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Integrative description of *Diosaccus koreanus* sp. nov. (Hexanauplia, Harpacticoida, Miraciidae) and integrative information on further Korean species

Byung-Jin Lim[1], Hyun Woo Bang[2], Heejin Moon[2], Jinwook Back[1]

1 *Department of Taxonomy and Systematics, National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea* **2** *Mokwon University, Daejeon, 35349, Korea*

Corresponding author: Jinwook Back (jinwookb@mabik.re.kr)

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Abstract

A new species of *Diosaccus* Boeck, 1873 (Arthropoda, Hexanauplia, Harpacticoida) was recently discovered in Korean waters. The species was previously recognized as *D. ezoensis* Itô, 1974 in Korea but, here, is described as a new species, *D. koreanus* **sp. nov.**, based on the following features: 1) second inner seta on exopod of fifth thoracopod apparently longest in female, 2) outer margin of distal endopodal segment of second thoracopod ornamented with long setules in male, 3) caudal seta VII located halfway from base of rami (vs. on anterior extremity in *D. ezoensis*), and 4) sixth thoracopod with three setae in female (vs. 2 setae in *D. ezoensis*). In addition, there is also a mitochondrial COI sequence difference of more than 19.93% with *D. ezoensis* registered in NCBI. A key to *Diosaccus* species of the world is also provided, and new morphological features and DNA sequences are presented for two other harpacticoid species, *Parathalestris verrucosa* Itô, 1970 and *Peltidium quinquesetosum* Song & Yun, 1999. In order to clearly identify harpacticoids at the species level, both morphological and DNA sequence characteristics should be considered.

Keywords

Key, mitochondrial cytochrome c oxidase subunit I, *Parathalestris verrucosa*, *Peltidium quinquesetosum*, taxonomy, 18S ribonucleic acid

Introduction

Harpacticoids (Arthropoda, Hexanauplia, Harpacticoida) are a group of benthic metazoans that are diverse in terms of both species and ecology. To date, ca 150 species of marine harpacticoids have been reported in Korean waters (Song et al. 2012). However, the diversity of harpacticoids in Korean waters is likely underestimated because many of these species have been identified on the basis of morphological characters, which are often insufficient for species identification owing to minor differences among closely-related taxa (Beheregaray and Caccone 2007; Vakati et al. 2019). In the case of *Tigriopus japonicus* Mori, 1938 collected from the Northwest Pacific Ocean, it is very difficult to identify its three cryptic species based on morphological characters, because there is no single morphological character that can distinguish among them (Karanovic et al. 2018). Several authors report species showing small morphological differences compared to the original descriptions, but have concluded that these are not sufficient for species differentiation (Chang 2007; Back and Lee 2011; Kim et al. 2011; Park and Lee 2011; Park et al. 2012; Kim et al. 2015). There is currently no clear way to distinguish between inter-species and intra-species differences.

In contrast to morphology-based taxonomy, recent advances in the cost and ease DNA sequencing and in the availability of public DNA sequence databases has facilitated the identification of numerous cryptic animal species (Hebert et al. 2003; Bhadury et al. 2006; DeSalle and Goldstein 2019), with the mitochondrial cytochrome c oxidase subunit I gene (*COI*) commonly used for species identification and the 18S ribonucleic acid gene (*18SrRNA*) commonly used for higher-level taxonomic grouping. Yet, to define new species on the basis of DNA sequences, accurate sequences of known species are needed, and few attempts have been made to assign DNA sequences to morphologically-defined harpacticoid species. Therefore, the aim of the present study is the integrative description of a newly discovered species, and to assign DNA sequences to a morphologically-defined species, and to identify previously unrecognized taxonomically informative morphological characteristics.

Material and methods

Sample collection

The samples were all collected from Korean waters which is part of the north-western Pacific Ocean (Table 1) and fixed in >95% ethanol. Harpacticoids were sorted from the samples using an M80 stereomicroscope (Leica, Wetzlar, Germany) and then frozen at -20 °C.

DNA extraction, amplification, sequencing, and analysis

Each specimen was rinsed in distilled water for 15 min to remove ethanol and then transferred, using a sterilized pipette tip or dissection needle, to a 1.5-mL tube that contained 20 mL Proteinase K and 180 mL ATL buffer for non-destructive DNA extraction (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany). After the specimens were incubated for 3 h in a thermoshaker (350 rpm, 56 °C), the 200 mL of lysis buffer (Proteinase K + ATL buffer) was moved to new 1.5-mL tubes under a stereomicroscope. Each 1.5-mL specimen tube was then filled with 70% ethanol to preserve the specimens for subsequent morphological identification and description, and DNA was isolated from the buffer samples following the protocol of the DNeasy Blood and Tissue Kit.

Both *COI* and *18Sr RNA* sequences were amplified from the sample DNAs using an AccuPower HotStart PCR PreMix (Bioneer, Daejeon, South Korea), gene-specific primers (Table 2), and the amplification procedure described by Vakati et al. (2019). The resulting PCR products were sequenced in both directions using an ABI PRISM 3730XL Analyzer (Macrogen, Inc., Seoul, Korea). Sequences were assembled using Geneious 10.1.3 (Biomatters Auckland, New Zealand) (Kearse et al. 2012). Pairwise distances were calculated using the Tamura and Nei distance model (Tamura and Nei 1993) in Geneious 10.1.3. The sequences from GenBank were aligned using the Muscle algorithm integrated in Geneious 10.1.3 (Edgar 2004).

Morphological characterization

After processing for molecular analysis, each specimen was dissected on several slides using lactophenol as a mounting medium and then observed using a Leica DM2500 microscope that was equipped with a drawing tube. Descriptive terminology was adopted from Huys et al. (1996).

Abbreviations used in the text are: **A1**: antennule; **A2**: antenna; **ae**: aesthetasc; **exp-1(2, 3)**: proximal (middle, distal) exopod; **enp-1(2, 3)**: proximal (middle, distal) endopod; **P1–P6**: first to sixth thoracopod; **seg-1(-5)**: first (to fifth) segment; **benp**: baseoendopod; **mxp**: maxilliped.

Taxonomy

Order Harpacticoida Sars, 1903
Family Miraciidae Dana, 1846
Genus *Diosaccus* Boeck, 1873

Diosaccus koreanus sp. nov.

http://zoobank.org/64547C65-0584-47D1-BEDF-AC6DDD748CB6
Figs 1–8

Material examined. ***Holotype.*** Republic Of Korea · Ulleungdo Island; 37°31'36.56"N, 130°49'41.77"E; 25 July 2017; B. Jinwook leg.; hand net, 0.5 m · 1 ♀ (MABIK CR00247255) was dissected on 14 slides (Table 1) · GenBank accession

Table 1. Collection information of morphologically-defined harpacticoid species.

