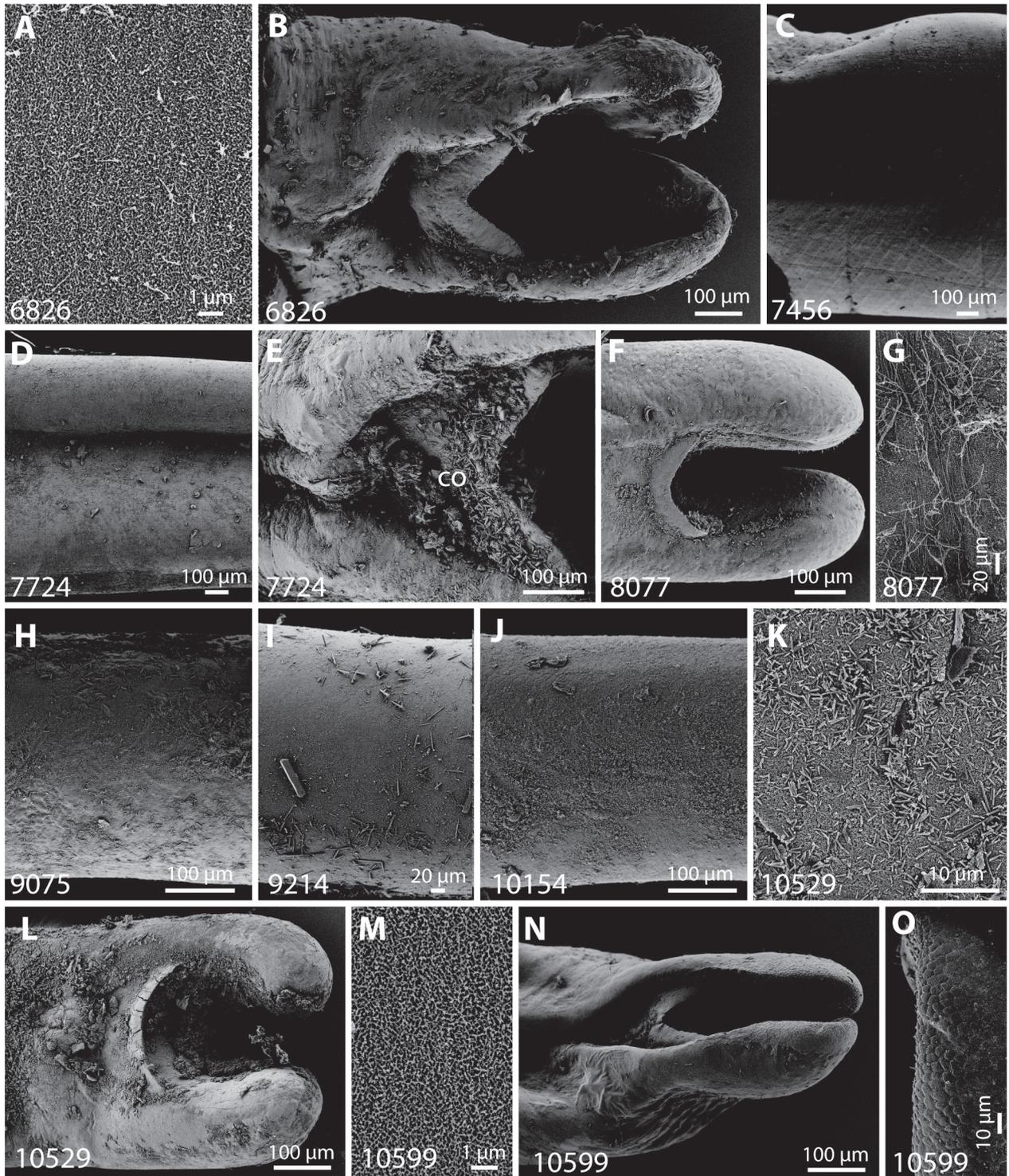
**Figure 1.** Specimens of *Gordius* sp., ventral view of posterior ends and cuticle in midbody sections. The specimen number is indicated in the lower left of each image. **A, B, G.** Posterior ends with postcloacal crescent (pcc) and cloacal opening (co). The cuticular surface (**C–F, H**) is in some cases covered with encrusting material (**C**) or dirt (**F, H**). Diagonal lines are created by muscular fibers underlying the cuticle.



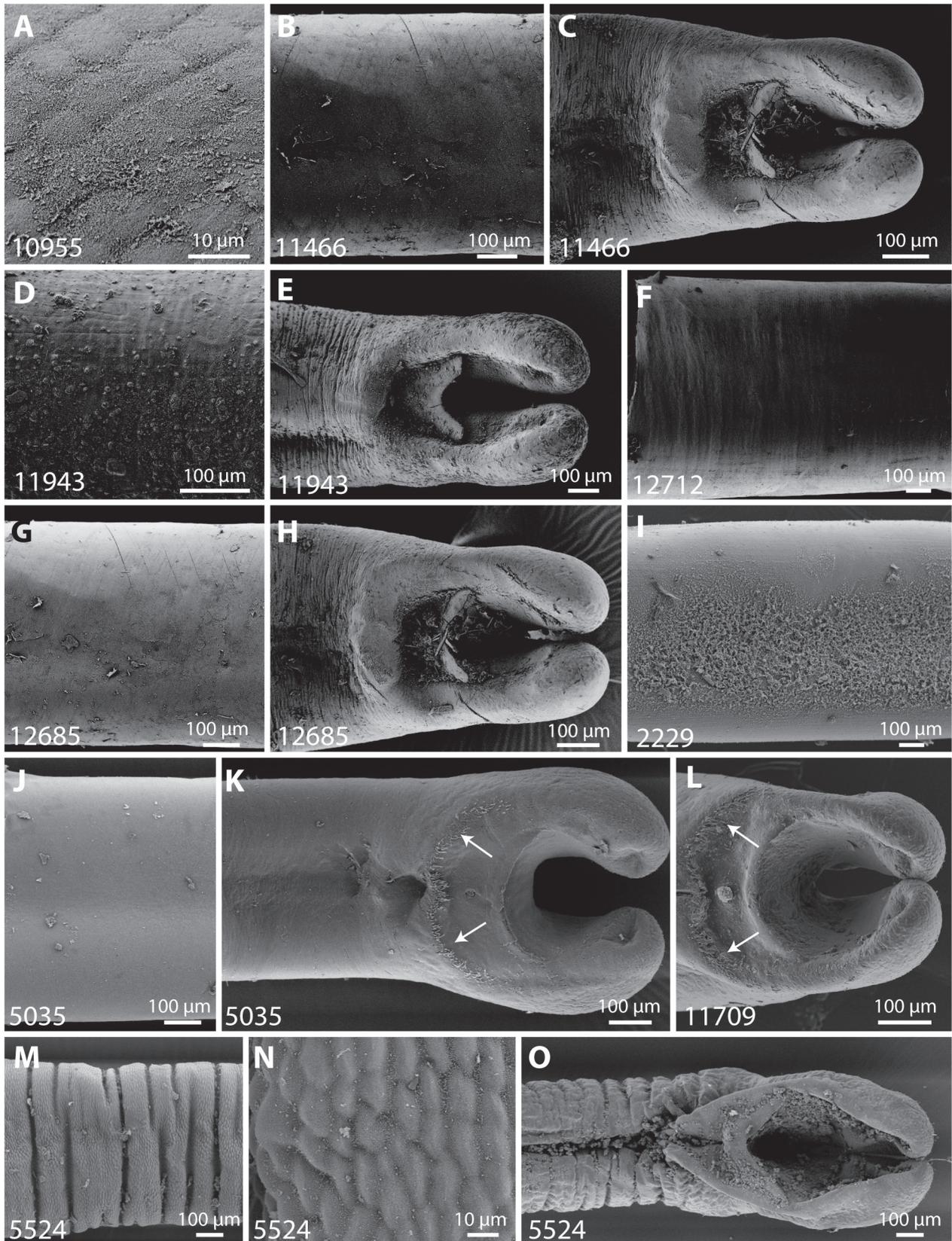
**Figure 2.** Specimens of *Gordius* sp., ventral view of posterior ends and cuticle in midbody sections. The specimen number is indicated in the lower left of each image. **A–E, G–I.** Cuticular surface. This is either smooth (**A–C, G**), has keel-like structures (**D**) or areoles (**E, H, I**). **F, J, K.** Posterior ends with postcloacal crescent (pcc) and cloacal opening (co). The posterior end of specimen V4611 has a fringed rim around the cloacal opening (**J, K**).



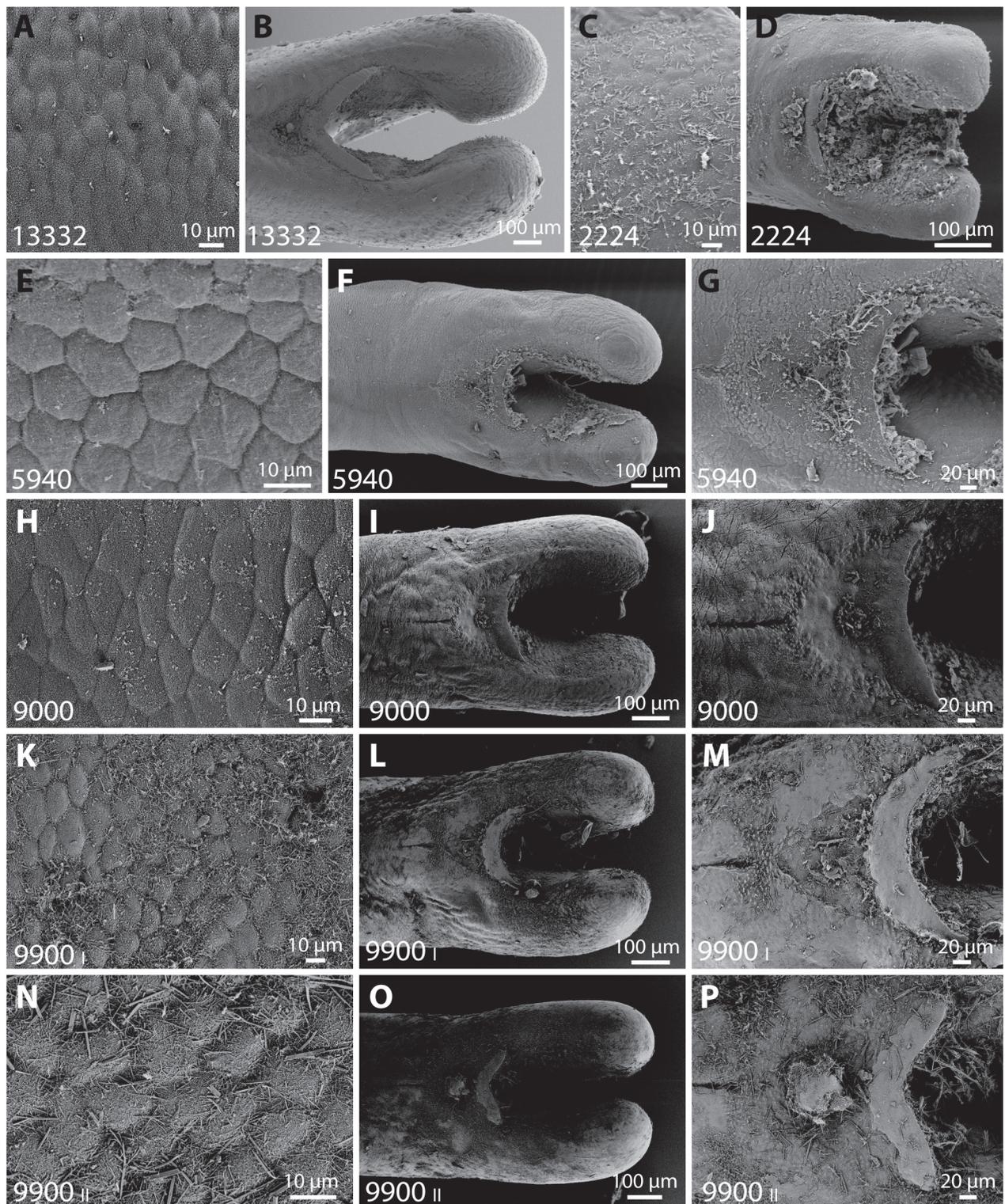
**Figure 3.** Specimens of *Gordius* sp., ventral view of posterior ends and cuticle in midbody sections. The specimen number is indicated in the lower left of each image. **A, D, E, G, H, J–L.** Cuticular surface, see text for explanation. **B, F, I, M.** Posterior ends with postcloacal crescent and cloacal opening (co). **C.** Magnification of short bristles on the tail lobes of V4981.



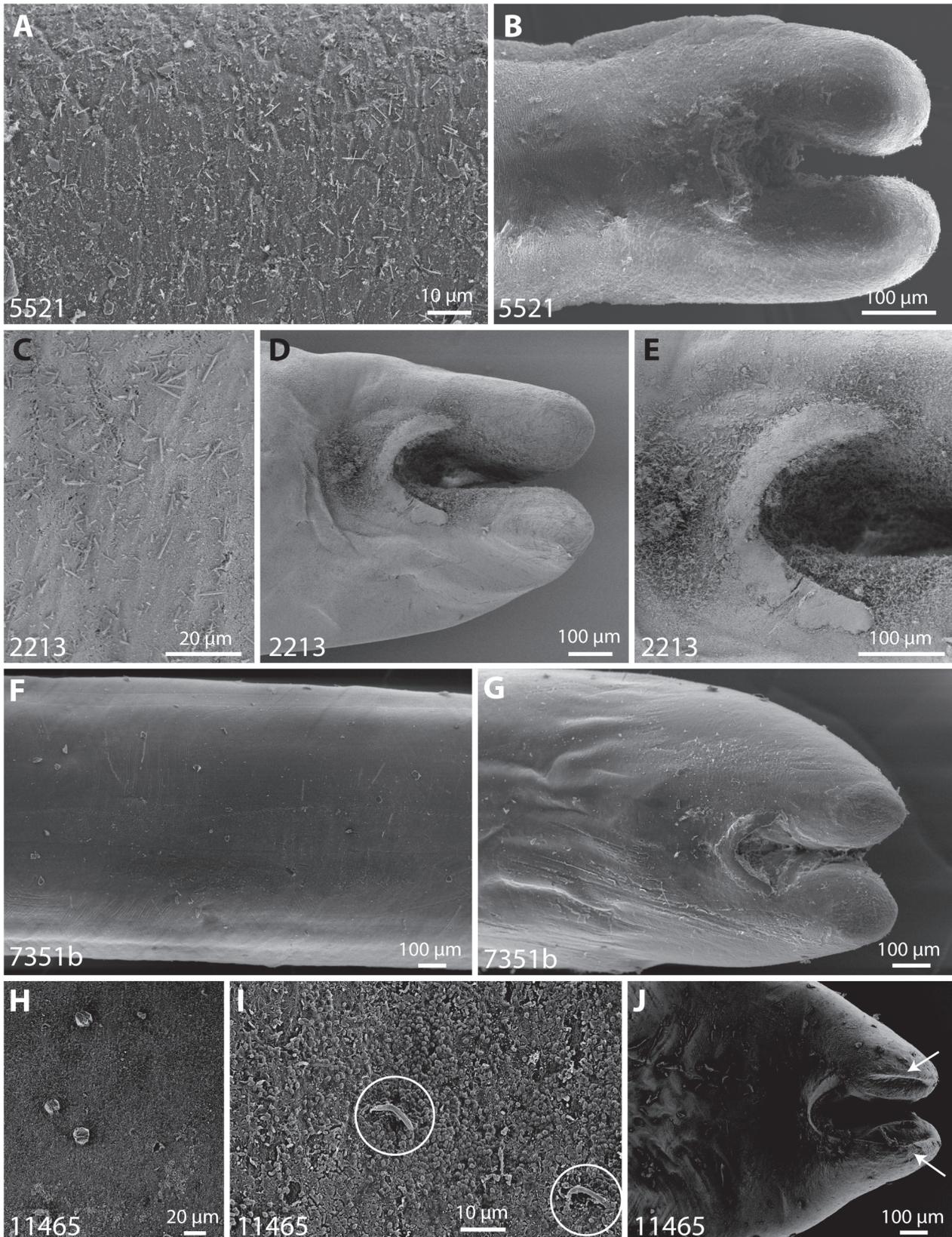
**Figure 4.** Specimens of *Gordius* sp., ventral view of posterior ends and cuticle in midbody sections. The specimen number is indicated in the lower left of each image. **A, C, D, G, H–K, M.** Cuticular surface, see text for explanation. **B, E, F, L, N.** Posterior ends with postcloacal crescent and cloacal opening (co). **O.** Areoles on the tail lobes of V10599.



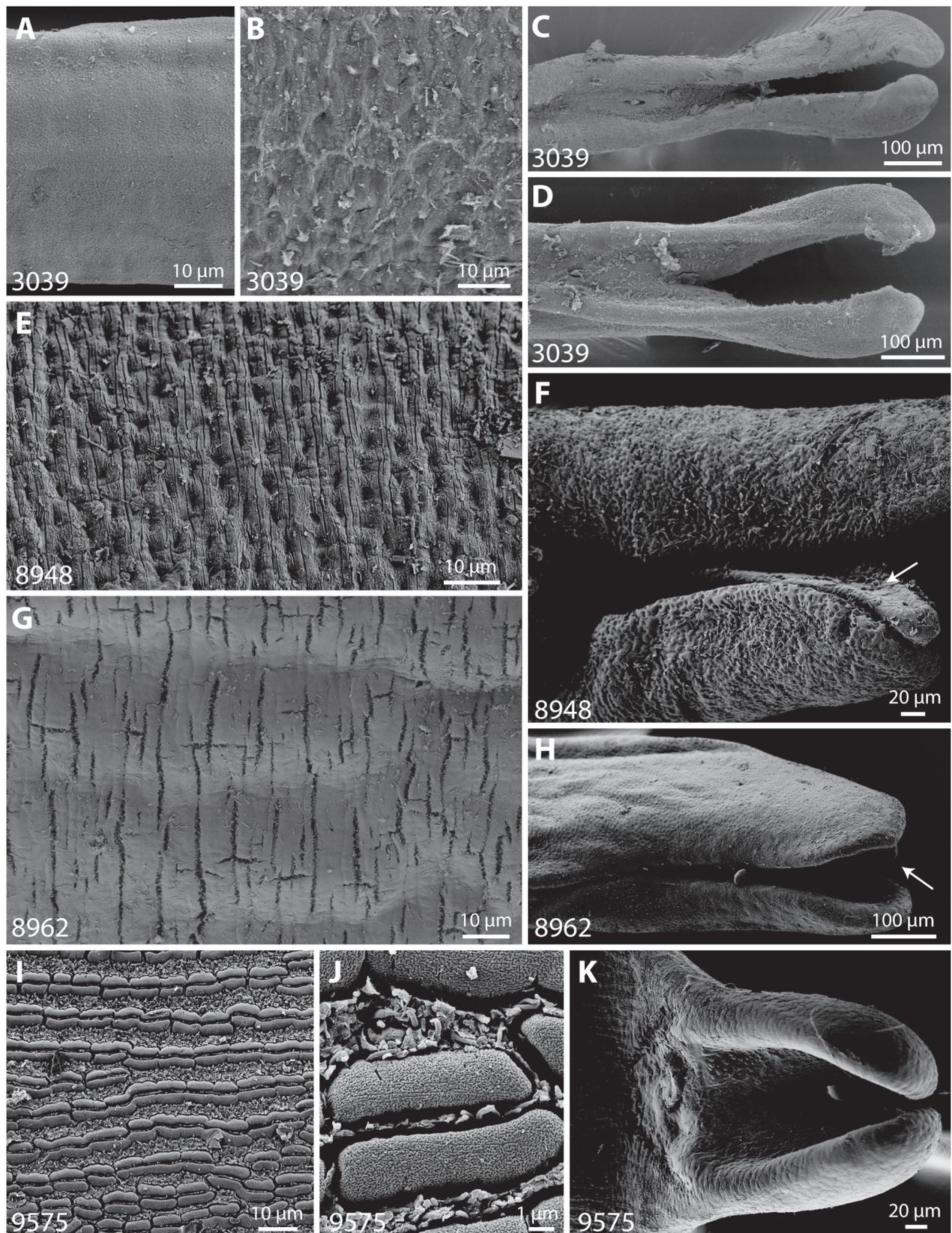
**Figure 5.** A–F. Specimens of *Gordius* sp., ventral view of posterior ends and cuticle in midbody sections. G, H. *Gordius aquaticus* (V12685), cuticle (G) and posterior end (H). I. Cuticular surface of *G. longissimus* (V2229). J, K. Cuticle (J) and posterior end (K) of *G. paranensis* (V5035). Note row of bristles on the posterior end (arrows in K). L. Posterior end of *G. paranensis* (V11709), arrows as in K. M–O. Cuticle (M, N) and posterior end (O) of *G. plicatissimus*. The specimen number is indicated in the lower left of each image.



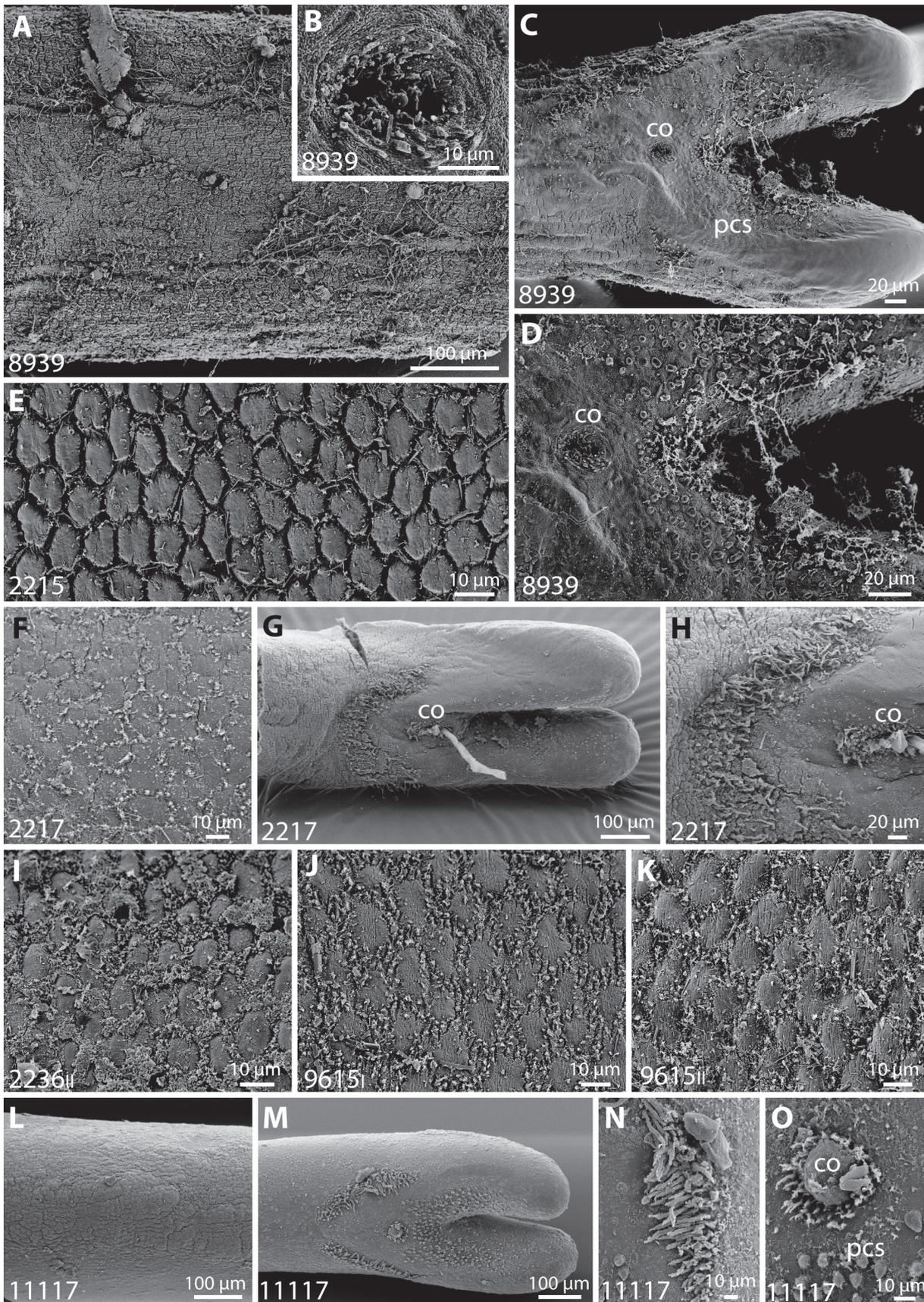
**Figure 6.** A, B. Cuticle (A) and posterior end (B) of *Gordius plicatissimus* (V13332). C–G. *Gordius setiger*, specimens V2224 (C, D) and V5940 (E–G). H–P. *Gordius cf. setiger*, specimens V9000 (H–J), V9900 male I (K–M) and V9900 male II (N–P).