Species	Date	Locality	Gear (depth)	Specimen nos.
Diosaccus koreanus sp. nov.	25–07–2017	37°31'36.56"N, 130°49'41.77"E	hand net (0.5 m)	CR00247255 CR00247256
	27–04–2018	35°18'39.0"N, 129°16'10.6"E	Grab (5 m)	CR00247257 CR00247258 CR00247259 CR00247260
Parathalestris verrucosa	19–07–2017	36°42'36.63"N, 129°28'31.69"E	light trap (2 m)	All specimens
Peltidium quinquesetosum	19–07–2017	36°42'36.63"N, 129°28'31.69"E	light trap (2 m)	All specimens

Table 2. Primer sequences and PCR conditions used in the present study.

Gene	References	Primer name	Primer sequence	PCR condition	Product size	Species
mt COI	Folmer et al. (1994)	LCO1490 (universal)	GGTCAACAAATCATAAAGATATTGG	94 °C, 300 s; 40 cycles × (94 °C, 60 s; 46 °C, 120 s; 72 °C, 180 s; 72 °C, 600 s)	658 658 661	*D. koreanus* sp. nov *Pa. verrucosa* *Pe. quinquesetosum*
		HCO2198 (universal)	TAAACTTCAGGGTGACCAAAAAATCA			
18S rRNA	Yamaguchi (2003)	18SF1 (universal)	TACCTGGTTGATCCTGCCAG	94 °C, 300 s; 40 cycle × (94 °C, 30 s; 50 °C, 30 s; 72 °C, 60 s); 72 °C, 420 s	1,756	*D. koreanus* sp. nov
		18SR9 (universal)	GATCCTTCCGCAGGTTCACCTAC		1,761	*Pa. verrucosa*
		18SF2 (internal)	CCTGAGAAACGGCTRCCACAT	These primers were used for primer walking to sequence over 1700 bp	1,763	*Pe. quinquesetosum*
		18SF3 (internal)	GYGRTCAGATACCRCCSTAGTT			
		18SF4 (internal)	GGTCTGTGATGCCCTYAGATGT			
		18SR6 (internal)	TYTCTCRKGCTBCCTCTCC			
		18SR7 (internal)	GYYARAACTAGGGCGGTATCTG			
		18SR8 (internal)	ACATCTRAGGGCATCACAGACC			

number for *COI* sequence: MN996281. ***Paratypes.*** Republic Of Korea (Table 1) · 1 ♂ (MABIK CR00247257) was dissected on 8 slides and observed · 4 ♀♀ (MABIK CR00247256, CR00247258 – CR00247260) were preserved in 99% alcohol · GenBank accession numbers: MN996277 to MN996280 (*COI*) and MT002900 to MT002902 (*18SrRNA*).

Description. Female. ***Body*** (Figs 1, 2): Total length, from anterior margin of rostrum to posterior margin of caudal rami, 1135 μm (*N* = 5, mean = 1133 μm; Fig. 1);

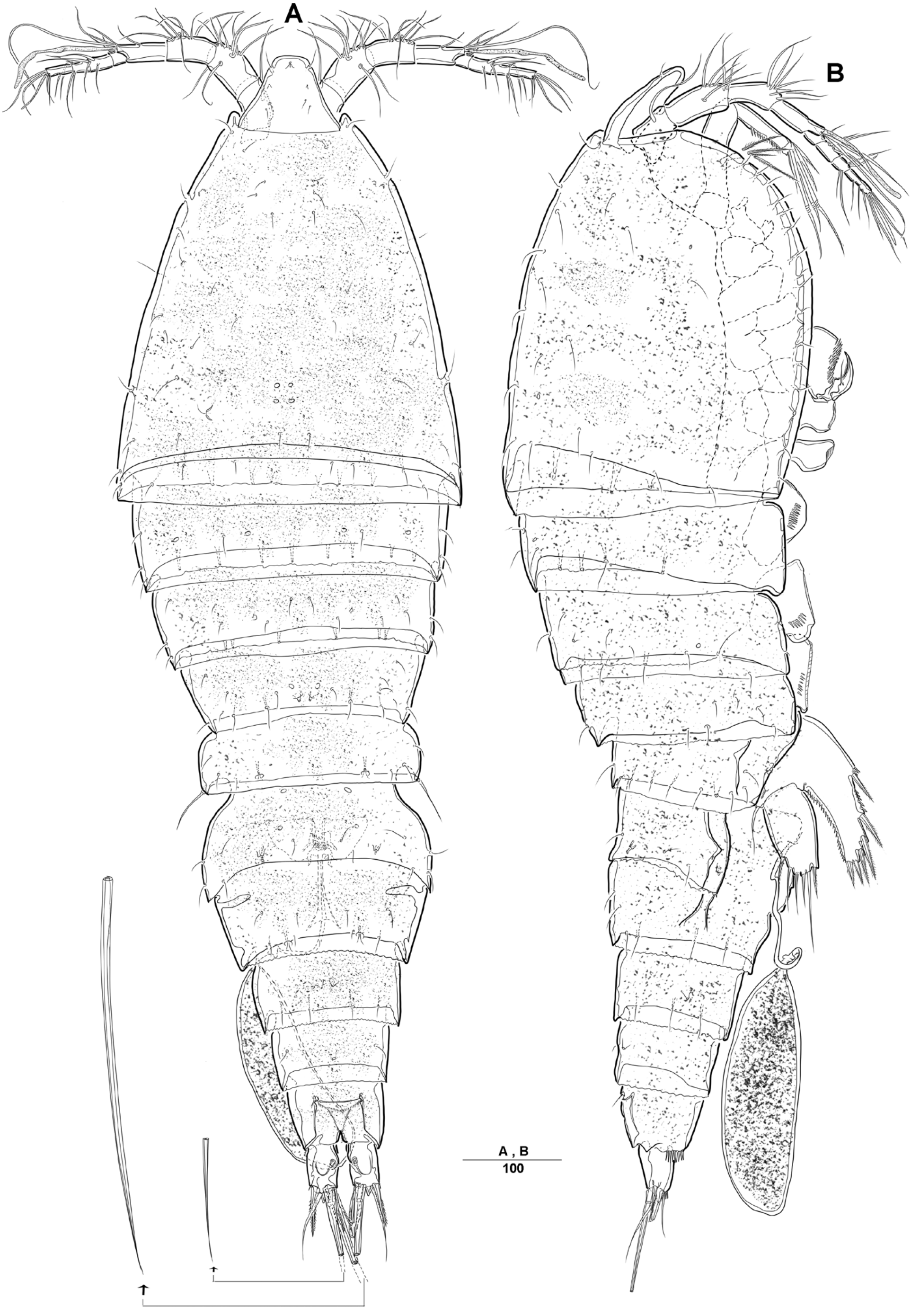

Figure 1. *Diosaccus koreanus* sp. nov., female **A** habitus, dorsal **B** habitus, lateral. Scale bars indicate length in μm.