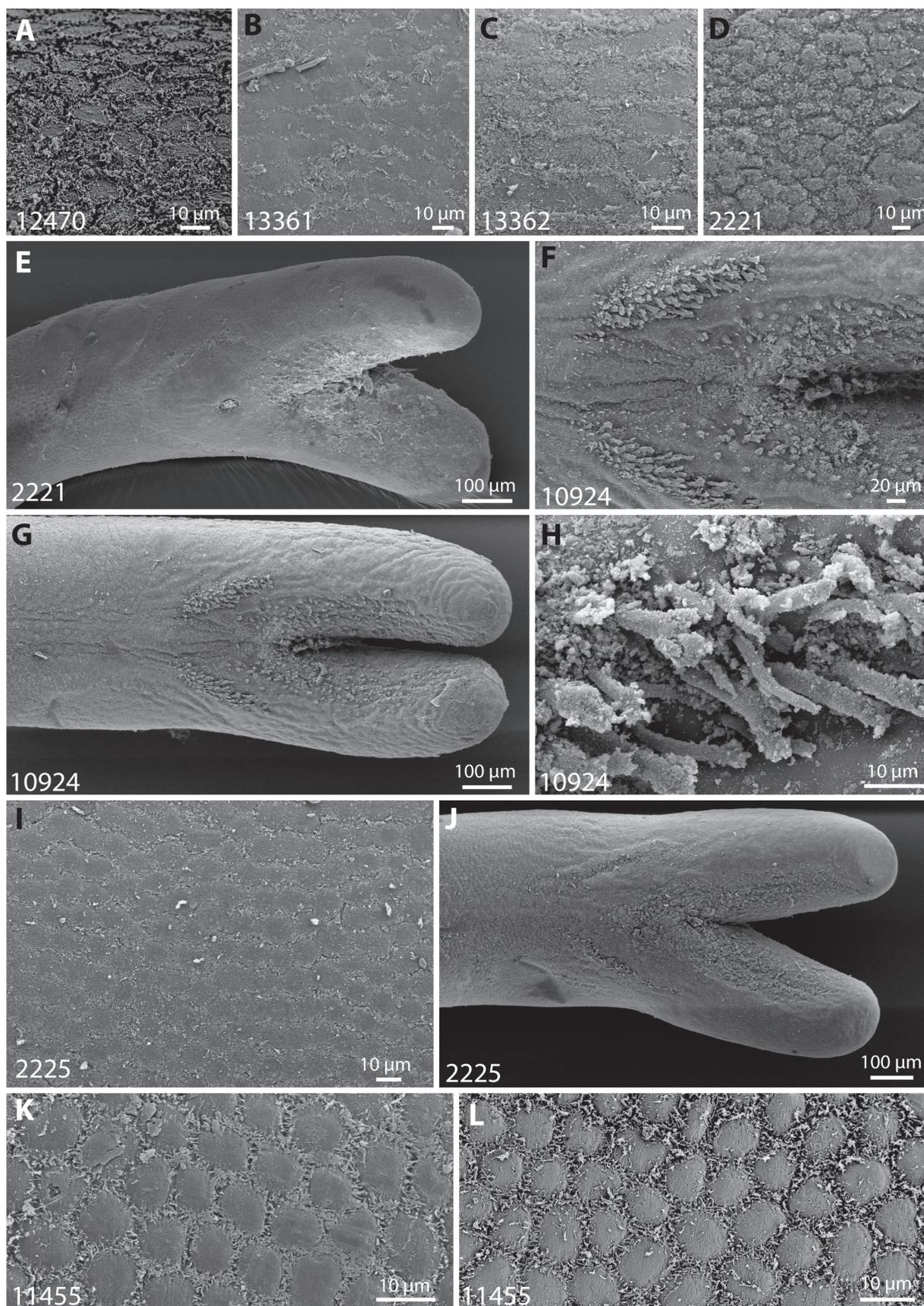
**Figure 7.** A, B. Cuticle (A) and posterior end (B) of *Gordius undulatus* (V5521). C–J. *Acutogordius cf. acuminatus*, specimens V2213 (C–E), V7351b (F, G) and V11465 (H–J). Note longitudinal crests or keels on tail lobes of V11465 (arrows in J) and scattered bristle-like structures on the cuticle (circles in I) of the same specimen (see text).



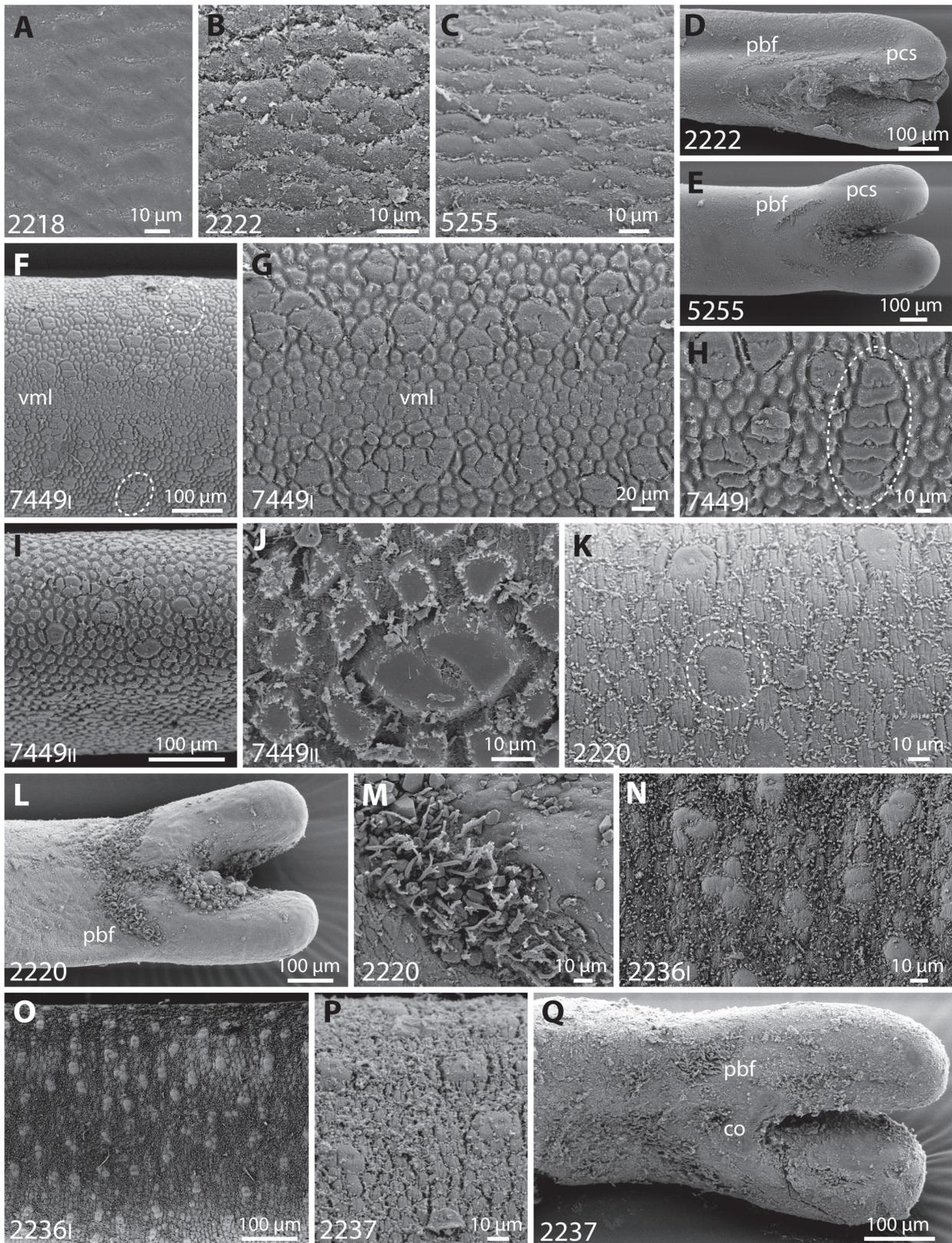
**Figure 8.** A–H. *Paragordius* sp., details of cuticle (A, B, E, G) and shape of the posterior ends of males (C, D; V3039) and females (F; V8948 and H; V8962). Arrow in F and H indicates the dorsal lobe. I–K. *Beatogordius* sp., longitudinal row of areoles (I), magnification of areoles (J) and posterior end (K).



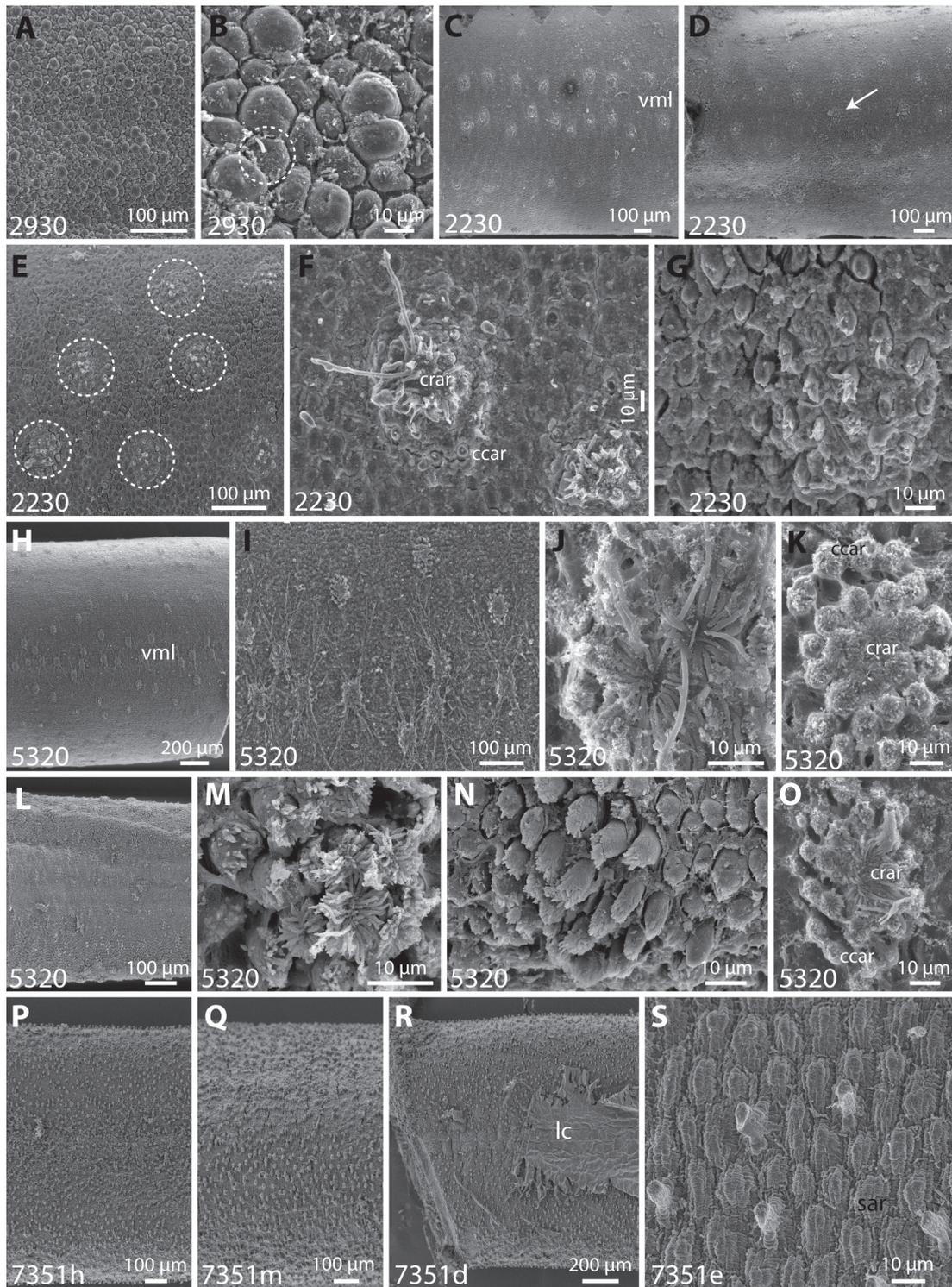
**Figure 9.** A–D. *Beatogordius* sp., cuticular pattern (A), detail of the cloacal opening (B), overview (C) and detail (D) of the posterior end, showing the cloacal opening (co) and the postcloacal spines (pcs). E–O. *Gordionus* sp., cuticular pattern and posterior ends of specimens V2215, V2217, V2236 (II), two specimens of V9615 (I and II) and V11117. H shows detail of precloacal bristles anterior to the cloacal opening (co). N shows precloacal bristles, O shows the cloacal opening (co) and some postcloacal spines (pcs).



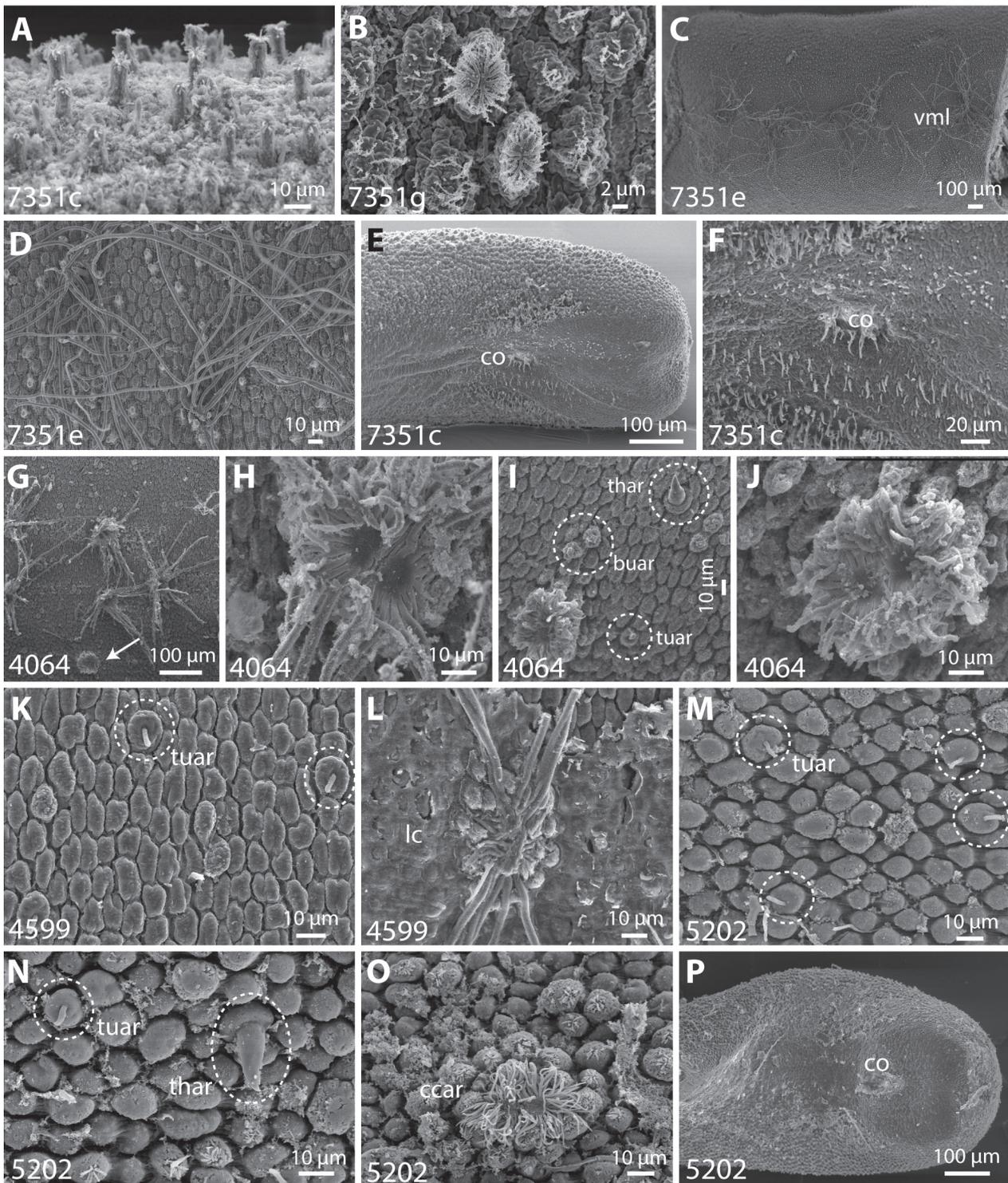
**Figure 10.** A–C. Cuticular surface of three specimens of *Gordionus* sp. (V12470, V13361, V13362). D–H. *Gordionus scaber*. Cuticular surface (D) and posterior end (E) of V2221, posterior end (G), magnification (F) and detail from the precloacal bristles (H) from V10924. I–L. *Gordionus violaceus*. Cuticular surface (I) and posterior end (J) of V2225, cuticular structure of two females of V11455 (K, L).



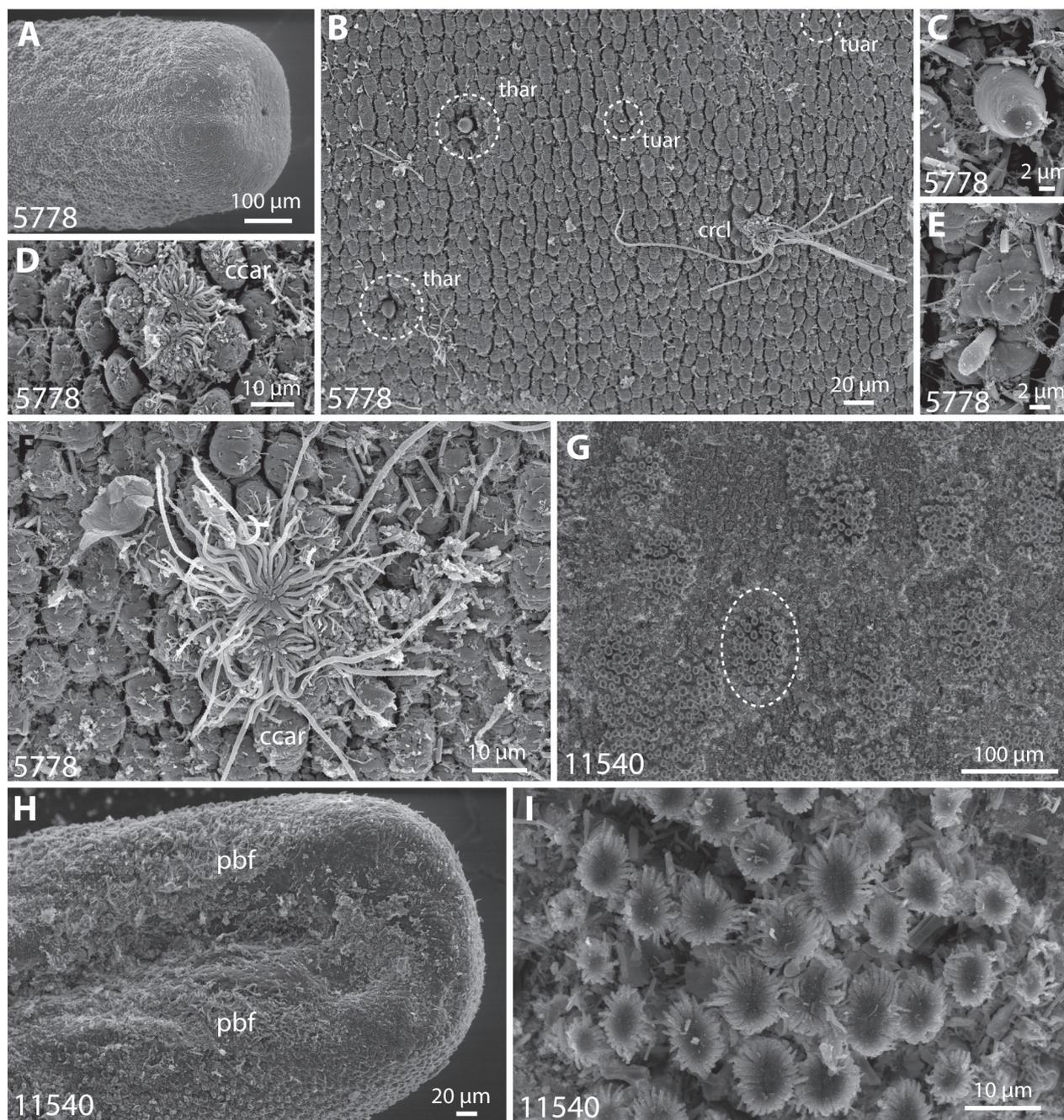
**Figure 11.** A–E. *Gordionus wolterstorffii*, cuticular surface (A–C) and posterior end (D, E) in specimens V2218, V2222 and V5255. Posterior end with precloacal bristlefields (pbf) and postcloacal spines (pcs). F–H. *Paragordionus vej dovskiy*, pattern of areoles on the cuticle. F. Overview with ventral midline (vml) and two types of areoles, simple areoles and larger megareoles in clusters (two of these are encircled). G. Magnification from F. H. Large cluster (encircled) of megareoles. I, J. *Paragordionus dispar* with smaller clusters of megareoles. K–Q. *Parachordodes tolosanus*. K. Superareoles, which are characteristic for *Parachordodes*, are encircled. L. Posterior end of V2220 with precloacal bristlefields (pbf), M. Magnification of precloacal bristlefields. N, O. Cuticular structure of V2236I with superareoles. P. Cuticle of V2237, Q. Posterior end of V2237 with precloacal bristlefields (pbf) and cloacal opening (co).