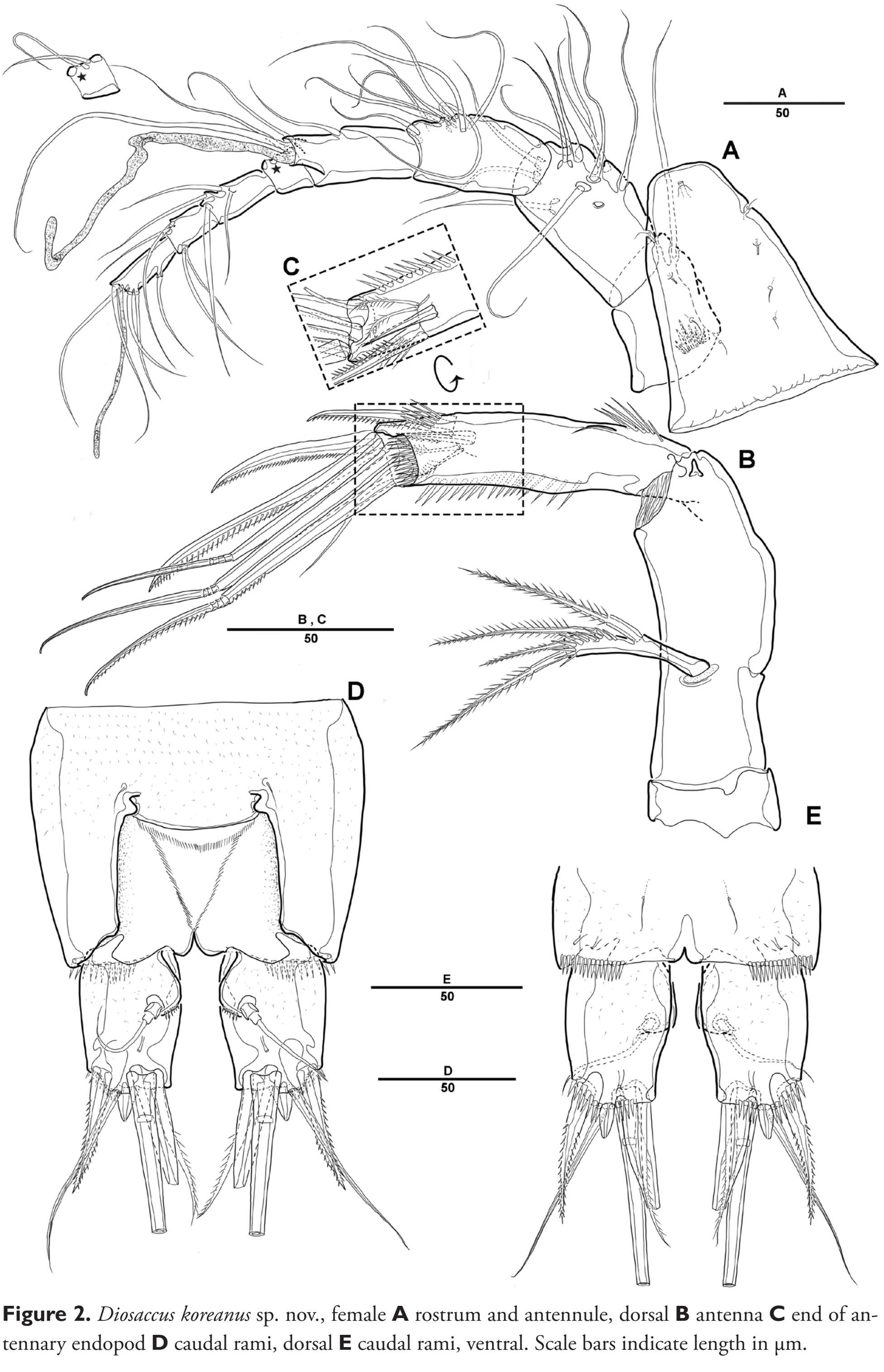

Figure 2. *Diosaccus koreanus* sp. nov., female **A** rostrum and antennule, dorsal **B** antenna **C** end of antennary endopod **D** caudal rami, dorsal **E** caudal rami, ventral. Scale bars indicate length in µm.

maximum width 340 µm, measured at distal cephalothorax; body cylindrical, not dorsoventrally depressed, and with minute dorsal sensilla; rostrum well developed, defined at base, trapezoid in shape, with round apex and 2 sensilla (Figs 1A, 2A); cephalothorax sub-triangle with sensilla and smooth margin; second and third urosomites fully fused ventrally, but with transverse ridge on dorsal and lateral surfaces indicating original segmentation (Figs 1A, B, 6B); anal operculum not well developed, with spinular tuft (Fig. 2D).

Caudal rami (Fig. 2D, E): Parallel, ca 1.5 times longer than maximum width, dorsal surface with small bumps; each ramus with 7 setae: seta I strong, pinnate; setae II bare on distal corner; seta III blunt spine; setae IV and V strong; seta VI pinnate; seta VII bare, triarticulate at base.

A1 (Fig. 2A): Slender, 8-segmented; seg-2 longest, ca 1.2 times as long as seg-3; seg-4 with sub-cylindrical pedestal armed with aesthetasc fused at base to 1 long bare seta; armature formula: 1–[1], 2–[11], 3–[9], 4–[3 + (1+ae)], 5–[2], 6–[4], 7–[4], 8–[3+acrothek]; apical acrothek of short aesthetasc fused basally to 2 bare setae.

A2 (Fig. 2B, C): 3-segmented, with coxa, allobasis, and free 1-segmented enp; coxa small and bare; allobasis without abexopodal seta; exp 1-segmented, with 2 lateral and 2 apical pinnate setae; free enp with 2 pinnate setae and 2 long spines laterally and with 1 bare seta, 2 spines, and 3 geniculate setae along distal margin.

Mandible (Fig. 3A): Gnathobase with several blunt teeth; palp basis with 2 inner pinnate setae; exp 1-segmented with 2 pinnate distal setae; enp with 2 lateral and 6 distal setae.

Maxillule (Fig. 3B, C): Praecoxa trapezoidal in shape, without ornamentation; arthrite well developed, with 2 juxtaposed setae near midpoint of anterior surface, 4 strong teeth-like spines and 3 tuft spines along distal margin; coxa fused with cylindrical endite, with 1 pinnate seta; basis fused with endite, with 1 bare and 5 pinnate setae; exp 1-segmented, with 2 pinnate setae distally; enp 1-segmented, with 4 pinnate setae along distal margin.

Maxilla (Fig. 3D): Syncoxa with 2 endites; proximal endite with 2 strong spines and 1 bare seta among distal margin; second endite with 1 strong spine, 1 bare seta, and 1 tuft-like seta; allobasis developed into cylindrical process, with 2 strong spines and 2 bare setae; enp 1-segmented, with 2 bare and 3 pinnate setae.

Mxp (Fig. 3E): 4-segmented, with syncoxa, basis, and 2-segmented enp; syncoxa with 2 pinnate setae distally; basis elongate and robust, with 2 small bare setae (Fig. 3E, arrow) and roughly ornamented with rows of spinules along inner margin; enp-1 with 1 bare and 1 pinnate setae; enp-2 forming strong claw ornamented with row of spinules among inner proximal half.

Swimming legs (Figs 4, 5): Biramous; P1–P4 with coxa, basis, and 3-segmented exp and enp; each ramus ornamented with setules or spinules along outer margins as figured.3

P1 (Fig. 4A, B): Coxa ornamented with inner spinules; basis with 1 outer and 1 inner pinnate setae; exp-1 with 1 outer spine; exp-2 with 1 outer spine and 1 inner pin-

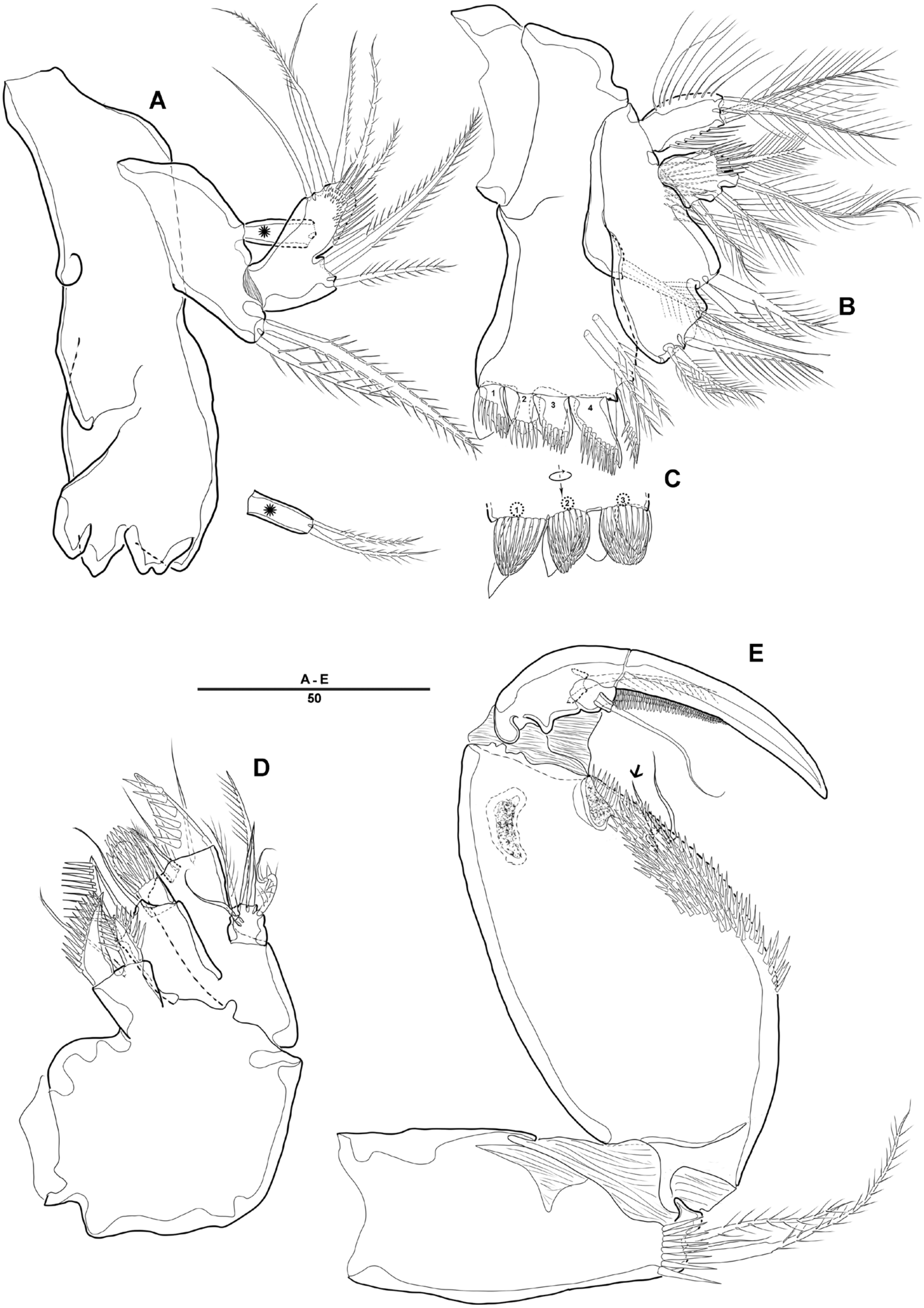

Figure 3. *Diosaccus koreanus* sp. nov., female **A** mandible **B** maxillule **C** shape of elements in praecoxal arthrite of maxillule **D** maxilla **E** maxilliped. Scale bars indicate length in μm.