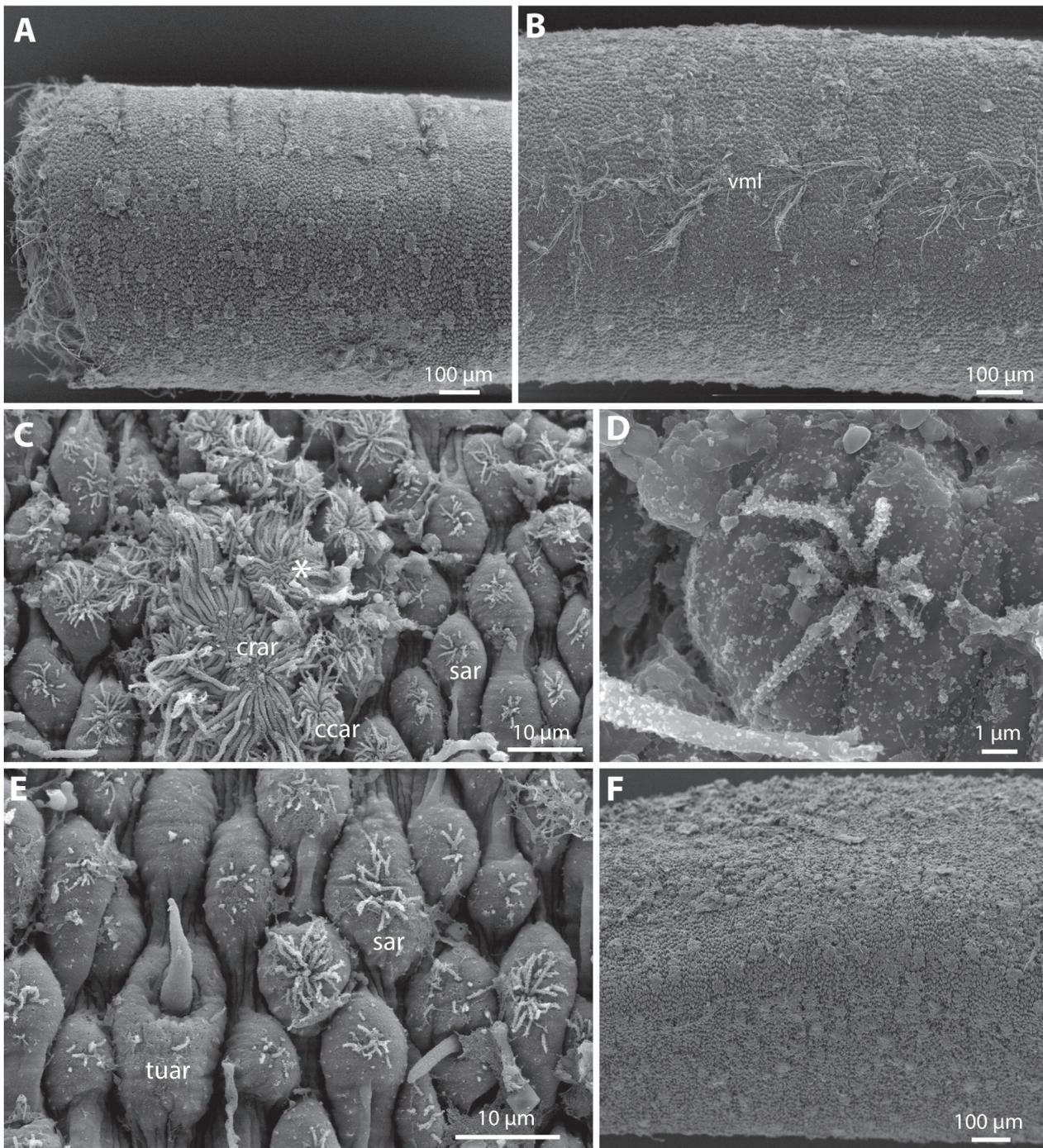
**Figure 12.** A, B. *Pseudochordodes meridionalis*, V2930. Cuticular structure in low (A) and higher (B) magnification, encircled in B is a tubercle areole. C–O. *Chordodes* sp., specimen V2230 (C–G) was formerly designated as holotype of *C. pilosus*. C. Overview onto the ventral side. Note elevated clusters on both sides of the ventral midline (vml). D. Overview onto the lateral side. Note scattered elevated clusters (arrow indicates one of these clusters). E. Higher magnification shows that the clusters are composed of elevated areoles. F. Clusters of crowned areoles (crar) and circumcluster areoles (ccar) are strongly eroded. Image shows a cluster from next to the ventral midline which includes crowned areoles with long apical filaments. G. Strongly eroded cluster from the lateral body side. H–O. Specimen V5320; H–K female specimen, L–O male specimen. H, I. Arrangement of crowned areole clusters with long filaments next to the ventral midline (vml). J. Two central crowned areoles within a cluster. K. Crowned areoles (crar) surrounded by circumcluster areoles (ccar). L–O. Cuticle and areoles in the male, see female for abbreviations. P–S. *Chordodes brasiliensis*, different specimens of number V7351. Cuticle shows scattered elevated crowned areoles among simple areoles (sar). Specimen V7351d has part of the cuticle covered by rests of the larval cuticle (lc).



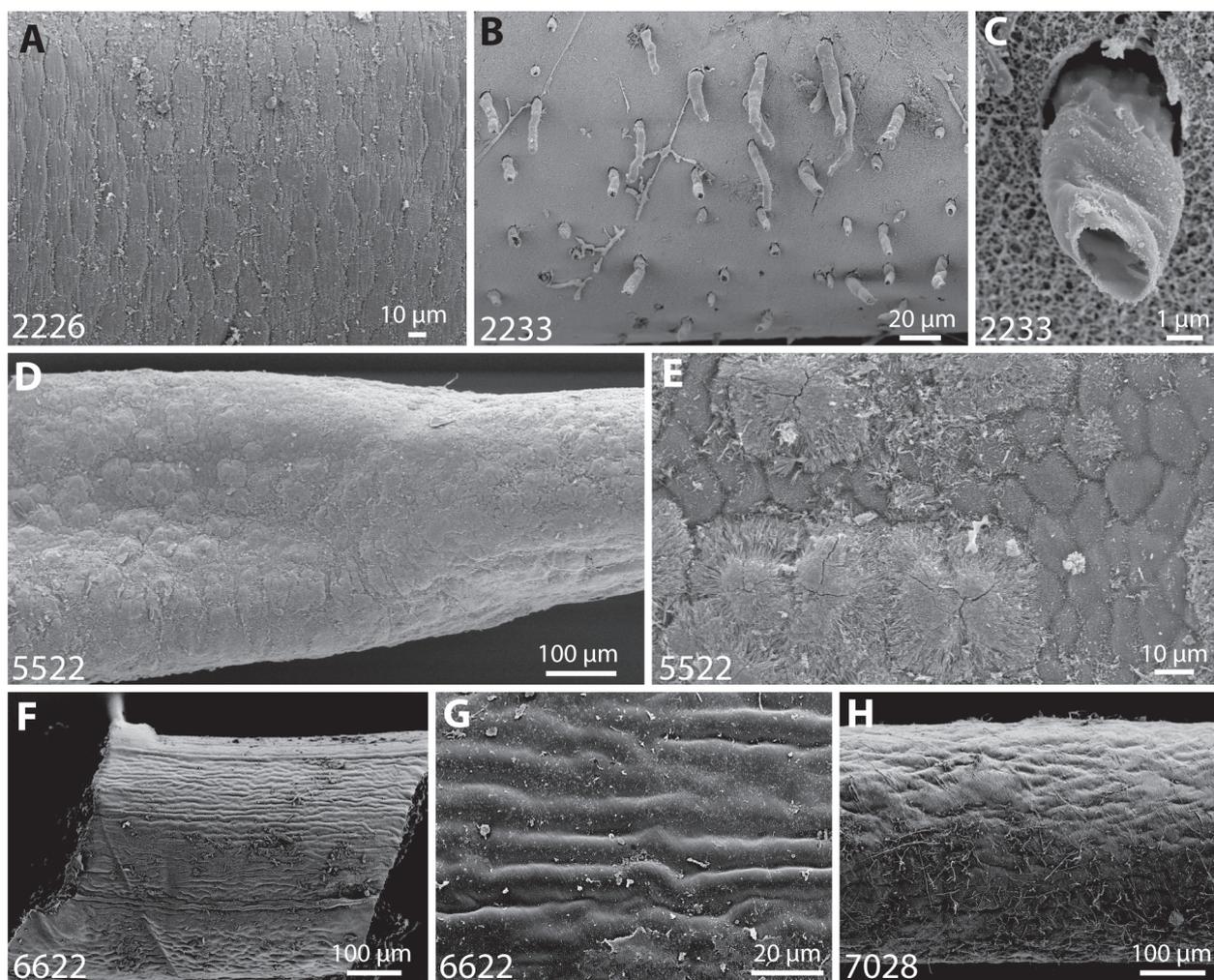
**Figure 13.** A–F. *Chordodes brasiliensis*, different specimens of number V7351. A, B. Higher magnification of crowned areoles. C, D. Crowned areoles with long apical filaments along the ventral midline (vml) in female specimen V7351e. E, F. Posterior end of a male (V7351c) with cloacal opening (co) and bristles around and in the region of the opening. G–J. *C. jandae* or *C. skorikovi*. G. Cuticular structures on the ventral side showing crowned areoles clusters with long filaments along the ventral midline and adjacent transition to clusters with short filaments (arrow). H, J. Magnification of crowned areoles. I. Different types of areoles: besides simple and crowned areoles (shown but not marked) there are bulging areoles (buar), thorn areoles (thar) and tubercle areoles (tuar). K, L. *Chordodes japonensis*, cuticular structure with tubercle areoles (tuar, encircled). Part of the cuticle is covered with larval cuticle (lc). M–P. *Chordodes cf. japonensis*, cuticle with different types of areoles: tubercle areoles (tuar, encircled in M), thorn areoles (thar) and circumcluster areoles (ccar) surrounding crowned areoles. Posterior end with cloacal opening (co).



**Figure 14.** A–F. *Chordodes* cf. *joyeuxi*. A. Female posterior end with terminal cloacal opening. B. Overview on cuticle with different types of areoles: tubercle areoles (tuar), thorn areoles (thar) and cluster of crowned and circumcluster areoles (crcl) (here a cluster with long filaments is present). C. Magnification of thorn areoles. E. Magnification of tubercle areoles. D, F. Clusters of central crowned areoles and circumcluster areoles (ccar). G–I. *Chordodes polycoronatus*. G. Abundant large crowned areole clusters (one cluster is encircled). H. Posterior end with precloacal bristlefields (pbf). I. Magnification showing similar structure of areoles in the clusters.



**Figure 15.** *Chordodes jelkae* sp. n.; A–E from holotype (V10960I). **A.** Overview on the cuticle from the lateral side showing elevated crowned areole clusters. **B.** Overview of the cuticle from the ventral side showing clusters with crowned areoles with long filaments along the ventral midline (vml). **C.** Fine structure of simple areoles (sar), crowned areoles (crar) and circumcluster areoles (ccar). Some areoles may be either type (\*). **D.** Magnification of simple areoles. **E.** Arrangement of simple areoles (sar) and structure of tubercle areoles (tuar). **F.** Cuticle from second specimen (V10960II).



**Figure 16.** Undetermined specimens. **A.** Areoles in V2226. **B, C.** Tubular structures in V2233. **D, E.** Encrusting material on V5522. **F, G.** Cuticle in V6622. **H.** Cuticle in V7028.

## Acknowledgements

Two persons have helped very much during this project. Vladimira Oldenburg made most of the SEM investigation during a student project. Helma Roggenbuck took care of handling the collection and updating everything according to new results. Many thanks to both of them.

## References

- Bolek MG, Schmidt-Rhaesa A, Hanelt B, Richardson DJ (2010) Redescription of the African *Chordodes albibarbatus* Montgomery, 1898, and description of *Chordodes janovyi* n. sp. (Gordiida, Nematomorpha) and its non-adult stages from Cameroon, Africa. *Zootaxa* 2631: 36–50.
- Camerano L (1897) *Monografia dei Gordii*. Memoire della Reale Accademia delle Science di Torino 47: 339–415.
- Carvalho JCM, Feio JLA (1950) Sobre alguns Gordiáceos do Brasil e da Republica Argentina (Nematomorpha, Gordioidea). *Anais da Academia Brasileira de Ciências* 22: 193–216.
- Creplin CH (1847) *Chordodes parasitus*, ein Schmarotzerwurm aus einer Heuschrecke. *Notizen aus dem Gebiete der Natur- und Heilkunde* 3: 161–166.
- De Miralles DB, De Villalobos LC (1996) Especies de *Neochordodes* de la Argentina (Gordiacea, Nematomorpha). *Iheringia, Sér. Zool.* 81: 145–150.
- De Miralles DB, De Villalobos LC (1998) Dos nuevas especies y una nueva combinación para el género *Acutogordius* (Nematomorpha: Gordiidae). *Neotropica* 44: 75–79.
- De Villalobos LC, Schmidt-Rhaesa A, Zanca F (2003) Revision of the genus *Beatogordius* (Gordiida, Nematomorpha). II. South American species with description of two new species. *Memorias do Instituto Oswaldo Cruz* 98: 115–128. doi: 10.1590/S0074-02762003000100015
- De Villalobos C, Zanca F, Ibara-Vidal H (2005) Redescription and new records of freshwater Nematomorpha (Gordiida) from Chile, with the description of two new species. *Revista Chilena de Historia Natural* 78: 673–686. doi: 10.4067/S0716-078X2005000400007
- De Villalobos C, Zanca F, Salas L (2008) Especies de Gordiida (Nematomorpha) en la Provincia de Catamarca. *Ciencia* 3: 47–56.
- De Villalobos C, Zanca F, Schmidt-Rhaesa A (2004) New data on South American species of *Chordodes* (Nematomorpha). *Arquivos do Museu Nacional, Rio de Janeiro* 62: 375–386.
- Hanelt B, Bolek MG, Schmidt-Rhaesa A (2012) Going solo: Discovery of the first parthenogenetic gordiid (Nematomorpha: Gordiida). *PLoS ONE* 7: e34472. doi: 10.1371/journal.pone.0034472

- Hanelt B, Thomas F, Schmidt-Rhaesa A (2005) Biology of the Phylum Nematomorpha. *Advances in Parasitology* 59: 243–305. doi: 10.1016/S0065-308X(05)59004-3
- Heinze K (1933) Revision von *Gordius flavus* Linstow 1906 und *Gordius flavus* G.W. Müller 1927. *Zoologischer Anzeiger* 105: 106–109.
- Heinze K (1934) Zur Systematik der Gordiiden. *Zoologischer Anzeiger* 106: 189–192.
- Heinze K (1935a) Über Gordiiden (Species inquirendae und Neubeschreibungen). *Zoologischer Anzeiger* 111: 23–32.
- Heinze K (1935b) Über das Genus *Parachordodes* Camerano 1897 nebst allgemeinen Angaben über die Familie Chordodidae. *Zeitschrift für Parasitenkunde* 7: 657–678. doi: 10.1007/BF02121327
- Heinze K (1937) Die Saitenwürmer (Gordioidea) Deutschlands. Eine systematisch-faunistische Studie über Insektenparasiten aus der Gruppe der Nematomorpha. *Zeitschrift für Parasitenkunde* 9: 263–344. doi: 10.1007/BF02119887
- Heinze K (1941) Saitenwürmer oder Gordioidea (Nematomorpha). In Dahl F (ed). *Die Tierwelt Deutschlands und der angrenzenden Meeresgebiete*. Gustav Fischer Verlag, Jena, 1–78.
- Heinze K (1952) Über Gordioidea, eine systematische Studie über Insektenparasiten aus der Gruppe der Nematomorpha. *Zeitschrift für Parasitenkunde* 15: 183–202.
- Inoue I (1952) On a new species of *Chordodes* (Gordiacea) from Japan. *Annotationes Zoologicae Japonensis* 25: 400–402.
- Janda J (1894) Beiträge zur Systematik der Gordiiden. I. Die Gordiiden Galiziens. - II. Ueber das Genus *Chordodes*. *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere* 7: 595–612.
- Kintsurashvili N, Schmidt-Rhaesa A, Gorgadze O (2011) *Chordodes parabipilus* (Nematomorpha: Gordiida), a new species of horsehair worms from Georgia. *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* 46: 235–241.
- Möbius K (1855) *Chordodes pilosus*, ein Wurm aus der Familie der Gordiaceen. *Zeitschrift für Wissenschaftliche Zoologie* 6: 428–431.
- Müller GW (1926) Über Gordiaceen. *Zeitschrift für Morphologie und Ökologie der Tiere* 7: 134–220. doi: 10.1007/BF00540721
- Römer F (1895) Die Gordiiden des Naturhistorischen Museums in Hamburg. *Zoologische Jahrbücher. Abteilung für Systematik* 8: 790–803.
- Römer F (1896) Beitrag zur Systematik der Gordiiden. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* 23: 249–295.
- Sato T, Watanabe K, Tamotsu T, Ichikawa A, Schmidt-Rhaesa A (2012) Diversity of nematomorphs and cohabiting nematode parasites in riparian ecosystems around Kii Peninsula, Japan. *Canadian Journal of Zoology* 90: 829–838. doi: 10.1139/z2012-048
- Schmidt-Rhaesa A (1997) Nematomorpha. In: Schwoerbel J, Zwick P (Eds) *Süßwasserfauna Mitteleuropas*. Gustav Fischer Verlag, Stuttgart, 1–124.
- Schmidt-Rhaesa A (2001) Variation of cuticular characters in the Nematomorpha: studies on *Gordionus violaceus* (Baird, 1853) and *G. wolterstorffii* (Camerano, 1888) from Britain and Ireland. *Systematic Parasitology* 49: 41–57. doi: 10.1023/A:1010600200665
- Schmidt-Rhaesa A (2002a) Are the genera of Nematomorpha monophyletic taxa? *Zoologica Scripta* 31: 185–200. doi: 10.1046/j.1463-6409.2002.00073.x
- Schmidt-Rhaesa A (2002b) Australian species of *Chordodes* (Nematomorpha) with a description of two new species, remarks on the genus and its life history. *Journal of Natural History* 36: 1569–1588. doi: 10.1080/00222930110059664
- Schmidt-Rhaesa A (2004) Japanese horsehair worms (Nematomorpha) from the Lake Biwa Museum. *Species Diversity* 9: 97–107.
- Schmidt-Rhaesa A (2005) Morphogenesis of *Paragordius varius* (Nematomorpha) during the parasitic phase. *Zoomorphology* 124: 33–46. doi: 10.1007/s00435-005-0109-z
- Schmidt-Rhaesa A (2010) Considerations on the genus *Gordius* (Nematomorpha, horsehair worms), with the description of seven new species. *Zootaxa* 2533: 1–35.
- Schmidt-Rhaesa A (2011) *Beatogordius chinensis* (Nematomorpha, Gordiida), a new species from China. *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* 46: 243–248
- Schmidt-Rhaesa A (2013) Nematomorpha. In: Schmidt-Rhaesa A (Ed.) *Handbook of Zoology*. De Gruyter, Berlin, 29–145.
- Schmidt-Rhaesa A, Brune S (2008) Description of one known and three new *Chordodes* species (Nematomorpha) parasitizing praying mantids (Mantoptera) in Malaysia, with a discussion of sexual dimorphism in *Chordodes*. *Zoosystematics and Evolution* 84: 57–66. doi: 10.1002/zoos.200700014
- Schmidt-Rhaesa A, Cieslak A (2008) Three new species of *Paragordionus* and *Gordionus* (Nematomorpha, Gordiida) from Spain and Turkey, with comments on the taxon *Semigordionus*. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* 105: 13–22.
- Schmidt-Rhaesa A, De Villalobos LC, Zanca F (2008) Summary of *Chordodes* species (Nematomorpha, Gordiida), with a discussion of their diagnostic characters. *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* 44: 37–114.
- Schmidt-Rhaesa A, Geraci CJ (2006) Two new species of *Acutogordius* (Nematomorpha), with a brief review of literature data of this genus. *Biodiversity and Systematics* 4: 427–433. doi: 10.1017/S1477200006001964
- Schmidt-Rhaesa A, Gusich VV (2010) A new *Gordionus* Müller, 1927 from Switzerland (Nematomorpha, Gordiida). *Revue Suisse de Zoologie* 117: 77–82. doi: 10.5962/bhl.part.117589
- Schmidt-Rhaesa A, Hanelt B, Reeves W (2003) Redescription and compilation of Nearctic freshwater Nematomorpha (Gordiida), with the description of two new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* 153: 77–117. doi: 10.1635/0097-3157(2003)153[0077:RACONF]2.0.CO;2
- Schmidt-Rhaesa A, Kieneke A, Muschiol D, Chung PR (2001) Tubular structures on the cuticle of two *Gordius* species (Nematomorpha, Gordioidea, Gordiidae) from Korea and New Zealand. *Korean Journal of Systematic Zoology* 17: 165–169.
- Schmidt-Rhaesa A, Kristensen P (2006) Horsehair worms (Nematomorpha) from the Baltic Island Bornholm (Denmark), with notes on the biology of *Gordius albopunctatus*. *Journal of Natural History* 40: 495–502. doi: 10.1080/00222930600761803
- Schmidt-Rhaesa A, Limatemjen, Yadav AK (2015) *Chordodes combiareolatus*, a new species of horsehair worms (Nematomorpha) from Nagaland, India, with further comments on *Chordodes moutoni*. *Zootaxa* 3925: 202–210. doi: 10.11646/zootaxa.3925.2.3
- Schmidt-Rhaesa A, Prous M (2010) Records of horsehair worms (Nematomorpha) in Estonia, with description of three new species from the genus *Gordius*. *Estonian Journal of Ecology* 59: 39–51. doi: 10.3176/eco.2010.1.03
- Schmidt-Rhaesa A, Sato T (2009) *Gordionus chinensis* (Villot, 1874) and *Gordionus kii* sp.n. (Nematomorpha, Gordiida), new reports of the genus *Gordionus* in Japan. *Species Diversity* 14: 61–67.

- 
- Schmidt-Rhaesa A, Thomas F, Poulin R (2000) Redescription of *Gordius paranensis* Camerano, 1892 (Nematomorpha), a species new for New Zealand. *Journal of Natural History* 34: 333–340. doi: 10.1080/002229300299516
- Schmidt-Rhaesa A, Urabe M (2009) New records of *Gordius* specimens from Japan (Nematomorpha, Gordiida). *Mitteilungen des Zoologischen Museums in Hamburg* 106: 1–6.
- Schmidt-Rhaesa A, Wagner FM (2013) Considerations on the genus *Paragordionus* (Nematomorpha, Gordiida). *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* 47: 173–183.
- Schmidt-Rhaesa A, Yadav A (2013) One new species and a new record of the genus *Chordodes* (Nematomorpha: Gordiida) from North-East India. *Zootaxa* 3693: 293–300. doi: 10.11646/zootaxa.3693.2.10
- Von Siebold CT (1848) Ueber die Fadenwürmer der Insecten (Zweiter Nachtrag). *Entomologische Zeitschrift* 10 (9. Jahrgang): 290–300.
- Zanca F, Schmidt-Rhaesa A (2006) Reinvestigation and new evaluation of representatives from the genera *Semigordionus*, *Euchordodes*, *Pantachordodes*, *Dacochordodes* and *Spinochordodes* (Nematomorpha). *Mitteilungen aus dem Museum für Naturkunde in Berlin, Zoologische Reihe* 82: 170–178. doi: 10.1002/mmnz.200600003
-

# An anocellar polistine wasp (Hymenoptera, Vespidae, Polistinae) from Texas

Volker Lohrmann<sup>1,2</sup>, George C. Waldren<sup>3</sup>, Martin Reiß<sup>2</sup>, Michael S. Engel<sup>4,5</sup>

1 *Übersee-Museum Bremen, Bahnhofplatz 13, D-28195 Bremen, Germany*

2 *Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Invalidenstraße 43, D-10115 Berlin, Germany*

3 *Department of Biology, Utah State University, Logan, Utah 84322, USA*

4 *Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024-5192, USA*

5 *Division of Entomology, Natural History Museum, and Department of Ecology & Evolutionary Biology, 1501 Crestline Drive – Suite 140, University of Kansas, Lawrence, Kansas 66045-4415, USA*

<http://zoobank.org/B3340BF9-B440-4D45-874A-54EFB8F3AF75>

Corresponding author: *Volker Lohrmann* (v.lohrmann@uebersee-museum.de)

## Abstract

Received 26 September 2016

Accepted 15 November 2016

Published 2 December 2016

Academic editor:

*Michael Ohl*

A remarkable teratological female of *Polistes (Fuscopolistes) dorsalis neotropicus* Bequaert, 1940 (Vespidae: Polistinae) is described and illustrated. The specimen lacks all three external dorsal ocelli but is normally developed in almost every other aspect. Additionally, similar findings in other Hymenoptera are briefly discussed, as are the consequences and the reasons that might cause the random loss of ocelli.