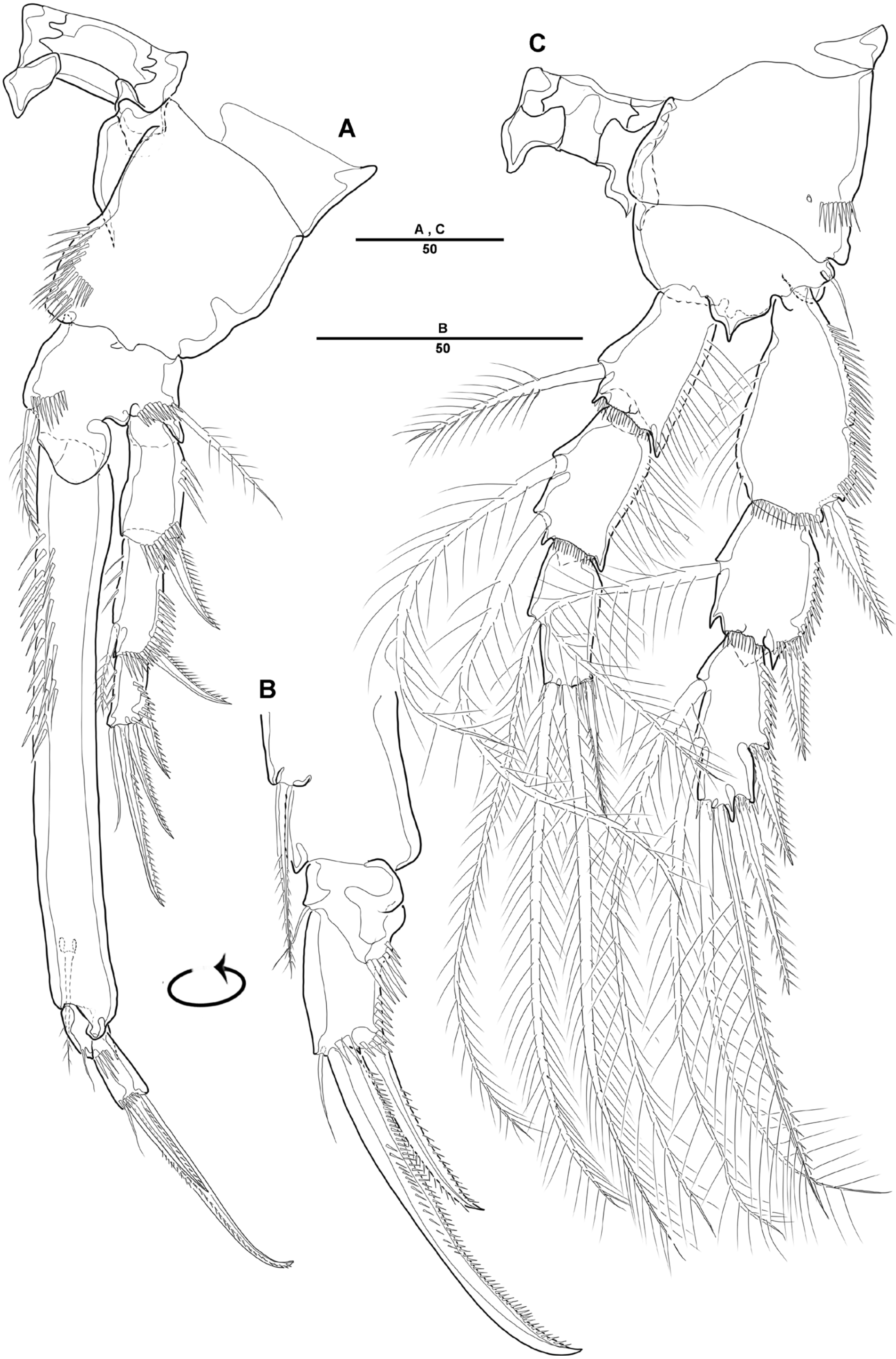

Figure 4. *Diosaccus koreanus* sp. nov., female **A** first thoracopod **B** middle and distal endopods of first thoracopod **C** second thoracopod. Scale bars indicate length in µm.

Figure 5. *Diosaccus koreanus* sp. nov., female **A** third thoracopod **B** fourth thoracopod. Scale bars indicate length in μm.

nate seta; exp-3 with 3 spines and 1 bare seta; enp-1 ornamented with row of spinules on inner proximal half, ca 2 times longer than exp, with 1 pinnate seta; enp-2 with 1 small bare seta on inner distal corner, enp-3 with 2 strong spines distally and 1 bare seta near inner distal corner.

P2 (Fig. 4C): Coxa ornamented with row of spinules on outer margin; basis with 1 outer bare seta near distal corner; exp-1 with 1 outer spine, ornamented with a row of long setules along inner margin; exp-2 with 1 outer spine and 1 inner pinnate seta, ornamented with row of setules along outer margin; exp-3 with 2 outer spines and 2 apical and 2 inner pinnate setae; enp-1 with 1 inner pinnate seta, ornamented with long setules along outer margin; enp-2 with 2 pinnate inner setae; enp-3 with 1 outer, 2 distal, and 1 inner pinnate setae.

P3–P4 (Fig. 5A, B): Coxa ornamented with rows of spinules on outer margin; basis with 1 outer bare seta near distal corner; exp-1 with 1 outer spine, ornamented with row of long setules along inner margin; exp-2 with 1 outer spine and 1 inner pinnate seta, ornamented with row of spinules along outer margin; exp-3 with 3 outer spines, 2 apical and 3 inner pinnate setae; enp-1 with 1 inner seta, ornamented with long setules among outer margin; enp-2 with 2 inner pinnate setae [P3] or 1 inner pinnate seta [P4]; enp-3 with 1 outer spine, 2 apical pinnate and 2 inner pinnate setae.

Armature formulae as follows:

	Exopod	**Endopod**
P1	0.1.112	1.1.120
P2	0.1.222	1.2.121
P3	0.1.323	1.2.221
P4	0.1.323	1.1.221

P5 (Fig. 6C): Defined at supporting somite; each side of endopodal lobe separated, with 6 spine-like setae; exp with 6 setae, second inner element longest.

P6 (Fig. 6A, B): Fused with supporting somite, with 3 bare setae, innermost seta longest.

Male. *Body* (Fig. 7A): Total length, from anterior margin of rostrum to posterior margin of caudal rami, 880 μm; maximum width 262 μm, measured at distal cephalothorax; general body shape, ornamentation, and sensilla pattern almost identical to those of female, but with sexual dimorphisms observed in A1, P1, P2, P5, P6, and genital somites.

A1 (Fig. 8C, D): Subchirocer 10-segmented, robust; seg-3 with aesthetasc fused at base to 1 bare seta; seg-5 swollen, with aesthetasc fused at base to 1 bare seta; armature formula: 1–[1], 2–[10], 3– [4+(1+ae)], 4–[2], 5–[4+(1+ae)], 6–[2 bare], 7–[1], 8–[1], 9–[4], 10–[5+(1+ae)].

P1 (Fig. 8A): General shape of P1 similar to that of female, except basis; basis with 1 outer pinnate seta and 1 wrinkled process near base of outer seta.

P2 (Fig. 8B): Enp 2-segmented; enp-1 with 1 inner bare seta and ornamented with row of long setules along outer margin; enp-2 with 1 inner bare seta on small disk