## Key Words

teratology

aberration

malformation

morphology

*Polistes dorsalis*

social wasp

paper wasp

## Introduction

In contrast to the multi-lensed compound eyes, the dorsal ocelli of insects are simple lens eyes forming the second visual system. Externally they consist of a single, usually round or oval aperture lens while internally hundreds of photoreceptors converge into a small suite of neurons targeted to neuropils (Berry et al. 2007, and citations therein). Many hypotheses have been proposed regarding the function of the ocelli, some of which suggest these simple eyes may play a direct or indirect role in a wide range of physiological processes, such as light response, flight stabilization, circadian rhythm, foraging, orientation, and navigation (e.g., Taylor 1981a, 1981b, Rence et

al. 1988, Schuppe and Hengstenberg 1993, Warrant 2006, Warrant et al. 2006, Schwarz et al. 2011a, 2011b, Viollet and Zeil 2013). Despite their obvious importance, ocelli have been reduced in number, size, and form in many groups of insects, e.g., Lepidoptera (if present, no more than two ocelli: Kristensen 2003), Coleoptera (most species lack ocelli, but if present, then never more than two: Gillott 2005), and Miridae (Hemiptera) (except for the basal Isometopinae, all members lack ocelli outright: Ferreira et al. 2015). Indeed, the loss of ocelli has occurred numerous times in various insect lineages, and in some there have been apparent secondary re-acquisitions of ocelli-like, photosensitive areas or lenses, but for which direct homology to true ocelli has not been conclusively

established (e.g., when present, the ‘ocelli’ or ‘ocelloids’ of worker termites: Engel et al. 2009).

As in most other insects, Hymenoptera usually have three slightly oval, convexly rounded dorsal ocelli: one median (anterior) and two lateral (posterior) (Huber and Sharkey 1993). But this general pattern has been modified multiple times within the order. For example, the number of ocelli has been reduced in several groups within Formicidae (most workers) or females of different mutillid subfamilies (Brothers 1975), while the ocelli have been morphologically modified in others, such as the hemispherical or gibbous forms of some Larrinae and Bembicini (apoid wasps) (Bohart and Menke 1976) or the greatly enlarged forms found in many nocturnal genera of bees and wasps e.g., *Megalopta* or *Apoica* (e.g., Kerfoot 1967, Warrant et al. 2006).

As with many anatomical structures, there are at times developmental aberrations or malformations that impact the ocellar system (e.g., Alfonsus 1931, Ohtani 1977). With respect to the dorsal ocelli there are a number of different malformations reported in the literature, e.g., supernumerary anterior or posterior ocelli (e.g., Tussac and Balazuc 1991, Engel et al. 2014, Lohrmann and Engel 2015); the duplication of a complete ocellar triangle (Tussac and Balazuc 1991); the reduction of one, two, or all three ocelli (e.g., Tussac and Balazuc 1991); or underdevelopment and misplacement of ocelli (e.g., Hopwood 2007, Gibbs 2010). Any of these developmental anomalies must certainly have dramatic consequences on the visual system of the individuals involved, and thereby on other aspects of the functional biology of the organism (e.g., impacts on flight, orientation, light sensitivity). Unfortunately, the study of such aberrations has been scarcely systematic and experimental manipulation of or functional investigation into these are virtually absent aside from earlier work in Orthoptera and Hymenoptera (e.g., Schremmer 1950, Schricker 1965, Taylor 1981a, 1981b, Schwarz et al. 2011a). Nonetheless, the documentation of these aberrant morphologies when they occur in sampled individuals that were seemingly undergoing typical lives is of interest and potential future value for understanding the use of ocelli and impacts of their loss or alteration.

In this context it is interesting to note that while recently discussing a malformed quadriocellar scoliid wasp (Lohrmann and Engel 2015), GCW mentioned the discovery of an anocellar female of a eusocial paper wasp (Vespidae: Polistinae: Polistini), which is described, illustrated, and discussed here.

## Materials and methods

Measurements were taken using a Zeiss SteREO Discovery.V20 combined with an ocular micrometer. The photographs were captured with a Nikon D800 digital camera with a Nikon AF-S Micro-NIKKOR 60 mm 1:2.8G ED lens in combination with the software programs Helicon Remote, Adobe Lightroom und Helicon Focus Pro. The

illustrations have been cleaned with Adobe Photoshop. The specimen, which is deposited in the entomological collection of the Übersee-Museum Bremen (UMB), was collected and identified by GCW and the identification was confirmed by Matthias Buck (Edmonton).

## Systematics

### *Polistes (Fuscopolistes) dorsalis neotropicus* Bequaert, 1940

‘Anocellar Deformity’

Figs 1–4

**Material.** ♀; USA, TX, Randall Co., Palo Duro Canyon, (34°56’37” N, 101°39’39” W), 21.viii.2008, F. Cliff Camp, G.C. Waldren (UMB).

**Measurements.** Total body length: 14.0 mm; head width: 3.2 mm; forewing length: 11.5 mm; hind wing length: 8.7 mm; mesoscutal width: 2.4 mm.

**Descriptive notes.** The female specimen, which seems to be normal in almost every other respect, shows a remarkably deformed head (Figs 3, 4). Most notably it completely lacks all three external dorsal ocelli. Additionally, the vertex shows a longitudinal median impression, giving the head a heart-like shape when seen from the front. The deepest point of the ocular sinus is shifted upwards and the upper end of the compound eye is slightly elongate mesally. As a result, the upper interorbital distance is much shorter than in normally developed specimens (UID : LID = 0.43 vs. 0.94; compare Figs 3 and 5). Finally, the lower inner orbits are rather moderately converging ventrally ( $\alpha = 11^\circ$  vs.  $25^\circ$ ), and the median keeled groove on the frons is lacking. However, this specimen is perfectly bilaterally symmetrical and shows no other malformations, nor any traces of stylopisation or other parasitic infestation.

**Comments.** *Polistes dorsalis* (Fabricius, 1775) is currently classified into five subspecies and occurs throughout the southern half of the US to Costa Rica (Buck et al. 2008). Similar specimens from the South Central US, the great majority of which without or with less well-defined mesoscutal stripes, are usually assigned to *P. dorsalis neotropicus* Bequaert, 1940 – a subspecies first described from Honduras. The present specimen belongs to this subspecies as it is currently circumscribed, although it remains to be determined by future work whether *P. d. neotropicus* should be further restricted, perhaps excluding Nearctic occurrences (Matthias Buck pers. comm.).

## Discussion

There are a number of similar findings of malformed Hymenoptera reported in the literature, among bees in par-



**Figures 1–6.** *Polistes (Fuscopolistes) dorsalis neotropicus* Bequaert, 1940. **1–4.** Anocellar female from Texas. **1.** Habitus in dorsal view. **2.** Habitus in lateral view. **3.** Head in anterior view. **4.** Ocellar area in dorsal view. **5.** Normal female from South Carolina; head in anterior view. Abbreviations: **LID** = Lower interorbital distance; **UID** = Upper interorbital distance. Photos: **1–4.** Matthias Haase. **5.** Used with permission from Buck et al. (2008), Illustration C75.4.

ticular. The most similar aberration has been reported by Tussac and Balazuc (1991, p. 51) who describe and figure a female specimen of *Epyris niger* Westwood, 1832 (Bethyridae) that lacks the dorsal ocelli and which has the dorsal aspects of the compound eyes more closely together but not fused. Like this specimen, most anocellar specimens show additional malformations of the head and it seems that the loss of ocelli often occurs in cyclopic (holoptic) individuals or “half-way” cyclops (specimens with completely or nearly fused compound eyes) (e.g., Alfonsus 1931, Miller 1936, Haydak 1948, Ohtani 1977). However, the absence of “external ocelli” does not necessarily mean that the ocelli are completely lost. Miller (1936) observed that the ocelli of cyclopic bees were well developed except for the external lenses, but are misplaced and concealed by a prominent lobe above the bases of the antennae. Thus, the individual of

*Polistes* reported herein may still have a well-developed set of ‘internal ocelli’, and almost assuredly retains the complete neural architecture for ocelli, despite the lack of externally functional components. However, since the specimen was dry-mounted the impact of dried preservation (e.g., desiccation and shrinking of tissues, etc.) does not allow a closer examination of the innervation through micro computed tomography (micro CT) or histological methods like semi-thin sections.

Whether ‘internal’ ocelli retain some minimal light-detecting function remains unclear. Indeed, the overall impact of such developmental malformations are challenging to predict given that the function of ocelli seem to differ between walking and flying insects (Schwarz et al. 2011a), made worse by the lack of a comprehensive understanding of ocellar function (Berry et al. 2007, Krapp 2009). It has been shown for

other social Hymenoptera that individuals with experimentally blinded ocelli might carry on relative normal lives. Most recently, Schwarz et al. (2011a) demonstrated that the red honey ant (*Melophorus bagoti* Lubbock, 1883) with blinded ocelli could readily orient towards the nest, and despite simultaneously showing that the ocelli contribute to the encoding of the celestial compass used in orientation. Similarly, it has been shown that blinding of one, two, or all three ocelli of honey bee workers (*Apis mellifera* Linnaeus, 1758) resulted in a narrower foraging period rather than in a termination of foraging behavior (Schrickler 1965; but see Renner and Heinzler (1979) for a counter argument), whereas Schremmer (1950) reported that bumble bees (*Bombus lucorum* (Linnaeus, 1761) and *B. hortorum* (Linnaeus, 1761)) with blinded ocelli rarely flew spontaneously. Thus, the impact of blinding the ocelli seems to have diverse, at times seemingly contrary, impacts on the behavior of the individuals involved, with no consistent pattern of diminished function. The *Polistes* reported herein did not show any kind of unnatural behavior before or while getting caught, nor does it seem that it had to deal with dramatically negative consequences caused by the loss of the ocelli.

Brachyptery or aptery is often associated with the reduction or loss of ocelli (e.g., Brothers 1975) — but what may cause the random loss of ocelli in exceptionally good flying insects, such as most Hymenoptera? Experimental studies with *Drosophila* have shown that mutations in the “eyes absent” (*eya*) gene (Bonini et al. 1998), mutations that reduce or abolish “orthodenticle” (*otd*) gene expression in the vertex primordium (Yorimitsu et al. 2011), elimination of the “hedgehog” (*Hh*) function during the third instar development (Amin et al. 1999), and clones of the “shaggy” (*sgg*) gene elsewhere in the head other than the eye field (Heslip et al. 1997), result in the loss of ocelli. Furthermore, flies homozygous for one of the “*Drosophila sine oculis*” (*so*) alleles *so*<sup>1</sup>, *so*<sup>2</sup>, or *so*<sup>5</sup> also result in the loss ocelli (Kawakami et al. 2000). However, since all of these experiments have been conducted only with flies it remains unclear the degree to which this can be translated to Hymenoptera and as potential mechanistic explanations for the malformation reported herein.

## Acknowledgements

We sincerely thank Matthias Haase (Bremen) for taking the photographs and Matthias Buck for confirming the identification of the species. The authors of Buck et al. (2008) are thanked for the kind permission to reuse the photograph of the normally developed specimen. Finally, we would like to thank Viktor Hartung (Berlin) for discussion, and the two reviewers, Lynn S. Kimsey and James M. Carpenter, for their valuable comments on the manuscript. This is a contribution of the Division of Entomology, University of Kansas Natural History Museum.

## References

- Alfonsus EC (1931) A one-eyed bee (*Apis mellifica*). *Annals of the Entomological Society of America* 24(2): 405–408. doi: 10.1093/aesa/24.2.405
- Amin A, Li Y, Finkelstein R (1999) Hedgehog activates the EGF receptor pathway during *Drosophila* head development. *Development* 126: 2623–2630. <http://dev.biologists.org/content/development/126/12/2623.full.pdf>
- Berry RP, Warrant EJ, Stange G (2007) Form vision in the insect dorsal ocelli: An anatomical and optical analysis of the locust ocelli. *Vision Research* 47(10): 1382–1393. doi: 10.1016/j.visres.2007.01.020
- Bohart RM, Menke AS (1976) Sphecids wasps of the world: a generic revision. University of California Press, Berkeley, ix + 695 pp.
- Bonini NM, Leiserson WM, Benzer S (1998) Multiple roles of the eyes absent gene in *Drosophila*. *Developmental Biology* 196(1): 42–57. doi: 10.1006/dbio.1997.8845
- Brothers DJ (1975) Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. University of Kansas Science Bulletin. <http://www.biodiversitylibrary.org/page/4386604#page/113/mode/1up>
- Buck M, Marshall SA, Cheung DKB (2008) Identification atlas of the Vespidae (Hymenoptera, Aculeata) of the northeastern Nearctic region. *Canadian Journal of Arthropod Identification* 5(1): 1–492. doi: 10.3752/cjai.2008.05
- Engel MS, Grimaldi DA, Krishna K (2009) Termites (Isoptera): their phylogeny, classification, and rise to ecological dominance. *American Museum Novitates* 3650: 1–27. doi: 10.1206/651.1
- Engel MS, Hinojosa-Díaz IA, Sagot P, Mérida J, Ayala R (2014) A pentocellar female of *Caenaugochlora inermis* from southern Mexico (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society* 87(4): 392–394. doi: 10.2317/JKES130907.1
- Ferreira PS, Henry TJ, Coelho LA (2015) Chapter 10. Plant Bugs (Miridae). In: Panizzi AR, Grazia J (Eds) *True Bugs (Heteroptera) of the Neotropics*. Springer, Dordrecht, xxii + 637 pp. doi: 10.1007/978-94-017-9861-7\_10
- Gibbs J (2010) An aberrant bee of the species *Lasioglossum (Dialictus) disparile* (Cresson) (Hymenoptera: Halictidae) with brief taxonomic notes on the species. *Journal of the Kansas Entomological Society* 83(1): 92–96. doi: 10.2317/JKES0806.16.1
- Gillott C (2005) *Entomology* (3rd edition). Springer, Dordrecht, xvii + 831 pp.
- Haydak MH (1948) Notes on biology of cyclopic bees. *Journal of Economic Entomology* 41(4): 663–664. doi: 10.1093/jee/41.4.663
- Heslip TR, Theisen H, Walker H, Marsh JL (1997) Shaggy and dishevelled exert opposite effects on Wingless and Decapentaplegic expression and on positional identity in imaginal discs. *Development* 124(5): 1069–1078. <http://dev.biologists.org/content/development/124/5/1069.full.pdf>
- Hopwood JL (2007) A “cyclops” of the bee *Lasioglossum (Dialictus) bruneri* (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society* 80(3): 259–261. doi: 10.2317/0022-8567(2007)80[259:ACOTBL]2.0.CO;2
- Huber JT, Sharkey MJ (1993) Chapter 3. Structure. In: Goulet H, Huber JT (Eds) *Hymenoptera of the world: An identification guide to families*. Agriculture Canada, Research branch, Publication 1894/E, 1993, vii + 668 pp.

- Kawakami K, Sato S, Ozaki H, Ikeda K (2000) Six family genes—structure and function as transcription factors and their roles in development. *BioEssays* 22(7): 616–626. doi: 10.1002/1521-1878(200007)22:7<616::AID-BIES4>3.0.CO;2-R
- Kerfoot WB (1967) Correlation between ocellar size and the foraging activities of bees (Hymenoptera; Apoidea). *The American Naturalist* 101(917): 65–70. doi: 10.1086/282470
- Krapp HG (2009) Ocelli. *Current Biology* 19(11): R435–R437. doi: 10.1016/j.cub.2009.03.034
- Kristensen NP (2003) Lepidoptera, Moths and Butterflies, vol 2: Morphology, Physiology, and Development. *Handbook of Zoology, Vol. IV. Arthropoda: Insecta. Part 36.* De Gruyter, Berlin, xii + 564 pp.
- Lohrmann V, Engel MS (2015) A quadriocellar scoliid wasp (Hymenoptera, Scoliidae) from Mallorca, with a brief account of supernumerary ocelli in insects. *Zoosystematics and Evolution* 91(2): 191–197. doi: 10.3897/zse.91.5463
- Miller FE (1936) A histological study of the eye and brain of a one-eyed bee. *Annals of the Entomological Society of America* 29(1): 66–69. doi: 10.1093/aesa/29.1.66
- Ohtani T (1977) Observations on the behaviour of a cyclopic worker honeybee. *Journal of Apicultural Research* 16(1): 34–40. doi: 10.1080/00218839.1977.11099857
- Rence BG, Lisy MT, Garves BR, Quinlan BJ (1988) The role of ocelli in circadian singing rhythms of crickets. *Physiological Entomology* 13(2): 201–212. doi: 10.1111/j.1365-3032.1988.tb00924.x
- Renner M, Heinzeller T (1979) Do trained honeybees with reliably blinded ocelli really return to the feeding site? *Journal of Apicultural Research* 18(3): 225–229. doi: 10.1080/00218839.1979.11099974
- Schremmer F (1950) Bemerkungen zur Ocellenfunktion bei Hummeln. *Österreichische Zoologische Zeitschrift* 2: 242–274. [http://www.zobodat.at/pdf/OEZ\\_02\\_0242-0274.pdf](http://www.zobodat.at/pdf/OEZ_02_0242-0274.pdf)
- Schricker B (1965) Die Orientierung der Honigbiene in der Dämmerung. *Zeitschrift für vergleichende Physiologie* 49(5): 420–458. doi: 10.1007/BF00298112
- Schuppe H, Hengstenberg R (1993) Optical properties of the ocelli of *Calliphora erythrocephala* and their role in the dorsal light response. *Journal of Comparative Physiology, A, Sensory, Neural, and Behavioral Physiology* 173(2): 143–149. doi: 10.1007/BF00192973
- Schwarz S, Albert L, Wystrach A, Cheng K (2011a) Ocelli contribute to the encoding of celestial compass information in the Australian desert ant *Melophorus bagoti*. *The Journal of Experimental Biology* 214(6): 901–906. doi: 10.1242/jeb.049262
- Schwarz S, Wystrach A, Cheng K (2011b) A new navigational mechanism mediated by ant ocelli. *Biology Letters* 7(6): 856–858. doi: 10.1098/rsbl.2011.0489
- Taylor CP (1981a) Contribution of compound eyes and ocelli to steering of locusts in flight: I. Behavioural analysis. *Journal of Experimental Biology* 93(1): 1–18. <http://jeb.biologists.org/content/93/1/1.full.pdf>
- Taylor CP (1981b) Contribution of compound eyes and ocelli to steering of locusts in flight: II. Timing changes in flight motor units. *Journal of Experimental Biology* 93(1): 19–31. <http://jeb.biologists.org/content/93/1/19.full.pdf>
- Tussac H, Balazuc J (1991) Anomalies de l'appareil visuel chez des Hyménoptères Apocrites. *L'Entomologiste* 47(1): 49–52.
- Viollet S, Zeil J (2013) Feed-forward and visual feedback control of head roll orientation in wasps (*Polistes humilis*, Vespidae, Hymenoptera). *Journal of Experimental Biology* 216(7): 1280–1291. doi: 10.1242/jeb.074773
- Warrant EJ (2006) Invertebrate vision in dim light. In: Warrant EJ, Nilsson D-E (Eds) *Invertebrate Vision*. Cambridge University Press, Cambridge, 83–126.
- Warrant EJ, Kelber A, Wallén R, Weislo WT (2006) Ocellar optics in nocturnal and diurnal bees and wasps. *Arthropod Structure & Development* 35(4): 293–305. doi: 10.1016/j.asd.2006.08.012
- Yorimitsu T, Kiritooshi N, Nakagoshi H (2011) Defective proventriculus specifies the ocellar region in the *Drosophila* head. *Developmental Biology* 356(2): 598–607. doi: 10.1016/j.ydbio.2011.06.015



# Species delimitation methods put into taxonomic practice: two new *Madascincus* species formerly allocated to historical species names (Squamata, Scincidae)

Aurélien Miralles<sup>1,2</sup>, Jörn Köhler<sup>3</sup>, Frank Glaw<sup>4</sup>, Miguel Vences<sup>1</sup>

<sup>1</sup> Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany

<sup>2</sup> ISYEB, UMR7205 MNHN-CNRS-UPMC-EPHE, Muséum National d'Histoire Naturelle, Département Systématique et Evolution, CP30, 25 rue Cuvier 75005 Paris, France

<sup>3</sup> Hessisches Landesmuseum Darmstadt, Friedensplatz 1, 64283 Darmstadt, Germany

<sup>4</sup> Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247 München, Germany

<http://zoobank.org/1B04381F-6D27-4365-A973-A47B4AB8841E>

Corresponding author: Aurélien Miralles (miralles.skink@gmail.com)

## Abstract

Received 20 July 2016  
Accepted 2 November 2016  
Published 6 December 2016

Academic editor:  
Johannes Penner

## Key Words

*Madascincus miafina* sp. n.  
*Madascincus pyrurus* sp. n.  
Madagascar  
phylogeny  
morphology  
integrative taxonomy  
species complex  
biogeography

In a previous study, Miralles & Vences (2013) compared seven different methods of species delimitation applied to the genus *Madascincus*. While focusing on methodological aspects their study involved an extensive data set of multilocus DNA sequences and of comparative morphology. On this basis they emphasized the need of revising the taxonomy of *Madascincus*, and revealed the existence of at least two well-supported candidate species. The present paper provides formal descriptions of these two taxa: (1) *M. miafina* sp. n., a species from dry areas of northern Madagascar, morphologically very similar to *M. polleni* (although both species are not retrieved as sister taxa), and (2) *M. pyrurus* sp. n., a montane species occurring >1500 m above sea level, endemic to the central highlands of Madagascar (Ibity and Itremo Massifs). Phylogenetically, *M. pyrurus* is the sister species of *M. igneocaudatus*, a taxon restricted to the dry littoral regions of the south and south-west of Madagascar in lowlands <500 m above sea level. To facilitate future taxonomic work, we furthermore elaborated an identification key for species of *Madascincus*. Finally, some aspects of the biogeographic patterns characterising the different main clades within the genus *Madascincus* are provided and discussed for the first time in the light of a robust phylogenetic framework.

## Introduction

The genus *Madascincus* represents a monophyletic group of skinks endemic to Madagascar (Crottini et al. 2009, Miralles and Vences 2013). These lizards likely have diversified during the early Oligocene (Miralles et al. 2015), giving rise to a dozen currently known species. In a recent study dealing with methodological aspects of species delimitation, Miralles and Vences (2013) applied and compared seven methods of delimitation to this genus, combining different approaches: (1) ITAX, the *Integrative Taxonomic approach*, which is based on the integration of as many lines of evidence as available to delimit

species (cf. Dayrat 2005, DeSalle et al. 2005, Padial et al. 2010, Miralles and Vences 2013); (2) MTMC, the *Mitochondrial Tree – Morphological Character approach*, which is based on the combination of evidence from DNA sequences and morphological data, considering as species those morphologically diagnosable units that are revealed by a mtDNA tree (cf. Riedel et al. 2013, Miralles and Vences 2013); (3) WP, the *Wiens and Penkrot protocol*, which is based on the identification of non-recombining molecular phylogenetic units (cf. Wiens and Penkrot 2002); (4) BAT, the *Bayesian Assignment Test*, which is based on the combination of population genetic and genealogical patterns across multiple loci, recognizing species

according to concordance between mtDNA clades and patterns of nuclear population structure (cf. Weisrock et al. 2010); (5) HW, the *Haplweb approach*, which is based on haplotype networks with additional connections between haplotypes found co-occurring in heterozygous specimens (haplowebs) to delineate species boundaries (cf. Doyle 1995, Flot et al. 2010); (6) BSD, the *Bayesian Species Delimitation*, which is based on coalescence theory, and uses bipartitions of specimens in gene trees that are shared across loci to infer species-level lineages (cf. Rannala and Yang 2003, Yang and Rannala 2010); and (7) GMYC, the *Generalized Mixed Yule-coalescent approach* which is based on a statistical model testing for the predicted change in branching rates at the species boundary of a single-locus phylogenetic tree (cf. Pons et al. 2006, Fontaneto et al. 2007, Monaghan et al. 2009). For details on each of the methods and their application to *Madascincus*, see Miralles and Vences (2013).