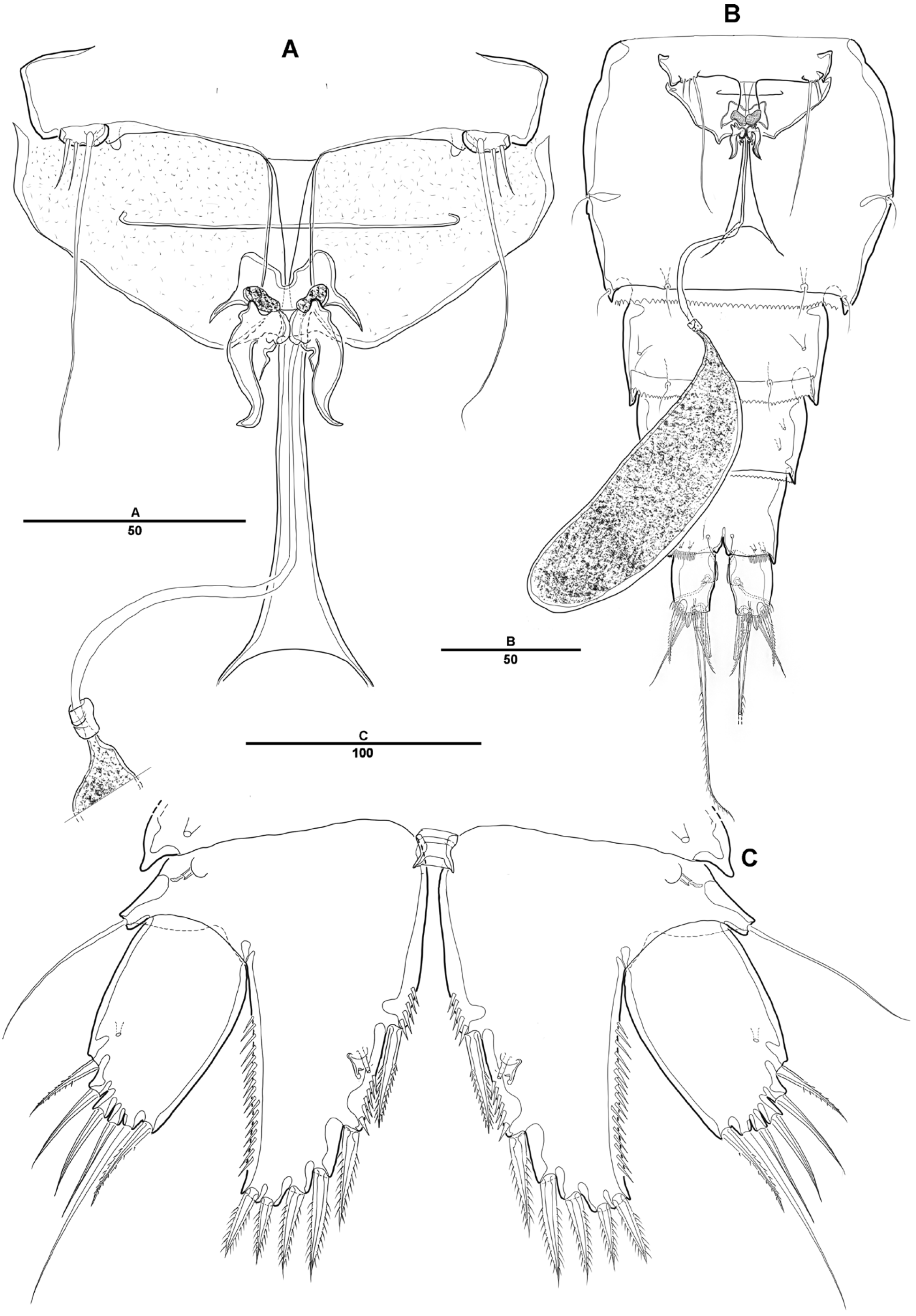

Figure 6. *Diosaccus koreanus* sp. nov., female **A** sixth thoracopod and genital field **B** urosomites, ventral **C** fifth thoracopod. Scale bars indicate length in μm.

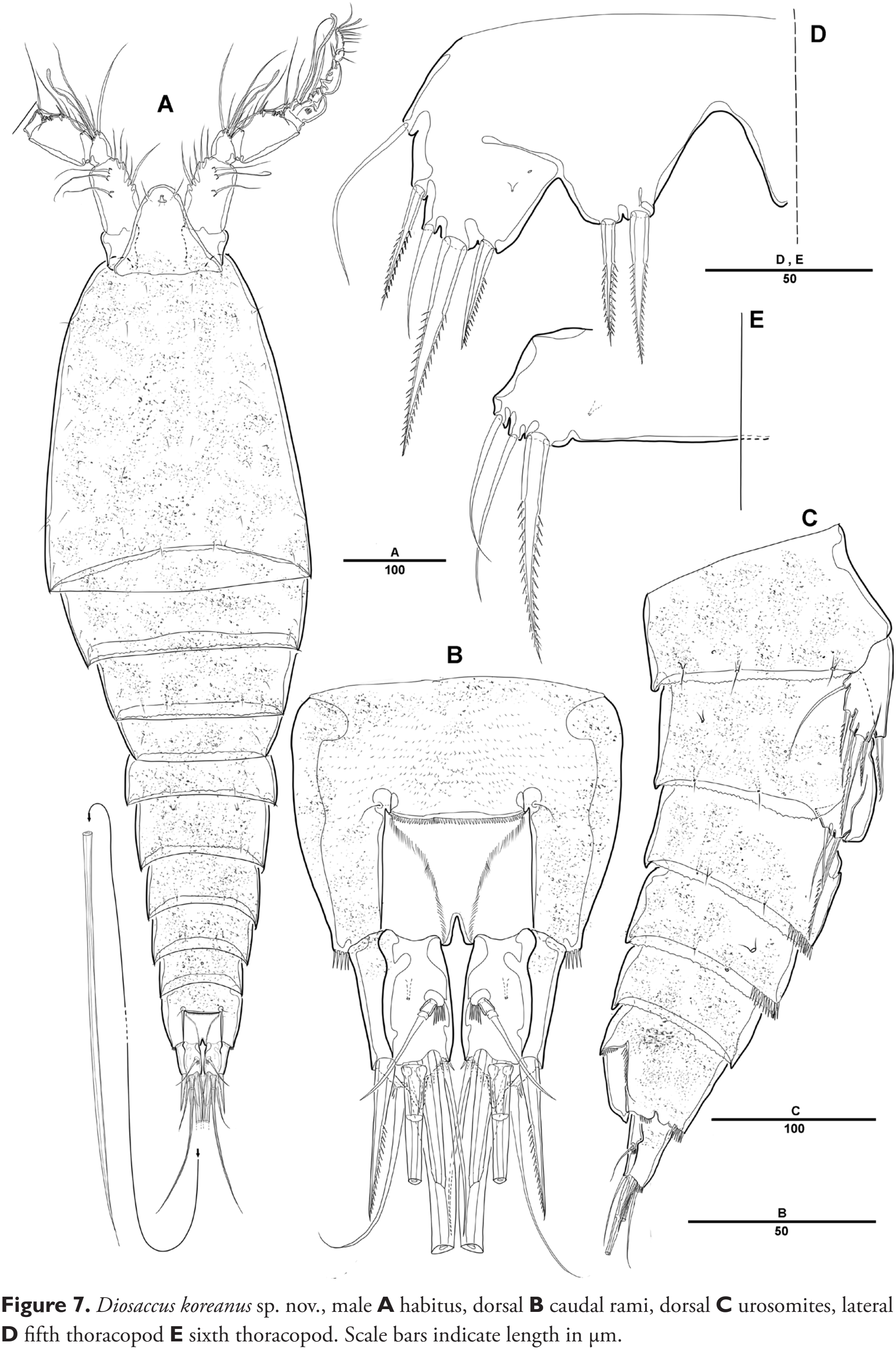

Figure 7. *Diosaccus koreanus* sp. nov., male **A** habitus, dorsal **B** caudal rami, dorsal **C** urosomites, lateral **D** fifth thoracopod **E** sixth thoracopod. Scale bars indicate length in μm.