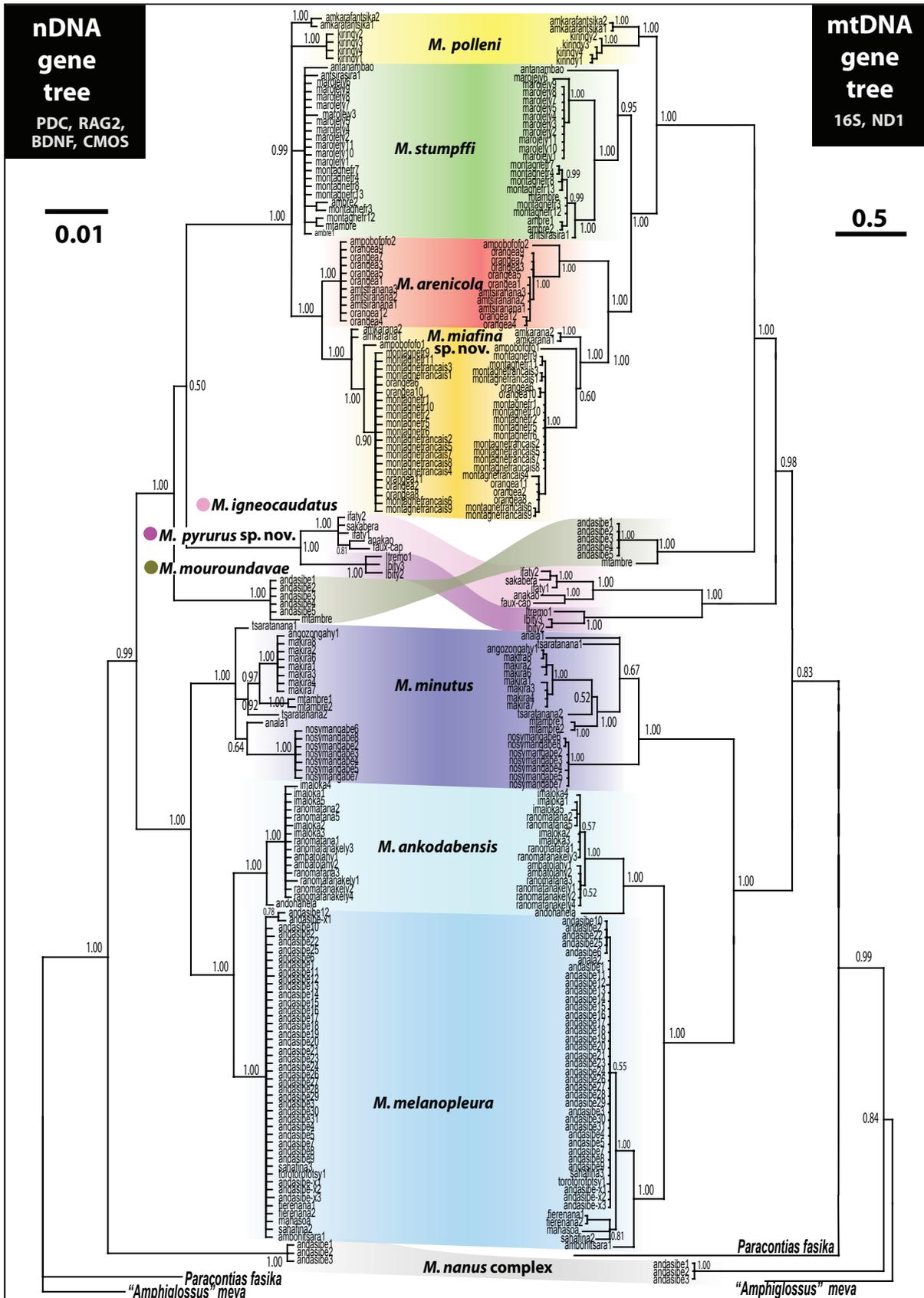
Miralles and Vences (2013) concluded that the taxonomy of the genus is in need of revision. Despite relevant incongruences between the various species delimitation approaches assessed, all of them agreed in suggesting at least two divergent lineages representing undescribed species: (1) a lineage referred to as *polleni*-N, with specimens morphologically very similar to *M. polleni* but phylogenetically more closely related to two morphologically distinct species, namely *M. arenicola* and *M. stumpffi*; and (2) a lineage referred to as *igneocaudatus*-C inhabiting the highland of central Madagascar, with specimens morphologically and ecologically distinguishable from *M. igneocaudatus* sensu stricto, its sister species endemic to the dry coast of southern and western Madagascar. Following up on the compelling evidence for a species status of these two lineages (Miralles and Vences 2013), the main aim of the present study is to provide their formal taxonomic description. We also took the opportunity of this work to discuss several aspects of biogeography and origins of this genus in a phylogenetic context.

## Material and methods

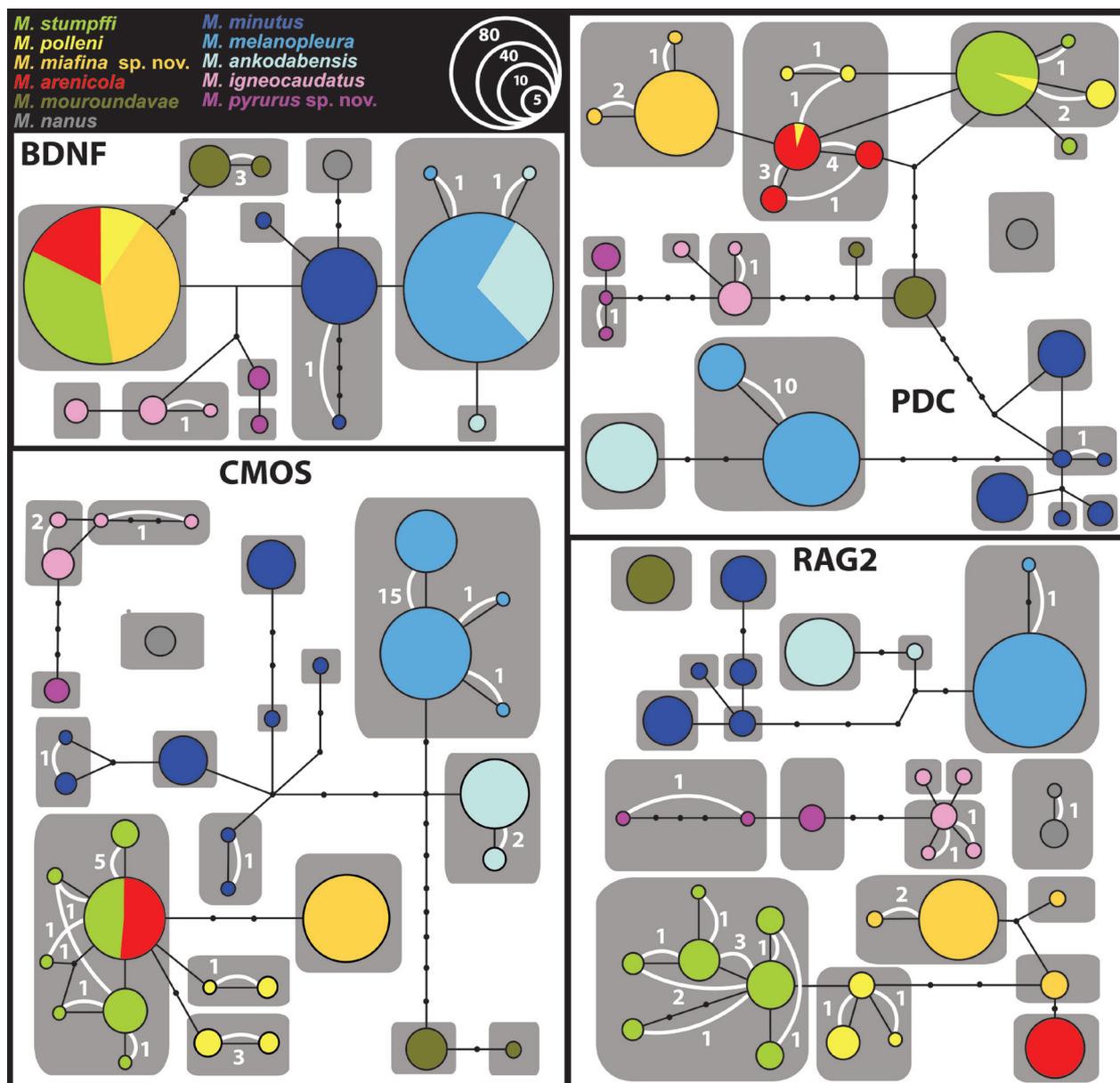
**Morphology.** The comparative morphology approach mostly relies on the data-set previously published by Miralles and Vences (2013). It involved morphological data composed of a total of 168 preserved specimens used in their molecular analyses, completed by 40 additional specimens having not been sequenced and complementary data previously published by Andreone and Greer (2002), Glaw and Vences (2007) and Miralles et al. (2011a). Specimens examined also included all the type specimens known for this genus, with exception of the types of *M. minutus* (UMMZ 192705) and *M. vulsini* (MCZ R-11869), both being unambiguously members of the *M. melanopleura* clade. All the examined specimens are deposited in the Muséum National d'Histoire Naturelle, Paris (MNHN), Museo Regionale di Scienze Naturali, Torino (MRSN), National Histo-

ry Museum, London (NHM), Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main (SMF), Université d'Antananarivo, Département de Biologie Animale (UADBA), and Zoologische Staatssammlung München (ZSM). In addition, where applicable, we also give for the specimens their respective field numbers, using the abbreviations: FGZC, FG/MV, MV, and MgF referring to Frank Glaw, Miguel Vences and Madagascar Frontiers field numbers (cf. Supplementary file 1). Some specimens deposited at UADBA have not yet been formally catalogued in that collection; we refer to these specimens as UADBA uncatalogued, followed by the respective field number which allows an unambiguous identification of the specimens in this collection. More specifically, the description of the two new species herein proposed involved the record of meristic, mensural and categorical morphological characters routinely used in the taxonomy of Scincidae, such as scale counts, presence or absence of homologous scale fusions, or color patterns (cf. Andreone and Greer 2002, Miralles et al. 2011a, b, c). The ventral scales are counted in a single row from the postmentals to the preanal scales which both are included in the count, while the mental scale is excluded. The paravertebrals are counted in a single row from the first scale posterior to a line connecting the posterior edges of the thighs held normal to the long axis of the body anteriorly to and including the nuchals. Nuchal scales (*sensu* Miralles 2006) are defined as enlarged scales of the nape, occupying transversally the place of two or more rows of dorsal cycloid scale. The frontal scale is considered *hourglass-shaped* when constricted by first supraocular, *bell-shaped* otherwise. Measurements of specimens were recorded to the nearest 0.1 mm using a dial caliper. Ranges are given for each meristic and mensural character, followed by the mean  $\pm$  the standard deviation, with sample size in parentheses. For some bilateral characters, the sample size has been noted as the number of sides rather than specimens (indicated after sample size).

**Phylogenetic analyses.** All molecular analyses of the present paper were directly taken and adapted from the work of Miralles and Vences (2013), i.e., the separate Bayesian analyses of the nuclear DNA (nDNA) data set (segments of the genes BDNF, CMOS, PDC and RAG2) and of the mitochondrial DNA (mtDNA) data set (segments of the genes ND1 and 16S) (Fig. 1) and the haplotype network reconstruction generated by TCS 1.21 (Clement et al. 2000) for the phased haplotypes of the four nuclear gene segments (Fig. 2). Additionally, uncorrected p-distances were estimated with MEGA 6 (Tamura et al. 2013) for the 16S and ND1 mtDNA segments to provide an overview of the genetic divergence among taxa (Table 3). See Miralles and Vences (2013) for detailed descriptions of all the molecular procedures, GenBank accession numbers, phylogenetic analyses and haplotype network reconstruction methods. The respective figures shown herein have been updated for species names and integrative species delimitation conclusions.



**Figure 1.** Comparison of phylogenetic trees of the genus *Madascincus* based on nDNA and mtDNA sequences (modified from Miralles and Vences 2013). Nuclear DNA tree inferred from BI analysis of concatenated BDNF, CMOS, PDC and RAG2 sequences compared to the mitochondrial DNA inferred from ND1 and 16S sequences (unpartitioned data set, posterior probabilities indicated for each node, see Miralles and Vences 2013 for details of analysis methods).



**Figure 2.** Haplotype network reconstructions for the four nuclear genes (BDNF, PDC, CMOS and RAG2). For each marker, circles represent haplotypes (size proportional to the number of individuals), black lines represent mutational steps and black dots missing haplotypes, white curves represent connections between haplotypes found co-occurring in heterozygous individuals, and white numbers represent the number of individuals in which the respective haplotypes were found co-occurring. Single locus fields of recombination (pools of co-occurring haplotypes) are represented by grey rectangles (redrawn from Miralles and Vences 2013).

**Taxonomic background.** The definition of the genus *Madascincus* herein follows previous molecular work (Schmitz et al. 2005, Crottini et al. 2009, Miralles et al. 2011c, Miralles and Vences 2013), encompassing all species of an exclusively four-legged clade that is sister to the legless genus *Paracontias*. The term “*Madascincus nanus* complex” designates a putative complex of species encompassing *M. nanus*, *M. macrolepis* and a candidate species previously referred as *Madascincus* sp. “baeus” by Glaw and Vences (2007). *Madascincus macrolepis* and *M. nanus* are very rarely encountered animals for which no topotypic molecular samples have so far become available. The three forms in the complex are almost certain-

ly closely related due to numerous morphological similarities, and we here provisionally follow Miralles and Vences (2013) in merging *M. sp.* “baeus” with *M. nanus*. The candidate species *Madascincus* sp. “vitreus”, known from a single juvenile individual from Kirindy (Glaw and Vences 2007) is here tentatively regarded as a juvenile specimen of *M. igneoaudatus* sensu stricto, pending molecular data and collection of further specimens for a more reliable assignment. For consistency we hereafter refer to the different species-level units as clades, following Miralles and Vences (2013) who used these provisional names to refer to units forming distinct clades in the mtDNA and nDNA tree. We also avoid the term “lineage”

which in other studies we have regularly used to refer to such species-level evolutionary units. We apply the same clade names as in Miralles and Vences (2013) and then assign Linnean names to them by either formally describing new taxa (two new species) or arguing for their conspecificity with previously described species. Concerning the usage of unscientifically and unethically erected taxon names we follow the recommendations of “censuring taxonomic vandals”, as proposed by Kaiser et al. (2013).

## Results

Despite numerous conflicts among the seven methods of species delimitation (ITAX, MTMC, WP, BAT, HW, BSD and GMYC) applied on *Madascincus* by Miralles and Vences (2013), all of them were in agreement on the specific distinctiveness of the two clades named *igneocaudatus-C* and *polleni-N* in that study. Some of these methods suggested a further splitting of these two units: BAT, BSD and GMYC recognized respectively two, two and three species within the *polleni-N* clade, whereas GMYC identified two species within *igneocaudatus-C* clade, corresponding to the population of Ibity and the population of Itremo. However, as argued by Miralles and Vences (2013) these approaches led to obvious oversplitting in several other *Madascincus* species, including objective errors such as assigning haplotypes co-occurring in the same population to different species, despite originating from specimens without differences in morphology or nuclear genes. We therefore do not propose a further subdivision of the *igneocaudatus-C* and *polleni-N* clades and consider each of them as one species in need of formal description.

Considering an integrative taxonomic approach, the distinctiveness of the two clades *igneocaudatus-C* and *polleni-N* is supported by the following independent lines of evidence. All results in the following are from Miralles and Vences (2013), with the exception of the genetic distances which have been newly calculated herein:

- Both clades represent monophyletic units fully supported by both the nDNA and the mtDNA data set (posterior probabilities of 1.00 for each clade and for each data set, cf. Fig. 1).
- The *igneocaudatus-C* clade possesses exclusive alleles for the four nuclear markers analysed and the *polleni-N* clade has exclusive alleles in three markers, only sharing one nuclear allele with *M. arenicola*, *M. stumpffi* and *polleni-S* for the very conserved BDNF segment (Fig. 2), suggesting no recent gene exchanges between these clades and their respective sister clades.
- Both clades are unambiguously morphologically diagnosable from their respective sister clade and from all the other species of *Madascincus* (Figs 3, 4, Tables 1, 2).
- The genetic distance values between these two clades and their respective sister clades are relatively high, with p-distances ranging from 7.3 to 9.0% (16S) and 16.2 to 17.7% (ND1) between *igneocaudatus-C* and *igneocaudatus-S*, and 2.5 to 3.4% (16S)

and 8.1 to 10.2% (ND1) between *polleni-N* and *M. arenicola*. These distances are consistent with inter-specific divergences observed between the other recognized species of *Madascincus* (Table 3), whereas intragroup divergence remains relatively low: 0.0 to 2.2% (16S) and 0.4 to 5.1% (ND1) within *igneocaudatus-C*, and 0.0 to 2.0% (16S) and 0.0 to 6.4% (ND1) within *polleni-N*.

### *Madascincus miafina* sp. n.

<http://zoobank.org/DAC928B3-73CF-4653-B8A3-B7A4E17DFF50>

Figs 3B, 4J, K

**Holotype.** ZSM 1562/2008 (FGZC 1658), adult male, from near Petit Tsingy, 12°57'25"S, 49°07'06"E, 90 m above sea level, Ankarana Special Reserve, Antsiranana province, north Madagascar, collected on 16 February 2008 by M. Franzen, F. Glaw, J. Köhler and Z. Nagy.

**Paratypes** (n=23, all from Antsiranana province, northern Madagascar). ZSM 242/2004 (FGZC 474), 245/2004 (FGZC 480), Montagne des Français, 12°19'34"S, 49°20'09" E, 334 m a.s.l., coll. on 23 and 24 February 2004 by F. Glaw, M. Puente and R.D. Randrianiaina; UADBA uncatalogued (FGZC, 1788, 1789), Montagne des Français, coll. by Frontier staff at unknown date; ZSM 1571/2008 (FGZC 1766), 1572/2008 (FGZC 1844), Baie des Sakalava (ca. 5 km SE Ramena), 12°16.371'S, 49°23.338'E, 28 m a.s.l., coll. on 22 and 26 February 2008 by S. Megson; ZSM 1573–1577/2008 (FGZC 1678, 1680, 1687, 1836, 1838), UADBA uncatalogued (FGZC 0481, 1677, 1684, 1762, 1763, 1835), Montagne des Français (pitfall lines 1, 2 & 5, no coordinates available), coll. on 19 and 25 February 2008 by N. D’Cruze and local collectors; ZSM 259/2004, Montagne des Français, coll. on 18–28 February 2004 by F. Glaw, M. Puente, R.D. Randrianiaina and A. Razafimanantsoa; ZSM 1570/2008 (FGZC 1917), Ampombofofo region, 12°05.571'S, 49°19.035'E (trapsite 5), coll. on 23 February 2007 by S. Megson; ZSM 1563/2008 (FGZC 1827), same data as holotype, but collected by a local assistant on 24 February 2008; UADBA uncatalogued (FGZC 1742, 1768, 1840), Orangea, coll. in February 2008 by S. Megson.

**Other specimens examined.** (n=2, not sequenced). MNHN 1897.31, Diego Suarez; MNHN 1980.1169, Bemanevika, Plateau Bealanana.

### Chresonyms.

*Scelotes intermedius* – Brygoo (1981, *partim*);  
*Amphiglossus intermedius* – Brygoo (1984, *partim*);  
*Madascincus intermedius* – Glaw & Vences (2007, *partim*);  
*Madascincus polleni* “clade 1” – Miralles et al. (2011b);  
*Madascincus polleni* “*polleni-N* clade” – Miralles & Vences (2013);  
*Madascincus* sp. “*polleni*” northern clade – Miralles et al. (2015).

**Table 1.** Comparison of the most relevant morphological characters, plus additional data on the altitudinal distribution and reproductive mode of *Madascincus* species. Ranges are given for meristic and mensural characters, followed by the mean  $\pm$  the standard deviation, with sample size in parentheses. For some bilateral characters, the sample size was noted as the number of sides rather than specimens. Data from Andreone and Greer 2002, Glaw and Vences 2007, Miralles et al. 2011a, Miralles and Vences 2013.

|   | igneocaudatus clade        |                        | melanopleura clade     |                        | mouroundavae           | nanus complex          | polleni clade          |                        |                       |                        |                        |                        |                         |
|---|----------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-----------------------|------------------------|------------------------|------------------------|-------------------------|
|   | igneocaudatus              | pyrurus (C)            | minutus (N)            | melanopleura (C)       |                        |                        | ankodabensis (S)       | arenicola              | miafina (N)           | polleni (S)            | stumpffi               |                        |                         |
| <b>N lamellae under 4<sup>th</sup> finger</b> | min-max:                   | 8-11                   | 8-11                   | 5-7                    | 5-8                    | 5-8                    | 8-11                   | 3-5                    | 6-7                   | 7-8                    | 6-9                    | 6-9                    |                         |
|   | mean $\pm$ SD:<br>n sides: | 9.0 $\pm$ 0.9<br>(50)  | 9.1 $\pm$ 0.9<br>(14)  | 6.0 $\pm$ 0.8<br>(13)  | 7.0 $\pm$ 0.6<br>(40)  | 6.3 $\pm$ 1.0<br>(9)   | 6.3 $\pm$ 1.0<br>(9)   | 9.8 $\pm$ 0.7<br>(16)  | 3.9 $\pm$ 0.6         | 6.4 $\pm$ 0.5<br>(11)  | 7.6 $\pm$ 0.5<br>(22)  | 7.5 $\pm$ 0.7<br>(26)  | 7.3 $\pm$ 0.8<br>(27)   |
| <b>N lamellae under 4<sup>th</sup> toe</b>    | min-max:                   | 15-22                  | 15-18                  | 9-13                   | 12-16                  | 12-15                  | 16-20                  | 5-8                    | 16-19                 | 18-23                  | 16-22                  | 15-20                  |                         |
|   | mean $\pm$ SD:<br>n sides: | 18.1 $\pm$ 1.4<br>(54) | 16.4 $\pm$ 1.3<br>(13) | 11.3 $\pm$ 1.5<br>(10) | 14.1 $\pm$ 1.2<br>(52) | 13.8 $\pm$ 1.1<br>(12) | 13.8 $\pm$ 1.1<br>(12) | 17.5 $\pm$ 1.2<br>(15) | 6.8 $\pm$ 1.3<br>(9)  | 17.5 $\pm$ 0.8<br>(13) | 20.6 $\pm$ 1.3<br>(20) | 18.5 $\pm$ 1.5<br>(22) | 17.9 $\pm$ 1.2<br>(28)  |
| <b>N ventral scale rows</b>                   | min-max:                   | 68-83                  | 73-78                  | 55-63                  | 56-61                  | 59-63                  | 63-66                  | 52-60                  | 75-80                 | 65-73                  | 74-78                  | 70-88                  |                         |
|   | mean $\pm$ SD:<br>n:       | 76.7 $\pm$ 4.4<br>(21) | 75.7 $\pm$ 1.8<br>(7)  | 58.3 $\pm$ 3.0<br>(7)  | 58.8 $\pm$ 1.2<br>(27) | 60.2 $\pm$ 1.5<br>(6)  | 60.2 $\pm$ 1.5<br>(6)  | 64.3 $\pm$ 1.0<br>(8)  | 57.6 $\pm$ 3.3<br>(5) | 77.9 $\pm$ 1.6<br>(7)  | 68.7 $\pm$ 2.1<br>(14) | 75.8 $\pm$ 1.2<br>(12) | 81.3 $\pm$ 4.0<br>(16)  |
| <b>N paravertebral scale rows</b>             | min-max:                   | 69-80                  | 71-79                  | 57-65                  | 51-62                  | 52-62                  | 60-65                  | 50-57                  | 74-81                 | 65-79                  | 71-81                  | 76-88                  |                         |
|   | mean $\pm$ SD:<br>n:       | 74.7 $\pm$ 3.0<br>(26) | 74.6 $\pm$ 3.7<br>(7)  | 59.7 $\pm$ 3.4<br>(7)  | 55.9 $\pm$ 2.9<br>(28) | 57.7 $\pm$ 3.1<br>(7)  | 57.7 $\pm$ 3.1<br>(7)  | 62.6 $\pm$ 2.1<br>(8)  | 53.6 $\pm$ 2.5<br>(5) | 79.0 $\pm$ 2.3<br>(7)  | 68.7 $\pm$ 3.3<br>(14) | 77.9 $\pm$ 2.6<br>(13) | 82.7 $\pm$ 3.2<br>(15)  |
| <b>N longitudinal scale rows at midbody</b>   | min-max:                   | 24-26                  | 22-24                  | 22-26                  | 24-26                  | 22-26                  | 28-30                  | 18-20                  | 26                    | 24-26                  | 24-26                  | 30-32                  |                         |
|   | mean $\pm$ SD:<br>n:       | 24.2 $\pm$ 0.6<br>(28) | 23.3 $\pm$ 1.0<br>(7)  | 24.0 $\pm$ 1.2<br>(7)  | 24.1 $\pm$ 0.4<br>(27) | 23.7 $\pm$ 1.5<br>(6)  | 23.7 $\pm$ 1.5<br>(6)  | 29.0 $\pm$ 4.2<br>(8)  | 19.6 $\pm$ 0.9<br>(5) | 26.0 $\pm$ 0<br>(7)    | 24.1 $\pm$ 0.5<br>(14) | 25.4 $\pm$ 0.9<br>(13) | 31.6 $\pm$ 0.8<br>(16)  |
| <b>Enlarged nuchal scales</b>                 | absent:                    | -                      | -                      | -                      | -                      | -                      | -                      | -                      | 42.9%                 | 92.3%                  | 56.3%                  | 81.3%                  |                         |
|   | one row:                   | -                      | -                      | -                      | 2%                     | 7.1%                   | 100%                   | 40.0%                  | 57.1%                 | 7.7%                   | 37.5%                  | 18.7%                  |                         |
|   | two rows:                  | 23.2%                  | 21.4%                  | 28.6%                  | 50%                    | 35.8%                  | -                      | -                      | 20.0%                 | -                      | -                      | -                      |                         |
|   | three rows:                | 71.4%                  | 78.6%                  | 57.1%                  | 48%                    | 57.1%                  | -                      | -                      | 40.0%                 | -                      | -                      | -                      |                         |
| <b>Postnasal</b>                              | four rows:                 | 5.4%                   | -                      | 14.3%                  | -                      | -                      | -                      | -                      | -                     | -                      | -                      | -                      |                         |
|   | n sides:                   | (56)                   | (14)                   | (14)                   | (58)                   | (14)                   | (14)                   | (16)                   | (10)                  | (14)                   | (26)                   | (32)                   |                         |
| <b>Frontal and interparietal</b>              | present:                   | 100%                   | 100%                   | 100%                   | 100%                   | 100%                   | 100%                   | 100%                   | -                     | 89.3%                  | 100%                   | 94.4%                  |                         |
|   | absent:                    | -                      | -                      | -                      | -                      | -                      | -                      | -                      | -                     | 100%                   | 10.7%                  | 5.6%                   |                         |
| <b>Frontal</b>                                | n sides:                   | (56)                   | (14)                   | (14)                   | (58)                   | (14)                   | (16)                   | (10)                   | (14)                  | (28)                   | (26)                   | (36)                   |                         |
|   | fused:                     | -                      | -                      | -                      | -                      | -                      | 87.5%                  | -                      | -                     | -                      | -                      | -                      |                         |
| <b>Snout-vent length (mm)</b>                 | separated:                 | 100%                   | 100%                   | 100%                   | 100%                   | 100%                   | 12.5%                  | 100%                   | 100%                  | 100%                   | 100%                   | 100%                   |                         |
|   | n:                         | (28)                   | (14)                   | (7)                    | (28)                   | (7)                    | (7)                    | (8)                    | (10)                  | (7)                    | (14)                   | (13)                   | (16)                    |
| <b>Frontal</b>                                | bell-shaped:               | 100%                   | 100%                   | -                      | -                      | -                      | -                      | -                      | 100%                  | 100%                   | 100%                   | 47.2%                  |                         |
|   | hour-glass shaped:         | -                      | -                      | 100%                   | 100%                   | 100%                   | 100%                   | 100%                   | -                     | -                      | -                      | 52.8%                  |                         |
| <b>Snout-vent length (mm)</b>                 | n:                         | (23)                   | (12)                   | (14)                   | (29)                   | (14)                   | (8)                    | (10)                   | (7)                   | (14)                   | (13)                   | (18)                   |                         |
|   | max:                       | 73.0                   | 54.2                   | 47.4                   | 53.5                   | 50.5                   | 50.5                   | 68.5                   | 33.6                  | 81.7                   | 61                     | 75                     |                         |
| <b>Snout-vent length (mm)</b>                 | mean $\pm$ SD:             | 56.3 $\pm$ 11.6<br>(9) | 52.3 $\pm$ 2.1<br>(4)  | 42.0 $\pm$ 5.1<br>(7)  | 49.5 $\pm$ 2.5<br>(21) | 48.0 $\pm$ 2.4<br>(5)  | 48.0 $\pm$ 2.4<br>(5)  | 60.1 $\pm$ 9.6<br>(7)  | 27.8 $\pm$ 8.2<br>(6) | 72.3 $\pm$ 6.1<br>(7)  | 54.9 $\pm$ 3.1<br>(14) | 66.0 $\pm$ 7.1<br>(13) | 89.6 $\pm$ 10.8<br>(14) |
|   | n:                         | (9)                    | (4)                    | (7)                    | (21)                   | (5)                    | (5)                    | (7)                    | (6)                   | (7)                    | (14)                   | (13)                   | (14)                    |

|                            | igneocaudatus clade |             | melanopleura clade |                  |                  | mouroundavae | nanus complex | polleni clade |             |             |          |         |         |
|----------------------------|---------------------|-------------|--------------------|------------------|------------------|--------------|---------------|---------------|-------------|-------------|----------|---------|---------|
|                            | igneocaudatus       | pyrurus (C) | minutus (N)        | melanopleura (C) | ankodabensis (S) |              |               | arenicola     | miafina (N) | polleni (S) | stumpffi |         |         |
| <b>Supraciliaries</b>      |                     |             |                    |                  |                  |              |               |               |             |             |          |         |         |
| <b>five:</b>               | –                   | 7.1%        | 7.1%               | –                | –                | –            | –             | –             | –           | –           | –        | –       | –       |
| <b>six:</b>                | 98.2%               | 92.9%       | 57.1%              | 8.6%             | 35.7%            | 93.8%        | 66.6%         | 92.9%         | 100%        | 87.5%       | –        | –       | –       |
| <b>seven:</b>              | 1.8%                | –           | 35.8%              | 79.3%            | 50.0%            | 6.2%         | 33.3%         | 7.1%          | –           | 12.5%       | –        | –       | n/a     |
| <b>eight:</b>              | –                   | –           | –                  | 12.7%            | 14.3%            | –            | –             | –             | –           | –           | –        | –       | –       |
| <b>n sides:</b>            | (56)                | (14)        | (14)               | (58)             | (14)             | (16)         | (6)           | (14)          | (2)         | (8)         | –        | –       | –       |
| <b>Subocular</b>           |                     |             |                    |                  |                  |              |               |               |             |             |          |         |         |
| <b>third SL:</b>           | 1.8%                | –           | –                  | –                | 7.1%             | –            | 100%          | –             | 3.6%        | –           | –        | –       | –       |
| <b>Fourth SL:</b>          | 98.2%               | 100%        | 100%               | 100%             | 92.9%            | 100%         | –             | 100%          | 96.4%       | 100%        | –        | –       | 100%    |
| <b>n sides:</b>            | (56)                | (14)        | (14)               | (58)             | (14)             | (24)         | (10)          | (14)          | (28)        | (24)        | –        | –       | (32)    |
| <b>Lower eyelid window</b> | spectacled          | spectacled  | spectacled         | spectacled       | spectacled       | scaly        | scaly         | scaly         | scaly       | scaly       | scaly    | scaly   | scaly   |
| <b>Reproduction</b>        | viviparous          | oviparous   | ?                  | oviparous?       | ?                | oviparous    | ?             | ?             | ?           | ?           | ?        | ?       | ?       |
| <b>Altitudinal range</b>   | ≤ 500 m             | ≥ 1500 m    | ≤ 1000 m           | ≤ 1000 m         | ≤ 1000 m         | ≤ 1000 m     | 500–1500 m    | ≤ 500 m       | ≤ 500 m     | ≤ 500 m     | ≤ 500 m  | ≤ 500 m | ≤ 500 m |

**Table 2.** Summary of the most relevant morphological characters differentiating each pair of species of *Madascincus*. Only objective and unambiguous diagnostic characters (e.g. fixed character states for qualitative characters or non-overlapping values for meristic characters) are reported; see complete data in Table 1. F: number of lamellae under 4th finger; T: number of lamellae under 4th toe; VR: number of ventral scale rows; PR: number of paravertebral scale rows; MR: number of longitudinal scale rows at midbody; N: number of enlarged nuchal scales; PN: presence or absence of postnasal scales; FS: shape of the frontal scale; SO: position of the subocular scale; EW: aspect of the lower eyelid window; R: reproduction mode (oviparity vs. viviparity).

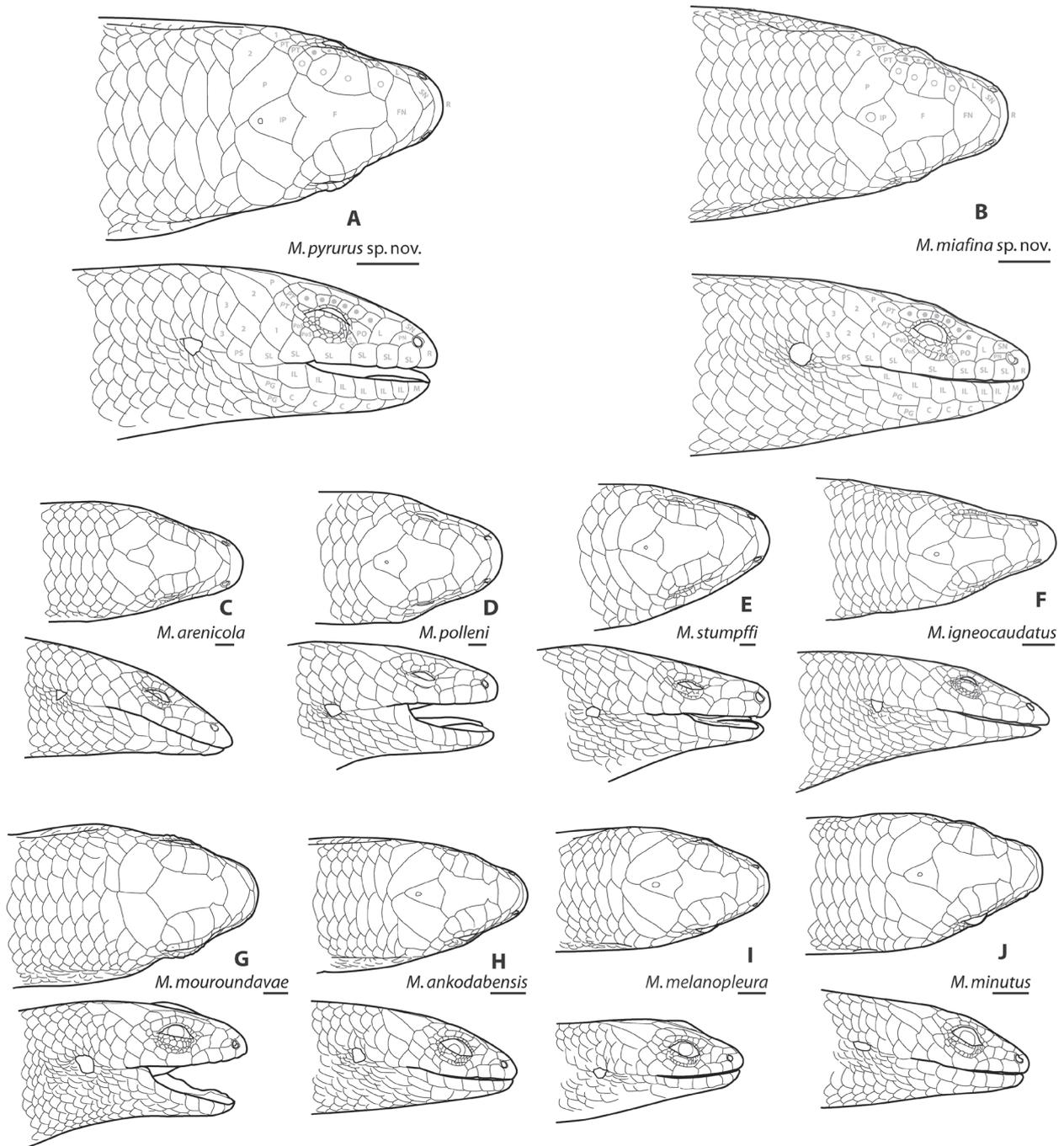
|                          | <i>nanus</i> complex         | <i>igneocaudatus</i>     | <i>pyrurus</i>               | <i>mouroundavae</i>       | <i>minutus</i>           | <i>melanopleura</i> | <i>ankodabensis</i>   | <i>miafina</i>       | <i>polleni</i>           | <i>stumpffi</i>      |
|--------------------------|------------------------------|--------------------------|------------------------------|---------------------------|--------------------------|---------------------|-----------------------|----------------------|--------------------------|----------------------|
| <i>M. arenicola</i>      | F, T, VR, PR, MR, PN, FS, SO | F, N, PN, EW             | F, MR, N, PN, EW             | F, VR, PR, MR, PN, FS, EW | T, VR, PR, N, PN, FS, EW | VR, PR, PN, FS, EW  | T, VR, PR, PN, FS, EW | VR                   | PN                       | MR                   |
| <i>M. nanus</i>          | –                            | F, T, VR, PR, MR, FS, EW | F, T, VR, PR, MR, FS, SO, EW | F, T, VR, PR, MR, SO      | T, MR, SO, EW            | T, MR, SO, EW       | T, MR, EW             | F, T, VR, PR, MR, FS | F, T, VR, PR, MR, FS, SO | F, T, VR, PR, MR, SO |
| <i>M. igneocaudatus</i>  | –                            | –                        | R                            | VR, PR, MR, N, FS, EW, R  | F, T, VR, PR, FS         | VR, PR, FS          | VR, PR, FS            | N, EW                | EW                       | MR, N, EW            |
| <i>M. pyrurus</i> sp. n. | –                            | –                        | –                            | VR, PR, MR, N, FS, EW     | F, T, VR, PR, FS         | VR, PR, FS          | VR, PR, FS            | N, EW                | EW                       | MR, N, EW            |
| <i>M. mouroundavae</i>   | –                            | –                        | –                            | –                         | F, T, MR, N, EW          | VR, MR, EW          | T, MR, EW             | MR, FS               | VR, PR, MR, FS           | VR, PR               |
| <i>M. minutus</i>        | –                            | –                        | –                            | –                         | –                        | none                | none                  | T, VR, N, FS, EW     | T, VR, PR, FS, EW        | T, VR, PR, MR, N, EW |
| <i>M. melanopleura</i>   | –                            | –                        | –                            | –                         | –                        | –                   | none                  | T, VR, PR, FS, EW    | VR, PR, FS, EW           | VR, PR, MR, EW       |
| <i>M. ankodabensis</i>   | –                            | –                        | –                            | –                         | –                        | –                   | –                     | T, VR, PR, FS, EW    | T, VR, PR, FS, EW        | VR, PR, MR, EW       |
| <i>M. miafina</i> sp. n. | –                            | –                        | –                            | –                         | –                        | –                   | –                     | –                    | VR                       | MR                   |
| <i>M. polleni</i>        | –                            | –                        | –                            | –                         | –                        | –                   | –                     | –                    | –                        | MR                   |

**Table 3.** Genetic divergences among *Madascincus* species: mean of the uncorrected p-distances for the ND1 (above diagonal) and the 16S (below diagonal) mtDNA segments estimated between and within the different species. The genetic distance values obtained between the two newly described species and their respective sister clades (in bold) are consistent with interspecific divergences observed between the other recognized species of *Madascincus*.

|                                 | Inter-specific distances (%) |              |              |              |              |              |              |              |              |              | Intra-specific distances (%) |     |     |
|---------------------------------|------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|------------------------------|-----|-----|
|                                 | <i>aren.</i>                 | <i>nanu.</i> | <i>pyru.</i> | <i>igne.</i> | <i>anko.</i> | <i>mela.</i> | <i>minu.</i> | <i>mour.</i> | <i>poll.</i> | <i>miaf.</i> | <i>stum.</i>                 | 16S | ND1 |
| <i>M. arenicola</i>             | -                            | 19.8         | 17.9         | 19.2         | 17.5         | 16.6         | 17.3         | 16.9         | 12.5         | <b>9.0</b>   | 10.5                         | 0.1 | 1.1 |
| <i>M. nanus</i>                 | 10.1                         | -            | 21.1         | 21.2         | 21.3         | 19.0         | 21.3         | 20.0         | 21.1         | 21.2         | 20.5                         | 0.0 | 0.0 |
| <b><i>M. pyrurus</i> sp. n.</b> | 8.2                          | 10.1         | -            | <b>17.0</b>  | 18.9         | 17.3         | 18.3         | 20.8         | 19.5         | 19.2         | 19.3                         | 1.5 | 3.5 |
| <i>M. igneocaudatus</i>         | 9.3                          | 9.9          | <b>8.3</b>   | -            | 20.5         | 19.3         | 20.1         | 18.8         | 19.3         | 20.0         | 19.3                         | 2.5 | 5.9 |
| <i>M. ankodabensis</i>          | 8.3                          | 10.9         | 9.8          | 11.2         | -            | 8.2          | 14.7         | 17.5         | 16.5         | 17.9         | 16.2                         | 0.5 | 1.0 |
| <i>M. melanopleura</i>          | 8.5                          | 10.6         | 9.7          | 11.6         | 3.4          | -            | 12.3         | 17.1         | 15.9         | 16.1         | 15.2                         | 0.5 | 0.7 |
| <i>M. minutus</i>               | 8.7                          | 11.3         | 10.9         | 12.5         | 8.5          | 8.0          | -            | 17.0         | 17.7         | 18.1         | 16.6                         | 3.1 | 5.7 |
| <i>M. mouroundavae</i>          | 7.0                          | 8.6          | 9.2          | 8.7          | 9.9          | 9.9          | 10.6         | -            | 15.8         | 17.6         | 15.6                         | 0.7 | 1.3 |
| <i>M. polleni</i>               | 3.4                          | 9.8          | 8.7          | 10.0         | 8.4          | 9.2          | 9.2          | 6.8          | -            | 10.5         | 9.5                          | 1.2 | 3.1 |
| <b><i>M. miafina</i> sp. n.</b> | <b>2.8</b>                   | 10.1         | 9.2          | 10.0         | 9.2          | 9.3          | 10.3         | 7.1          | 3.4          | -            | 8.9                          | 0.5 | 1.5 |
| <i>M. stumpffi</i>              | 3.4                          | 8.7          | 9.1          | 9.4          | 7.6          | 8.6          | 9.3          | 6.7          | 3.0          | 3.8          | -                            | 1.1 | 2.7 |

**Diagnosis.** A member of the genus *Madascincus* based on its molecular phylogenetic relationships (see Fig. 1). Within the genus *Madascincus*, *M. miafina* is distinguishable from all its congeners by the following combination of characters: medium body size with a maximum snout-vent length (SVL) of 61.0 mm (versus, in smaller species, a maximum SVL of 33.6 mm in *M. nanus* complex, 47.4

mm in *M. minutus*, 50.5 mm in *M. ankodabensis*, 53.5 mm in *M. melanopleura*); 65–79 rows of paravertebral scales (versus 51–62 in *M. melanopleura*, 57–65 in *M. minutus*, 52–62 in *M. ankodabensis*, 60–65 in *M. mouroundavae*, and 50–57 in *M. nanus* complex); 65–73 rows of ventral scales (versus 55–63 in *M. minutus*, 56–61 in *M. melanopleura*, 52–60 in *M. nanus* complex, 59–63 in



**Figure 3.** Drawings of the lateral and dorsal views of the heads of most of the species of *Madascincus*, including the holotypes of the two new species described herein. **A:** *Madascincus pyrurus* sp. n., holotype ZSM 520/2001 (MV 2001-445), Mont Ibity; **B:** *M. miafina* sp. n., holotype ZSM 1562/2008 (FGZC 1658), Ankarana Special Reserve; **C:** *M. arenicola*, holotype ZSM 1565/2008 (FGZC 1703), Baie des Sakalava; **D:** *M. polleni*, holotype MNHN 1895.210, “Mouroundava” (= Morondava); **E:** *M. stumpffi*, holotype SMF 16019, “Nossibé” (=Nosy Be); **F:** *M. igneocaudatus*, ZSM 1600/2010 (ZCMV 12888), Anakao; **G:** *M. mouroundavae*, ZSM 13/2005 (ZCMV 2254), Andasibe; **H:** *M. ankodabensis*, ZSM 355/2006 (ZCMV 2907), Ranomafana; **I:** *M. melanopleura*, ZSM 20/2005 (ZCMV 2266), Andasibe; **J:** *M. minutus*, ZSM 400/2005 (ZCMV 2166), Nosy Mangabe. Dwarf species of *Madascincus*, namely *M. nanus* and *M. macrolepis*, not represented. Scale bars = 2 mm. Abbreviations used for indication of scales (**A**, **B**) follow those defined by Miralles et al. (2011b).

*M. ankodabensis*, 63–66 in *M. mouroundavae*, 73–78 in *M. pyrurus*, 74–78 in *M. polleni* and 75–80 in *M. arenicola*); 18–23 subdigital lamellae under the fourth toes (versus 5–8 in *M. nanus* complex, 9–13 in *M. minutus*,

12–15 in *M. ankodabensis*, 12–16 in *M. melanopleura* and 15–18 in *M. pyrurus*); 24–26 rows of scales around midbody (versus in *M. nanus* complex, 28–30 in *M. mouroundavae*, 22–24 in *M. pyrurus* and 30–32 in *M. stumpf-*

*fi*); pentadactyl forelimbs (versus 3–5 digits in *M. nanus* complex); and most often (89.3%) the presence of postnasal scales (always absent in *M. arenicola*). The frontal is bell-shaped (versus hourglass-shaped in *M. nanus*, *M. minutus*, *M. melanopleura*, *M. ankodabensis*, *M. mouroundavae*, and in half (52.8%) of the specimens examined of *M. stumpffi*); the frontal is always separated from the interparietal (versus most often (87.5%) fused in *M. mouroundavae*). The lower eyelid window is scaly (versus spectacled in *M. igneocaudatus*, *M. pyrurus*, *M. minutus*, *M. melanopleura* and *M. ankodabensis*); absence in most specimens (92.3%) of a single row of enlarged nuchal scales (versus presence of at least two rows in *M. igneocaudatus*, *M. pyrurus* and *M. minutus*). More generally, *M. miafina* can be distinguished from all the other species (with exception of *M. polleni*) by its apparently very conserved pattern of coloration, characterized by a single pair of lateral dark brown stripes relatively large and well-defined anteriorly, then progressively breaking up into two parallel very thin dashed lines posteriorly to forelimbs, hardly distinguishable from the rest of the dots covering the body.