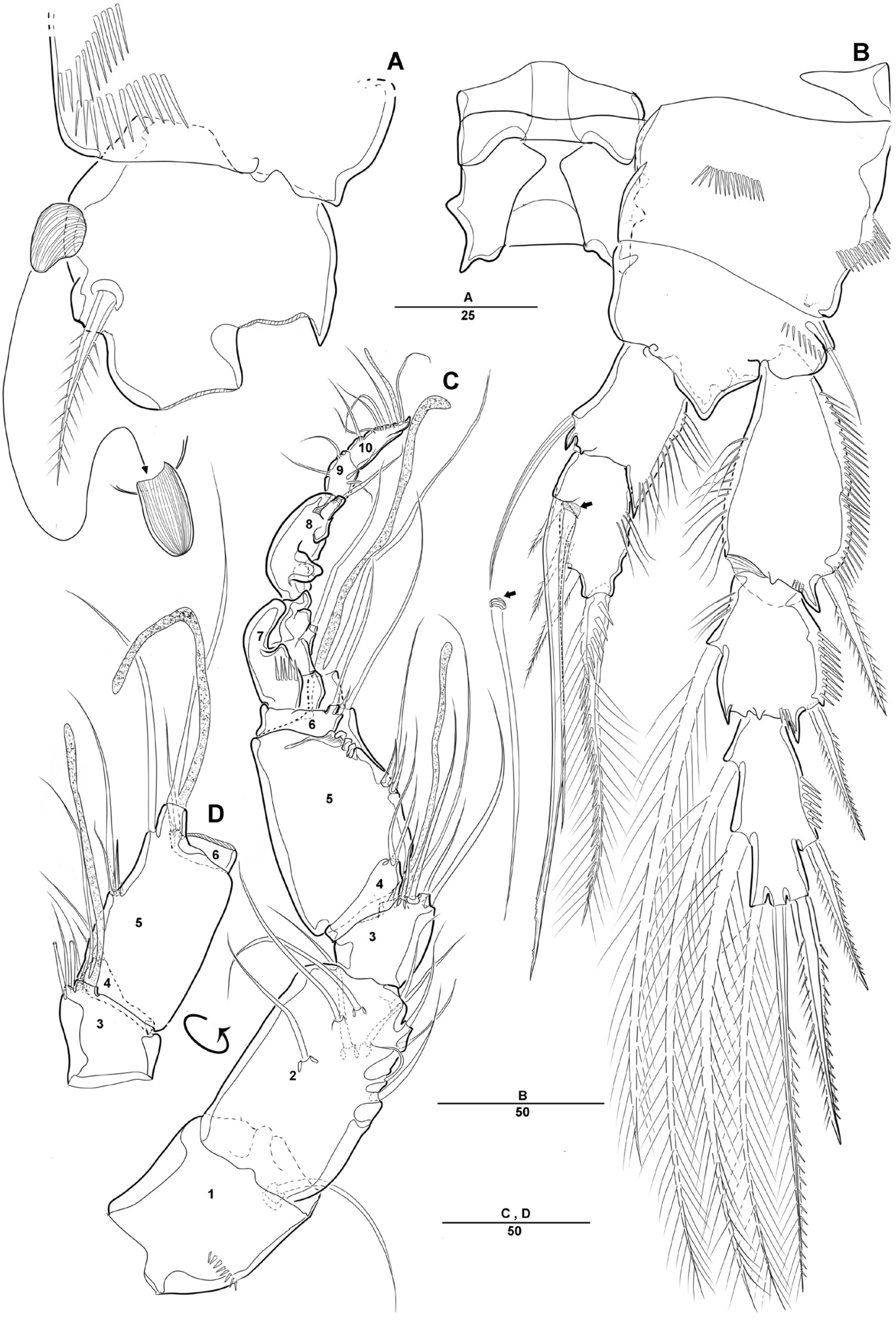

Figure 8. *Diosaccus koreanus* sp. nov., male **A** base of first thoracopod **B** second thoracopod **C** antennule **D** antennule segments 3–6. Scale bars indicate length in μm.

Table 3. Pairwise distances (Tamura-Nei distance) between *COI* sequences from species in genus *Diosaccus*. Numbers in parentheses indicate the Genbank accession numbers.

		1	2	3	4	5	6	7	8
1	*D. koreanus* sp. nov. (CR00247255, CR00247258)								
2	*D. koreanus* sp. nov. (CR00247256)	1.52							
3	*D. koreanus* sp. nov. (CR00247257 CR00247260)	0.91	0.91						
4	*D. koreanus* sp. nov. (CR00247259)	2.28	1.67	1.67					
5	*D. ezoensis* (KR049013)	19.93	20.62	20.62	20.79				
6	*D.spinatus* (MH242730)	20.36	21.28	21.28	22.04	19.76			
7	*D.spinatus* (MH242731)	20.67	21.59	21.59	22.34	19.42	1.06		
8	*D.spinatus* (HQ966504)	20.06	20.97	20.97	21.73	19.93	1.06	0.61	

Table 4. Pairwise distances (Tamura-Nei distance) based on 1,756 bp between *18SrRNA* sequences from species in genus *Diosaccus*.

	Species (Genbank accession number)	1	2	3
1	*Diosaccus koreanus* sp. nov (MT002900 – MT002902)			
2	*D. ezoensis* (KR048740)	1.46		
3	*Diosaccus* sp. (EU380290)	7.24	8.55	

(Fig. 8B, arrow) of which middle inner edge and 1 longest bare seta, 3 pinnate inner setae, and 1 strong spinulose seta apically.

P5 (Fig. 7D): Fused medially; plate of benp fused each side; basal part with 1 bare seta; endopodal lobe with 2 spinulose pinnate setae; exp fused at base, with 3 spinulose setae and 1 bare seta.

P6 (Fig. 7E): Fused at base, with 2 bare and 1 spinulose setae.

Etymology. Species name refers to the type locality (i.e., Republic of Korea).

DNA sequences. In regards to pairwise distances (Tamura-Nei distance) among the 582-bp *COI* sequences, *D. koreanus* sp. nov. exhibited intra-specific variation of 0–2.28%, and inter-specific distances of 19.42–22.34% were observed among all three *Diosaccus* species (Table 3). In regards to the *18SrRNA* sequences, intra- and inter-specific variations of 0% and 1.46–8.55% were observed (Table 4).