*Madascincus miafina* differs from its sister species *M. arenicola* by a paler coloration, with lateral lines well defined anteriorly, becoming one – or two parallel – very thin dashed line posteriorly to forelimbs (versus a very contrasted coloration in *M. arenicola*, characterized by the presence of a pair of two-scale wide dark lateral lines extending from snout to hindlimbs, well defined all along the body) and by a relatively shorter snout, rounded in lateral aspect (versus a relatively long snout, acute in lateral aspect, in *M. arenicola*). It also differs by a lower number of ventral scales (65–73 vs. 75–80 in *M. arenicola*). Moreover, *M. miafina* is one of the few species (together with *M. pyrurus* and *M. igneocaudatus*) in which the tail might be bright red in some specimens (see also Tables 1 and 2). Morphologically, the species most similar to *M. miafina* is *M. polleni* (including its junior synonym *M. intermedius*); this species is identical in coloration, body shape, and body size to *M. miafina* despite not being the direct sister species, differing only by the number of ventrals (see above).

**Description of the holotype (Fig. 3B).** ZSM 1562/2008 (FGZC 1658). Adult male, with both hemipenes everted. Good state of preservation, with exception of a little circular sampling incision on the left flank (ca. 5 mm of diameter). SVL (57.0 mm) 7.5 times head length (7.6 mm), almost as long as tail (79.1 mm, apparently entire and not regenerated). Limbs relatively short: SVL 5.6–5.8 times front limb length (9.9–10.2 mm) and 3.2–3.3 times hind limb length (17.3–18.0 mm). Snout relatively short and rounded on lateral aspect, with a rostral tip bluntly rounded in dorsal aspect. Rostral wider than high/long, contacting first supralabials, nasals, and supranasals. Paired supranasals in median contact, contacting loreals. Frontonasal roughly triangular, wider than long, contacting loreals, first supraciliaries and first supraoculars. Prefrontals absent. Frontal approximately as wide as long, wider

posteriorly, in contact with frontonasal, supraoculars, parietals and interparietal. Supraoculars four, all of them in contact with frontal; subequal in size, except for the posteriormost pair that is significantly smaller; the first pair not constricting frontal (frontal bell-shaped sensu Andreone and Greer 2002). Frontoparietals absent. Interparietal longer than wide, well separated from supraoculars; parietal eyespot present with parietal eye evident. Parietals contact posterior to interparietal. Absence of enlarged nuchals. Nasals just slightly larger than nostrils; contacting rostral, first supralabials, postnasals and supranasals. Postnasals present, separating supranasals from first supralabials, and nasals from loreals. Loreal single, slightly higher than long. Preocular trapezoidal, longer than high, single. Presubocular roughly square, single. Six supraciliaries on both sides, in continuous row; first and last pairs significantly larger and longer than the intermediate ones; last pair projecting onto supraocular shelf. Upper palpebrals small except for last which projects dorsomedially. Pretemporals two, both contacted by parietal. Postsuboculars two; upper contacting lower pretemporal; both contacting penultimate supralabial. Lower eyelid moveable, scaly; lower palpebrals small, longer than high, interdigitating with large polygonal scales of central eyelid. Contact between upper palpebrals and supraciliaries seemingly direct but flexible, i.e. palpebral cleft narrow. Primary temporal single. Secondary temporals two; upper long, contacting lower pretemporal anteriorly and overlapping lower secondary temporal ventrally. Two tertiary temporals bordering lower secondary temporal. Supralabials six, with the fourth being the enlarged subocular, contacting scales of the lower eyelid. Postsupralabial single. External ear opening round, without lobules. Mental wider than long, posterior margin convex. Postmental wider than long, contacting first two pairs of infralabials. Infralabials six. Three pairs of large chin scales, both members of first pair in contact, both members of second pair separated by a single median scale, and members of third pair separated by three scale rows. No scales extending between infralabials and large chin scales; two asymmetrical postgenials posterolaterally in contact with the third pair of chin scales. Gulars similar in size and outline to ventrals. All scales, except head shields and scales on palms, soles, and digits, cycloid, smooth, and imbricate; longitudinal scale rows at midbody 24; paravertebrals 68, similar in size to adjacent scales; ventrals 68. Inner preanals overlapped by outer. Both pairs of limbs pentadactyl; fingers and toes relatively short, clawed; relative length of toes in the following order: I<II<V<III<IV. Subdigital lamellae smooth, single, with 8 under right fourth finger and 7 under left fourth finger, 18 under right fourth toe, and 20 under left fourth toe.

Coloration of the holotype in preservative with a pair of lateral dark brown stripes (about two scales wide on the neck) relatively large and well defined anteriorly (overlapping rostral, mental, first four supralabials, loreals, and presuboculars), then progressively breaking up into two parallel very thin dashed lines posteriorly



**Figure 4.** Photographic plate showing most of the recognized species of *Madascincus* (picture not available for *M. macrolepis*), highlighting the chromatic polymorphism (red tail and brown tail morphs) for *M. miafina* sp. n., *M. pyrurus* sp. n. and *M. igneocaudatus*. Picture I depicts the sole specimen known from Kirindy that in Glaw and Vences (2007) was assigned to a candidate species *M. sp.* “vitreus”, and O depicts a specimen that these authors assigned to a candidate species *M. sp.* “baeus”.

to forelimbs, hardly distinguishable from the rest of the dots covering the body. Dorsum and dorsal sides of forelimbs, hindlimbs and tail light bronze. The bronze dorsal field and flanks are covered by numerous little dark dots, each of them in the middle of a dorsal scale, in contact with its posterior edge; resulting in many thin dash lines (14 to 16 at midbody, including the dark lateral stripes), darker and more contrasted in the posterior part of the dorsum, then posteriorly becoming progressively indistinguishable from the background coloration of tail, and laterally, becoming progressively indistinguishable from the light coloration of the ventral field. No distinct border

between the background coloration of the dorsal and the ventral sides. Immaculate whitish ventral field extending from lower side of head (mental excluded), throat, lower side of limbs and venter, to the ventral side of tail. Palms and soles barely darker than venter. Coloration in life was almost identical to the coloration in preservative, with the only significant difference being the presence of iridescent glints of scales and a venter with some violet-pinkish tint (*cf.* Figs 4J, K).

**Variation.** For variation in measurements and scale characters see Table 1. Some variation is evident with respect

to overall dorsal and tail coloration. The paratype from Ankarana was more or less similar to the holotype described above, with a bronze dorsal color predominating the dorsum and tail. At Montagne des Français, specimens were generally darker, with a bronze-brown dorsal coloration and a reddish brown tail dorsally and red-orange tail ventrally.

**Etymology.** The specific epithet *miafina* is the Malagasy word for “secretive”. The name refers to the secretive habits of the species, as all specimens were exclusively trapped by pitfalls and never observed in situ, as well as to the fact that this species was hidden behind several other taxon names in use and could only be discovered by an integrative taxonomic approach. The name is treated as an invariable noun in apposition.

**Distribution, habitat and habits.** The species is known from northernmost Madagascar including at least four localities (see localities of type specimens above and Fig. 5) with karstic outcrops and sandy soils. The species apparently has nocturnal and secretive habits, as all specimens were exclusively caught by pitfall trapping overnight in forest or shrub areas. The species can therefore be considered to represent a leaf litter dweller. The new species occurs in sympatry with *M. arenicola* and *M. stumpffi*. Hence, it appears to tolerate a rather wide range of habitat conditions, whereas *M. arenicola* exclusively occurs on sandy soils and *M. stumpffi* seems to be restricted to forests. Nothing else is known on the natural history of the new species.

#### *Madascincus pyrurus* sp. n.

<http://zoobank.org/2A2D2E29-6FE3-4964-A17D-624BAEF8C842>

Figs 3A, 4D, E

**Holotype.** ZSM 520/2001 (MV 2001-445), adult male from Mont Ibity, approximately at 20°14'S, 47°03'E, 1700–1800 m above sea level, Fianarantsoa province, central Madagascar, collected on 10 March 2001 by M. Vences, D.R. Vieites, L. Raharivololoniaina and D. Rakotomalala.

**Paratypes (n=7).** MNHN 1980-1217, Mont Ibity, Fianarantsoa province, central Madagascar, coll. by Y. Thérézien and R. Capuron; ZSM 518–519/2001 (MV 2001-441, 2001-444), UADBA uncatalogued (MV2001.442 and 443), same data as holotype; ZSM 521/2001 (MV 2001-611), UADBA uncatalogued (MV2001.610), Itremo (camp, 20°36'08"S, 46°34'16"E, 1648 m a.s.l.), Fianarantsoa province, coll. on 10 March 2001 by M. Vences, D.R. Vieites, L. Raharivololoniaina and D. Rakotomalala.

#### Chresonyms.

*Scelotes igneocaudatus* – Blanc (1967), Brygoo (1981, *partim*);

*Amphiglossus igneocaudatus* – Brygoo (1984, *partim*), Whiting et al. (2004), Schmitz et al. (2005);

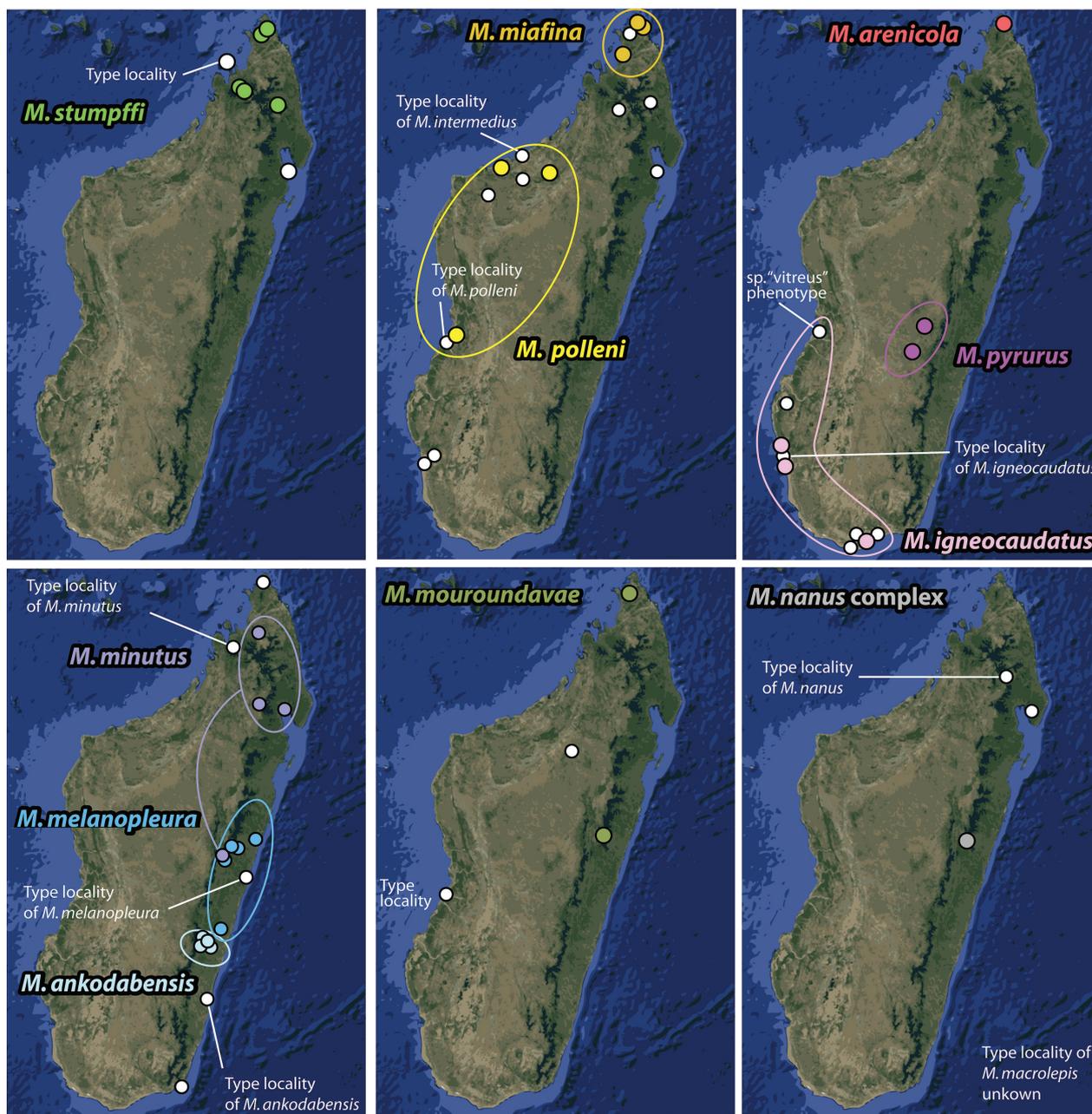
*Madascincus igneocaudatus* – Glaw and Vences (2007, *partim*), Crottini et al. (2009; *partim*), Miralles et al. (2011a, c, *partim*; 2011b);

*Madascincus igneocaudatus* “*igneocaudatus*-C clade” – Miralles and Vences (2013);

*Madascincus* sp. “*igneocaudatus*” central clade – Miralles et al. (2015).

**Diagnosis.** A member of the genus *Madascincus* based on its molecular phylogenetic relationships (see Fig. 1). Within the genus *Madascincus*, *M. pyrurus* is distinguished from all its congeners by the following combination of characters: medium body size with a maximum snout-vent length (SVL) of 54.2 mm (versus, in smaller species, a maximum SVL of 33.6 mm in *M. nanus* complex); 71–79 rows of paravertebral scales (versus 51–62 in *M. melanopleura*, 57–65 in *M. minutus*, 52–62 in *M. ankodabensis*, 60–65 in *M. mouroundavae*, and 50–57 in *M. nanus* complex); 73–78 rows of ventral scales (versus 55–63 in *M. minutus*, 56–61 in *M. melanopleura*, 52–60 in *M. nanus* complex, 59–63 in *M. ankodabensis*, 65–73 in *M. miafina*, 63–66 in *M. mouroundavae*); 15–18 subdigital lamellae under the fourth toes (versus 5–8 in *M. nanus* complex, 9–13 in *M. minutus*, 18–23 in *M. miafina* and 12–15 in *M. ankodabensis*); 22–24 rows of scales around midbody (versus 18–20 in *M. nanus* complex, 28–30 in *M. mouroundavae*, 24–26 in *M. igneocaudatus* and 30–32 in *M. stumpffi*); pentadactyl forelimbs (versus 3–5 digits in *M. nanus* complex; the presence of postnasal scales (always absent in *M. arenicola*); the frontal is bell-shaped (versus hourglass shaped in *M. nanus*, *M. minutus*, *M. melanopleura*, *M. ankodabensis*, *M. mouroundavae*, and in half (52.8%) of the specimen examined of *M. stumpffi*); the frontal is always separated from the interparietal (versus most often (87.5%) fused in *M. mouroundavae*); lower eyelid window is spectacled (versus scaly in *M. arenicola*, *M. nanus*, *M. mouroundavae*, *M. polleni*, *M. miafina* and *M. stumpffi*), the presence of two (21.4%) or three (78.6%) rows of enlarged nuchal scales (versus absence or presence of a single row in *M. nanus*, *M. mouroundavae*, *M. miafina* and *M. stumpffi*). More specifically, *M. pyrurus* differs from its sister species *M. igneocaudatus* in having a shorter and rounder snout (versus a relatively long and pointed snout usually characterizing semi-fossorial species found in sandy habitat) and in being oviparous (versus viviparous). More generally, *M. pyrurus* can also be easily distinguished from all the other members of the genus *Madascincus* by its very characteristic pattern of coloration, being the only species with six well-defined very dark stripes (a pair of thin dorsal, a pair of wide upper lateral and a pair of thin lower lateral stripes) running along the body, and one of the few species (together with *M. miafina* and *M. igneocaudatus*) in which the tail might be bright red in some specimens (see also Tables 1 and 2).

**Description of the holotype.** ZSM 520/2001 (MV 2001-445, Fig. 3A). Adult male, with both hemipenes everted.



**Figure 5.** Distribution maps for *Madascincus* species. Colored dots are representing localities sampled in the molecular studies by Miralles and Vences (2013), whereas white dots are representing localities of specimens only identified by morphology, and/or type localities. For *M. polleni*, another locality has been added based on a recent paper from Rakotoarison et al. (2015), who have collected an additional specimen (ZCMV 14157) in Mitsinjo forest in north-western Madagascar (16°02'54.5"S, 45°47'24.1"E). The identity of this specimen is confirmed by both its morphological characteristics and its phylogenetic position inferred from a segment of the 16S rRNA gene (nested within the *M. polleni* clade, and closely related to the population of Ankarafanstika, GenBank accession number KR025911). Note that for some species, especially *M. mouroundavae*, additional verified localities exist but these are here excluded for consistency because no samples were examined in the framework of the present study.

Good state of preservation, with exception of a little incision on the right flank (ca. 10 mm). SVL (49.0 mm) 7.1 times head length (6.9 mm), shorter than the tail (58.0 mm, apparently regenerated). Limbs relatively short: SVL 6.2–6.5 times front limb length (7.5–7.9 mm) and 3.4 times hind limb length (14.4–15.0 mm). Snout relatively short and rounded on lateral aspect, with a rostral tip bluntly rounded in dorsal aspect. Rostral wider

than high/long, contacting first supralabials, nasals, and supranasals. Paired supranasals in median contact, contacting loreals. Frontonasal roughly pentagonal, wider than long, contacting loreals, and first suproculars. Pre-frontals absent. Frontal longer than wide, wider posteriorly, in contact with frontonasal, supraoculars, parietals and interparietal. Supraoculars four, all of them in contact with frontal; the second anteriormost pair larger and the

posteriormost pair significantly smaller; the first pair not constricting frontal (bell-shaped). Frontoparietals absent. Interparietal longer than wide, well separated from supraoculars; parietal eyespot present but poorly marked. Parietals contact posterior to interparietal. Two pairs of primary nuchal scales (with two cycloids scales inserted between the parietal and the first nuchal, on the right side). Nasals slightly larger than nostrils; contacting rostral, first supralabials, postnasals and supranasals. Postnasals present, separating supranasals from first supralabials, and nasals from loreals. Loreal single, as high as long. Preocular higher than long, single. Presubocular single. Six supraciliaries on both sides, in continuous row; last pair slightly larger than the others, and projecting onto supraocular shelf. Pretemporals two, both contacted by parietal. Postsuboculars two; upper contacting lower pretemporal; both contacting penultimate supralabial. Lower eyelid moveable, with a transparent and undivided disc, and one row of small scales across its dorsal edge. Contact between upper palpebrals and supraciliaries seemingly direct but flexible, i.e. palpebral cleft narrow. Primary temporal single. Secondary temporals two; the upper one elongated, contacting lower pretemporal anteriorly and overlapping lower secondary temporal ventrally. Two tertiary temporals bordering lower secondary temporal. Supralabials six, with the fourth being the enlarged subocular contacting scales of the lower eyelid. Postsupralabial single. External ear opening roughly triangular, without lobules. Mental wider than long, posterior margin straight. Postmental wider than long, contacting first two pairs of infralabials. Infralabials six. Three pairs of large chin scales, both members of first pair in contact, both members of second pair separated by a single median scale, and members of third pair separated by three scale rows. No scales extending between infralabials and large chin scales; two asymmetrical postgenials posterolaterally in contact with the third pair of chin scales. Gulars similar in size and outline to ventrals. All scales, except head shields and scales on palms, soles, and digits, cycloid, smooth, and imbricate; longitudinal scale rows at midbody 22; paravertebrals 71, similar in size to adjacent scales; ventrals 73. Inner preanals overlapped by outer. Both pairs of limbs pentadactyl; fingers and toes relatively short, clawed; relative length of toes in the following order: I<II=V<III<IV. Subdigital lamellae smooth, single, 10 under fourth finger of both left and right manus, 17 under right fourth toe, and 15 under left fourth toe.

Coloration in preservative with upper side of the head, neck, back, limbs, and tail dark bronze. Venter, lower side of head, throat, lower side of limbs, and tail whitish/cream. Lateral borders on the ventral side maculated by very small dark dots. Six very well defined very dark stripes run along the body, continuing along the first third of the tail, then abruptly ending where the tail is regenerated. Two thin blackish dorsal stripes formed by succession of contiguous dots start on the supranasal; at midbody, each dorsolateral stripe is less than one scale wide and both are separated by two rows of dorsal

scales. Two wide dark brown upper lateral stripes; margins slightly darker and very sharp; about two scales wide at midbody and overlying three rows of scales; starting from the rostral, where the stripes all meet, extending on the upper half of each supralabial, the loreals, around the eyes, above ear opening, and above forelimbs and hindlimbs. Two thin dark lower lateral stripes, starting on the last infralabials, extending through the forelimb and hindlimb insertion. At midbody, each lower lateral stripe is less than one scale wide, with irregular margin. Four very light stripes run along the body, continuing along the first third of tail, then abruptly ending where the tail is regenerated. Two whitish dorsolateral stripes separate dark dorsal stripes from the upper lateral dark stripes; about one scale wide at midbody and overlying two rows of scales. Two whitish lateral stripes separating the dark upper lateral stripes from the dark lower lateral stripes; about one scale wide at midbody and overlying two rows of scales. Regenerated part of the tail cream, maculate with many small dark dots on the dorsal side. Palms and soles darker than the ventral side.

Life coloration for the holotype has not been documented, but it is apparently very similar to the coloration in preservative, with exception of the tail which is usually bright red, or pinkish brown in some specimens (cf. Fig. 4D, E).

**Variation.** For variation in measurements and scale characters see Table 1. Some variation is evident with respect to tail coloration which may be bronze or reddish (cf. Fig. 4D, E) and ventral coloration which may be maculated by small black dots or uniformly whitish (Blanc and Blanc 1967).

**Etymology.** The specific epithet *pyrurus* is based on Greek roots *pûr* (πῦρ) and *ourá* (οὐρά), respectively meaning “fire” and “tail”. This word is here treated as invariable noun and has the same meaning as an other specific epithet in the genus, *igneocaudatus*, which is based on Latin roots. This epithet has been chosen to highlight the morphological similarity of *M. igneocaudatus* and *M. pyrurus*, both these sister species being characterized by a tail which may be red and reminding fire.

**Distribution, habitat and habits.** The species is known from the dry environments on two massifs in the central highlands of Madagascar, in Mont Ibity and in Itremo (Fig. 5), dominated by rock outcrops and tapia woodlands (loose forests of *Uacapa bojeri* trees). This montane lizard has been found at altitudes ranging from 1648 to 1922 m a.s.l. in Itremo, and up to 2252 m a.s.l. in Ibity (Brygoo 1984, present study). It can be commonly found under stones on usually somewhat sandy substrate (quartz sand especially on Mont Ibity), in open areas. On Mont Ibity, 6 eggs of *M. pyrurus* have been collected at the end of December which measured 18 × 12 mm, the hatched juveniles measuring 25 mm SVL (Blanc and Blanc 1967, Brygoo 1984). In contrast, in *M. igneocaudatus*, developed juveniles have been found in a female dissected from Ifaty, indicating that the sister species of *M. pyrurus*, endemic to the dry low-

lands of the western coast, is viviparous (Glaw and Vences 2007). Interestingly, the different modes of reproduction observed in these two sister species appear to be in contradiction with the trend usually observed in lizard taxa

having both oviparous and viviparous species/populations: typically, evolution to viviparity in these groups is interpreted as local adaptation to cold climates, either at high altitude or latitude (Pianka and Vitt 2003).

**Synopsis and identification key**

A list of the species currently recognized, including information supporting their respective taxonomic validity is presented in Table 4. To facilitate future taxonomic work, we furthermore elaborated the following identification key for species of *Madascincus*. The key is based on the selection of the features we considered to be the most diagnostic and easily observable.

- a postnasal always absent.....*M. arenicola*
- a' postnasal mostly present (rarely absent in *M. miafina* and *M. stumpffi*).....b
- b 28 or more scale rows at midbody.....c
- b' 26 or less scale rows at midbody.....d
- c 30–32 scale rows at midbody, 76–88 paravertebral scale rows, 70–88 ventral scale rows, frontal and interparietal separated together ..... *M. stumpffi*
- c' 28–30 scale rows at midbody, 60–65 paravertebral scale rows, 63–66 ventral scale rows, frontal and interparietal mostly fused together .....*M. mouroundavae*
- d 18–20 scale rows at midbody, 8 or less lamellae under 4<sup>th</sup> toe, frequently less than 5 fingers, adult SVL < 35 mm, an atypical head shape with a short and acuminate snout and relatively large eyes ..... *M. nanus* complex
- d' 22–26 scale rows at midbody, 9 or more lamellae under 4<sup>th</sup> toe, always 5 fingers, SVL in adult > 40 mm .....e
- e Frontal bell-shaped, 65 or more ventral scale rows, 65 or more paravertebral scale rows, tail frequently red colored.....f
- e' Frontal hour-glass shaped, 63 or less ventral scale rows, 65 or less paravertebral scale rows, tail always brown colored.....*M. melanopleura* complex (including also *M. ankodabensis* and *M. minutus*)
- f Two lateral dark brown stripes relatively large and well defined anteriorly, then progressively breaking up into two parallel very thin dashed lines posteriorly to forelimbs, hardly distinguishable from the rest of the dots covering the body, lower eyelid scaly .....g
- f' Four or six well defined and brightly contrasted dark stripes running along the body, lower eyelid spectaclled.....h
- g 65–73 ventral scale rows ..... *M. miafina*
- g' 74–78 ventral scale rows ..... *M. polleni*
- h A relatively short and rounded snout, six well defined and contrasted stripes running along the body, 22–24 scale rows around midbody..... *M. pyrurus*
- h' A relatively long and pointed snout, four well defined and contrasted stripes running along the body, 24–26 scale rows around midbody..... *M. igneocaudatus*

**Discussion**

**Taxonomy in the genus *Madascincus*.** By applying the results supported by the ITAX approach of Miralles and Vences (2013), eleven distinct species within their *Madascincus* sampling can presently be recognized for this genus. This number might be increased to 12 if *M. macrolepis* is included. It is worthwhile to remind here that we are following Miralles and Vences (2013, Supporting information 1) who synonymized *Madascincus intermedius* (Boettger, 1913) - type locality “Majunga” = Mahajanga, with *Madascincus polleni* (Grandidier, 1869) - type locality “Morondava”, both type localities unambiguously corresponding to conspecific populations of the *polleni*-S clade.

**Phylogenetic relationships.** In their previous work, Miralles and Vences (2013) published multilocus phylogenetic results used to delimit species but did not discuss the results from biogeographic and phylogeographic perspectives.

The different phylogenetic inferences applied (separated phylogenetic Bayesian analysis based on the

mtDNA; (Fig. 1), retrieved the monophyly of the 11 recognized species sampled in this study. These different approaches also agreed on the existence of five main clades strongly supported within the genus *Madascincus*: (1) the *M. polleni* clade (*M. arenicola*, *M. stumpffi*, *M. polleni* and *M. miafina*); (2) *M. mouroundavae*; (3) the *M. igneocaudatus* clade (*M. igneocaudatus* and *M. pyrurus*); (4) the *M. melanopleura* clade (*M. ankodabensis*, *M. melanopleura* and *M. minutus*); and (5) *M. nanus*. Their tree derived from the concatenated nuclear data set was congruent in topology with the mtDNA tree, with only two exceptions: (1) the relative positions of the *M. igneocaudatus* clade and *M. mouroundavae* clade are inverted and (2) the monophyly of *Madascincus* is recovered with exclusion of *Paracontias*. The species tree presented by Miralles and Vences (2013, Supporting information S6e), inferred by \*BEAST and combining mtDNA and nDNA agreed with the mtDNA Bayesian tree.

Two of these clades reveal a relevant biogeographical component: (1) The *M. polleni* clade includes four species

**Table 4.** List of the species presently recognized in the genus *Madascincus*, with the different lines of evidences supporting their distinctiveness. MSD = Methods of species delimitation.

| Taxa   | Morphological diagnosability   | Monophyly   | Species delimitation methods  |
|--|--|---|---|
| <b><i>M. arenicola</i> Miralles, Köhler, Glaw and Vences, 2011</b> | Unambiguously diagnosable  | Monophyletic (mtDNA / nDNA)   | Supported by six of seven MSD, HW merging <i>M. arenicola</i> , <i>M. polleni</i> , and <i>M. stumpffi</i> into a single species.   |
| <b><i>M. mouroundavae</i> (Grandidier, 1872)</b>                   | Unambiguously diagnosable  | Monophyletic (mtDNA / nDNA)   | Supported by all seven MSD.   |
| <b><i>M. stumpffi</i> (Boettger, 1882)</b>                         | Unambiguously diagnosable  | Monophyletic (mtDNA / nDNA)   | Supported by six of the seven MSD, HW merging <i>M. arenicola</i> , <i>M. polleni</i> , and <i>M. stumpffi</i> into a single species.   |
| <b><i>M. nanus</i> (Andreone and Greer, 2002)</b>                  | Unambiguously distinguishable from most of the species (see below <i>M. macrolepis</i> )<br><i>Taxonomic comment:</i> Miralles and Vences (2013) treated <i>M. sp.</i> “baeus” provisionally as conspecific with <i>M. nanus</i> , pending molecular analysis of topotypic <i>M. nanus</i>   | Monophyletic (mtDNA / nDNA)   | Supported by all seven MSD.   |
| <b><i>M. macrolepis</i> (Boulenger, 1888)</b>                      | Unambiguously distinguishable from most of the species (see species delimitation)<br><i>Taxonomic comment:</i> Almost certainly closely related to <i>M. nanus</i> due to numerous morphological similarities, it seems to differ from the latter by several morphological characters (Andreone and Greer, 2002), leading us to consider both taxa as distinct pending more detailed investigations.   | Not tested  | Rare species so far not studied using molecular methods and therefore not included in Miralles and Vences (2013).   |
| <b><i>M. polleni</i> (Grandidier, 1869)</b>                        | Unambiguously distinguishable from almost all other species of <i>Madascincus</i> , but superficially very similar to <i>M. miafina</i> . Both species only differ by a single character, the number of ventral scale rows (65–73 in <i>M. miafina</i> and 74–78 in <i>M. polleni</i> ) which is likely to become less diagnostic once a larger sampling will reveal the true extent of intraspecific variation of this character<br><i>Taxonomic comment:</i> Referred to as <i>polleni</i> -S clade in Miralles and Vences (2013). According to type examinations and/or type localities, and in application of the principles of nomenclature (ICZN 1999), the name <i>M. polleni</i> , type locality (TL) “Morondava”, is here restricted to the <i>polleni</i> -S clade. <i>Scelotes intermedius</i> Boettger, 1913, type locality “Majunga” = Mahajanga, has been considered as a subjective junior synonym of <i>M. polleni</i> by Miralles and Vences (2013).  | Monophyletic (mtDNA / nDNA), not closely related to <i>M. miafina</i> despite very similar phenotypes | Supported by six of the seven MSD, HW merging <i>M. arenicola</i> , <i>M. polleni</i> , and <i>M. stumpffi</i> into a single species.   |
| <b><i>M. miafina</i> sp. n.</b>                                    | Unambiguously distinguishable from almost all other species of <i>Madascincus</i> , but superficially very similar to <i>M. polleni</i> (see above)<br><i>Taxonomic comment:</i> referred as <i>polleni</i> -N clade in Miralles and Vences (2013). Herein described as a new species.   | Monophyletic (mtDNA / nDNA).  | Supported by all seven MSD.   |
| <b><i>M. igneocaudatus</i> (Grandidier, 1867)</b>                  | Unambiguously diagnosable<br><i>Taxonomic comment:</i> Referred to as <i>igneocaudatus</i> -S clade in Miralles and Vences (2013). The type material fits morphologically very well with the specimens sequenced from this clade, and its type locality is unambiguously nested within its distribution area (see Fig. 5). According to type examinations and/or type localities, and in application of the principles of nomenclature (ICZN 1999), the name <i>M. igneocaudatus</i> , type locality “Tuléar” (= Toliara), is here restricted to the <i>igneocaudatus</i> -S clade.  | Monophyletic (mtDNA / nDNA).  | Supported by all seven MSD.   |
| <b><i>M. pyrusus</i> sp. n.</b>                                    | Unambiguously diagnosable<br><i>Taxonomic comment:</i> Referred as <i>igneocaudatus</i> -C clade in Miralles and Vences (2013). Herein described as a new species.   | Monophyletic (mtDNA / nDNA)   | Supported by all seven MSD.   |
| <b><i>M. melanopleura</i> (Günther, 1877)</b>                      | Species of the <i>M. melanopleura</i> complex are well diagnosable from all the other species of <i>Madascincus</i> , but the three species of this complex are apparently not diagnosable by their morphology alone (see. <i>M. ankodabensis</i> and <i>M. minutus</i> )<br><i>Taxonomic comment:</i> Referred as <i>melanopleura</i> -C clade in Miralles and Vences (2013). According to type examinations and/or type localities, and in application of the principles of nomenclature (ICZN 1999), the name <i>Madascincus melanopleura</i> , type locality “Anzahamaru” (close to Mahanoro in the central eastern lowlands), is here restricted to the <i>melanopleura</i> -C clade (including <i>Sepsina vulsini</i> Barbour, 1918, type locality “eastern forest between Tamatave and Tananarive”, as a junior synonym). This type locality is indeed nested very centrally within the distribution area of the C-clade, making it very probable that the type specimen of <i>Madascincus melanopleura</i> is a member of this clade predominantly present in that central region (cf. Fig. 5). It should nevertheless be highlighted that due to the presence of one outlier sample in the Miralles and Vences (2015) data set, which unambiguously clustered with the northern clade but geographically is nested within the central clade distribution area (in An’Ala, a locality relatively close to the type locality of <i>M. melanopleura</i> ), we cannot totally discard the possible – past or present – sympatric occurrence of <i>M. minutus</i> at Anzahamaru. | Monophyletic (mtDNA / nDNA)   | Supported by five of the seven MSD, MTMC and WP merging all the species of the <i>M. melanopleura</i> species complex (namely <i>M. melanopleura</i> , <i>M. ankodabensis</i> and <i>M. minutus</i> ) into a single species. Miralles and Vences (2013) have nevertheless found evidence supporting the distinctiveness of these three taxa (e.g., absence of gene flow between <i>M. melanopleura</i> and <i>M. minutus</i> despite their sympatric occurrence in An’Ala). |

| Taxa  | Morphological diagnosability   | Monophyly                   | Species delimitation methods   |
|---|--|-----------------------------|--|
| <b><i>M. ankodabensis</i> (Angel, 1930)</b>             | Species of the <i>M. melanopleura</i> complex are well diagnosable from all the other species of <i>Madascincus</i> , but the three species of this complex are apparently not diagnosable by their morphology alone (see. <i>M. melanopleura</i> and <i>M. minutus</i> )  | Monophyletic (mtDNA / nDNA) | Supported by five of the seven MSD (see discussion on <i>M. melanopleura</i> above).   |
|   | <i>Taxonomic comment:</i> Referred as <i>melanopleura</i> -S clade in Miralles and Vences (2013). In application of the principles of nomenclature (ICZN 1999), the name <i>Madascincus ankodabensis</i> , type locality “Ankodabe”, is here applied to the <i>melanopleura</i> -S clade based on the fact that (1) the morphology of the type material fits remarkably well with the sequenced specimens of this clade and (2) the S clade is the most southern one put into evidence by Miralles and Vences (2013) and therefore the only one fitting with this type locality (see Fig 5). |                             |  |
| <b><i>M. minutus</i> (Raxworthy and Nussbaum, 1993)</b> | Species of the <i>M. melanopleura</i> complex are well diagnosable from all the other species of <i>Madascincus</i> , but the three species of this complex are apparently not diagnosable by their morphology alone (see. <i>M. ankodabensis</i> and <i>M. melanopleura</i> )   | Monophyletic (mtDNA / nDNA) | Supported by five of the seven MSD (see discussion on <i>M. melanopleura</i> above). In contrast with other species of the <i>M. melanopleura</i> species complex, the taxonomy of the <i>melanopleura</i> -N clade remains insufficiently understood. The ITAX approach suggested the existence of at least two genetically distinct but morphologically cryptic species within this group, whereas other approaches suggested up to six cryptic species, all occurring in allopatry but partly in close spatial proximity. A more complete sampling (both in terms of number of localities and number of samples per population) is required to investigate more into detail the taxonomy of this species complex. |
|   | <i>Taxonomic comment:</i> Referred to as <i>melanopleura</i> -N clade in Miralles and Vences (2013). Due to sampling gaps, Miralles and Vences (2013) were unable to assign the name <i>M. minutus</i> (Raxworthy and Nussbaum, 1993), type locality “Manongarivo”, to one of these identified clades and therefore proposed conservatively to consider the whole <i>melanopleura</i> -N group as a single species <i>M. minutus</i> , pending a more detailed taxonomic revision.   |                             |  |

apparently restricted to the western and northern regions of Madagascar, and a diversification concentrated in the northern tip of the island (Fig. 5). All species in this clade inhabit leaf-litter of dry deciduous forests or shrubs, or in more open and sandy areas. On the contrary, (2) the *M. melanopleura* clade, which includes at least three species morphologically extremely similar, is mostly restricted to the eastern part of Madagascar, with species essentially inhabiting rainforest leaf litter.

In contrast, the *M. igneocaudatus* clade does not present any obvious shared biogeographic characteristic. *Madascincus igneocaudatus* is indeed endemic to the dry lowlands of the south-western and southern coasts of Madagascar, whereas *M. pyrurus* is a montane species only known from the central highlands of Madagascar. The ranges of *M. nanus* and of *M. mouroundavae* are less accurately understood, and more studies will be necessary to better elucidate the systematics and the biogeography of these taxa. Nevertheless, both groups appear to be restricted to the rainforests and transitional forests in the northern half of Madagascar. The holotype of *M. mouroundavae* has been described by Grandidier (1872) from Morondava, on the central west coast of Madagascar, but no other specimens of this species have ever been collected subsequently in this area, suggesting that its type locality might be erroneous. Taking into account these data and the internal topology of the phylogenetic tree (Fig. 1), we hypothesize that the genus *Madascincus* has likely originated in the humid central-eastern part of Madagascar. Subsequently, diversification led to species colonizing the dry lowlands of the western, southern and northern parts of the island, where the other quadrupedal Malagasy scincine

skinks (genus *Amphiglossus* sensu stricto, and two new genera which will be described by Erens et al. in press) are absent or significantly under-represented (Glaw and Vences 2007). More specifically, the northern part of the island represents a center of diversification for the *M. polleni* species complex (sensu Miralles et al. 2011a) with a diversification trend oriented toward the north. Similar biogeographical patterns are also observed in the sister lineage of *Madascincus* (genus *Paracontias*, Miralles et al. 2016) and in several other squamates (Brown et al. 2014, 2016).

## Acknowledgments

Research in Madagascar was made possible in the framework of collaboration agreements among the Université d’Antananarivo (Faculté des Sciences – Mention Biologie Animale), the Direction des Eaux et Forêts, Madagascar, the Zoologische Staatssammlung München, and the Zoological Institute of TU Braunschweig. We are indebted to the Malagasy institutions for granting research and export permits, to M. Kondermann and G. Keunecke who assisted with labwork, to P. Bora, N. D’Cruze, H. Enting, M. Franzen, A. Knoll, S. Megson, Z. Nagy, L. Raharivololoniaina, D. Rakotomalala and D. R. Vieites for help with fieldwork, and to various curators for providing access to collections under their care. We thank A. Bauer, O. Hawlitschek, J. Nopper and A. Schmitz for critically reviewing the manuscript. We are grateful to the Volkswagen Foundation for supporting fieldwork in Madagascar, and to the Humboldt Foundation for a post-doctoral fellowship to AM.

## References

- Andreone F, Greer AE (2002) Malagasy scincid lizards: descriptions of nine new species, with notes on the morphology, reproduction and taxonomy of some previously described species (Reptilia, Squamata: Scincidae). *Journal of Zoology* 258: 139–181. doi: 10.1017/S0952836902001280
- Blanc CP, Blanc F (1967) Observations biologiques (adultes, oeufs, jeunes) sur quelques Sauriens du Mont Bity. *Annales Université Madagascar (Sciences)* 5: 67–74.
- Brown JL, Cameron A, Yoder AD, Vences M (2014) A necessarily complex model to explain the biogeography of the amphibians and reptiles of Madagascar. *Nature Communications* 5: e5046. doi: 10.1038/ncomms6046
- Brown JL, Sillero N, Glaw F, Bora P, Vieites DR, Vences M (2016) Spatial biodiversity patterns of Madagascar's amphibians and reptiles. *PLoS ONE* 11: e0144076. doi: 10.1371/journal.pone.0144076
- Brygoo ER (1981) Systématique des lézards scincidés de la région malgache. IX. Nouvelles unités taxinomiques pour les *Scelotes* s. l. *Bulletin du Muséum national d'Histoire naturelle. Section A*, 3(4): 1193–1204.
- Brygoo ER (1984) Systématique des lézards scincidés de la région malgache. XV. *Gongylus igneocaudatus* A. Grandidier, 1867, et *Scelotes intermedius* Boettger, 1913. Les *Amphiglossus* du groupe *igneocaudatus*. *Bulletin du Muséum national d'Histoire naturelle. Section A*, 6(3): 779–789.
- Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657–1660. doi: 10.1046/j.1365-294x.2000.01020.x
- Crottini A, Dordel J, Köhler J, Glaw F, Schmitz A, Vences M (2009) A multilocus phylogeny of Malagasy scincid lizards elucidates the relationships of the fossorial genera *Androngo* and *Cryptoscincus*. *Molecular Phylogenetics and Evolution* 53: 345–350. doi: 10.1016/j.ympev.2009.05.024
- Dayrat B (2005) Toward integrative taxonomy. *Biological Journal of the Linnean Society* 85: 407–415. doi: 10.1111/j.1095-8312.2005.00503.x
- DeSalle R, Egan MG, Siddal M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B* 360: 1905–1916. doi: 10.1098/rstb.2005.1722
- Doyle J (1995) The irrelevance of allele tree topologies for species delimitation, and a non-topological alternative. *Systematic Botany* 20: 574–588. doi: 10.2307/2419811
- Erens J, Miralles A, Glaw F, Chatrou L, Vences M (in press) Extended molecular phylogenetics and revised systematics of Malagasy scincine lizards. *Molecular Phylogenetic and Evolution*.
- Flot J-F, Couloux A, Tillier S (2010) Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus *Pocillopora* in Clipperton. *BMC Evolutionary Biology* 10: e372. doi: 10.1186/1471-2148-10-372
- Fontaneto D, Herniou E, Boschetti C, Caprioli M, Melone G, Ricci C, Barraclough TG (2007) Independently evolving species in asexual bdelloid rotifers. *PLoS Biology* 5: e87. doi: 10.1371/journal.pbio.0050087
- Glaw F, Vences M (2007) A field guide to the amphibians and reptiles of Madagascar. Third edition. Vences & Glaw Verlag, Cologne, 496 pp.
- Grandidier MA (1872) Description de quelques Reptiles nouveaux, découverts à Madagascar en 1870. *Annales des Sciences naturelles (Zoologie)* (5) 15 art. 20: 6–11.
- ICZN (1999) International Code of Zoological Nomenclature. The International Trust for Zoological Nomenclature, London.
- Kaiser H, Crother BI, Kelly CMR, Luiselli L, O'Shea M, Ota H, Passos P, Schleich WD, Wüster W (2013) Best practices: in the 21st Century, taxonomic decisions in herpetology are acceptable only when supported by a body of evidence and published via peer-review. *Herpetological Review* 44: 8–23.
- Miralles A (2006) A new species of *Mabuya* (Reptilia, Squamata, Scincidae) from the isolated Caribbean island of San Andrés, with a new interpretation of nuchal scales, character of systematic importance. *Herpetological Journal* 16: 1–7.
- Miralles A, Hipsley CA, Erens J, Gehara M, Rakotoarison A, Glaw F, Müller J, Vences M (2015) Distinct patterns of desynchronized limb regression in Malagasy scincine lizards (Squamata, Scincidae). *PLoS ONE* 10(6): e0126074. doi:10.1371/journal.pone.0126074
- Miralles A, Jono T, Mori A, Gandola R, Erens J, Köhler J, Glaw F, Vences M (2016) A new perspective on the reduction of cephalic scales in fossorial legless skinks (Squamata, Scincidae). *Zoologica Scripta* 45: 380–393. doi: 10.1111/zsc.12164
- Miralles A, Köhler J, Glaw F, Vences M (2011a) A molecular phylogeny of the "*Madascincus polleni* species complex", with description of a new species of scincid lizard from the coastal dune area of northern Madagascar. *Zootaxa* 2876: 1–16.
- Miralles A, Köhler J, Vieites DR, Glaw F, Vences M (2011c) Developing hypotheses on rostral shield evolution in head-first digging squamates from a molecular phylogeny and new species of the genus *Paracontias* (Scincidae). *Organisms, Diversity and Evolution* 11: 135–150. doi: 10.1007/s13127-011-0042-6
- Miralles A, Raselimanana AP, Rakotomalala D, Vences M, Vieites DR (2011b) A new large and colorful skink of the genus *Amphiglossus* from Madagascar revealed by morphology and multilocus molecular study. *Zootaxa* 2918: 47–67.
- Miralles A, Vences M (2013) New metrics for comparison of taxonomies reveal striking discrepancies among species delimitation methods in *Madascincus* lizards. *PLoS ONE* 8(7): e68242. doi: 10.1371/journal.pone.0068242
- Monaghan MT, Wild R, Elliot M, Fujisawa T, Balke M, Inward DJ, Lees DC, Ranaivosolo R, Eggleton P, Barraclough TG, Vogler AP (2009) Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology* 58: 298–311. doi: 10.1093/sysbio/syp027
- Padial JM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. *Frontiers in Zoology* 7: 16. doi: 10.1186/1742-9994-7-16
- Pianka ER, Vitt LJ (2003) *Lizards: Windows to the Evolution of Diversity*. University of California Press, Berkeley, 348 pp.
- Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler A (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595–609. doi: 10.1080/10635150600852011
- Rakotoarison A, Erens J, Ratsoaovina FM, Vences M (2015) Amphibian and reptile records from around the Betsiboka delta area in North-Western Madagascar. *Herpetology Notes* 8: 535–543.
- Rannala B, Yang Z (2003) Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164: 1645–1656.
- Riedel A, Sagata K, Suhardjono YR, Tänzler R, Balke M (2013) Integrative taxonomy on the fast track - towards more sustaina-

- bility in biodiversity research. *Frontiers in Zoology* 10: e15. doi: 10.1186/1742-9994-10-15
- Schmitz A, Brandley MC, Mausfeld P, Vences M, Glaw F, Nussbaum RA, Reeder TW (2005) Opening the black box: phylogenetics and morphological evolution of the Malagasy fossorial lizards of the subfamily “Scincinae”. *Molecular Phylogenetics and Evolution* 34: 118–133. doi: 10.1016/j.ympev.2004.08.016
- Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. doi: 10.1093/molbev/mst197
- Weisrock DW, Rasoloarison RM, Fiorentino I, Ralison JM, Goodman SM, Kappeler PM, Yoder AD (2010) Delimiting species without nuclear monophyly in Madagascar’s mouse lemurs. *PLoS ONE* 5: e9883. doi: 10.1371/journal.pone.0009883
- Whiting AS, Sites JW Jr, Bauer AM (2004) Molecular phylogenetics of Malagasy skinks (Squamata: Scincidae). *African Journal of Herpetology* 53(2): 135–146. doi: 10.1080/21564574.2004.9635506
- Wiens JJ, Penkrot TA (2002) Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* 51: 69–91. doi: 10.1080/106351-502753475880
- Yang Z, Rannala B (2010) Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences USA* 107: 9264–9269. doi: 10.1073/pnas.0913022107

## Supplementary material 1

### List of specimens examined morphologically

Authors: Aurélien Miralles, Jörn Köhler, Frank Glaw, Miguel Vences

Data type: Adobe PDF file

Explanation note: Specimens examined are listed including collection number, locality and collector information.

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.