Family Thalestridae Sars G.O., 1905
Genus *Parathalestris* Brady & Robertson D., 1873

Parathalestris verrucosa Itô, 1970

Figs 9–15

Material examined. Republic Of Korea · 1 ♀ (MABIK CR00246555) was dissected on 13 slides · 1 ♂ (MABIK CR00246552) was dissected on 9 slides · 11 ♀♀

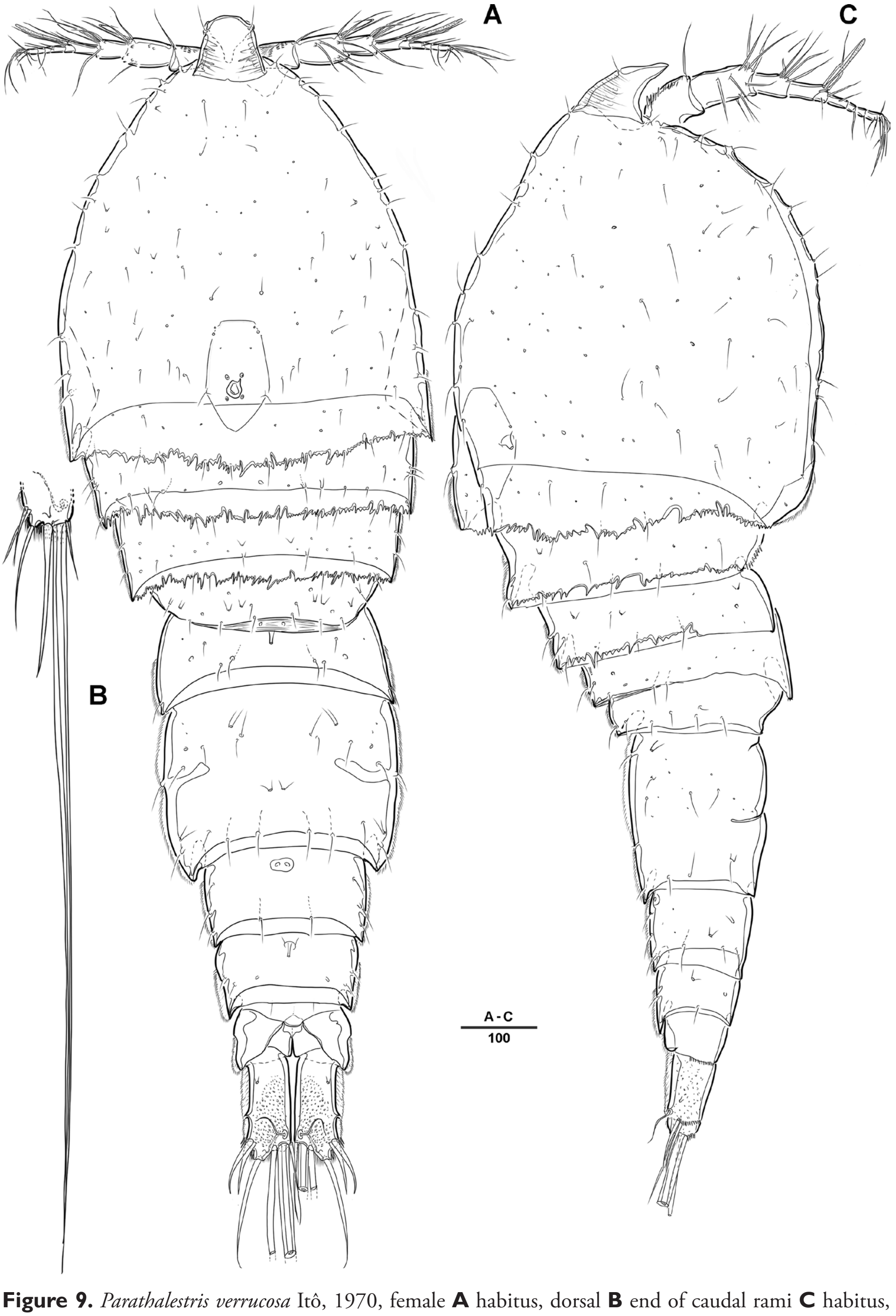

Figure 9. *Parathalestris verrucosa* Itô, 1970, female **A** habitus, dorsal **B** end of caudal rami **C** habitus, lateral. Scale bars indicate length in µm.

(MABIK CR00246553, CR00246554, CR00246556 to CR00246560, CR00246562 to CR00246565) and 1 ♂ (MABIK CR00246561) were preserved in 99 % alcohol · GenBank accession numbers: MN996282 to MN996293 (*COI*) and MT002906 to MT002909 (*18SrRNA*).

Description. *Parathalestris verrucosa* Itô, 1970 (p. 211–218, Figs 1–4), see also Chang and Song (1997).

Note. Chang and Song (1997) reported that *P. verrucosa* collected from Korea differed from Itô's description in regards to three characteristics (length of caudal rami, segmentation of A2 exp, and presence of rows of spines along posteroventral margin), and the specimens analyzed in the present study also varied in this manner. In particular, the base of the second lateral seta of the A2 exp was protruding and could be seen as two segments, depending on the angle. In addition, the male specimens analyzed in the present study also differed from Itô's original description in regards to A1 segmentation. More specifically, the A1 of Itô's specimen possessed a small seg-3 and swollen seg-4, whereas that of the present study's specimens possessed small seg-3 and seg-4 and a swollen seg-5.

Family Peltidiidae Claus, 1860
Genus *Peltidium* Philippi, 1839

Peltidium quinquesetosum Song & Yun, 1999
Figs 16–22

Peltidium quinquesetosum Song & Yun, 1999: 67–74, figs 1–3

Material examined. Republic Of Korea (Table 1) · 1 ♀ (MABIK CR00246774) was dissected on 10 slides · 1 ♀ (MABIK CR00246775) was dissected on 6 slides · 1 ♂ (MABIK CR00246787) was dissected on 10 slides · 11 ♀♀ (MABIK CR00246776 to CR00246786) were preserved in 99% alcohol · GenBank accession numbers: MT006218 to MT006229 (*COI*) and MT002903 to MT002905 (*18SrRNA*).

Note. There was no remarkable difference between the original description and the specimens analyzed in the present study. However, additional details of sensilla on the surface, the structure of mouthparts and appendages, and the rows of spinules and setules were added in the figures.

Discussion

Relationships among *Diosaccus* spp.

The new species (*D. koreanus* sp. nov.) was placed in the genus *Diosaccus* on the basis of several characteristics (A2 exp with 4 setae, P2 exp-2 with 2 inner setae, P2 exp-1 without inner seta, and P4 enp 3-segmented) and was most closely related to *D. ezoensis*

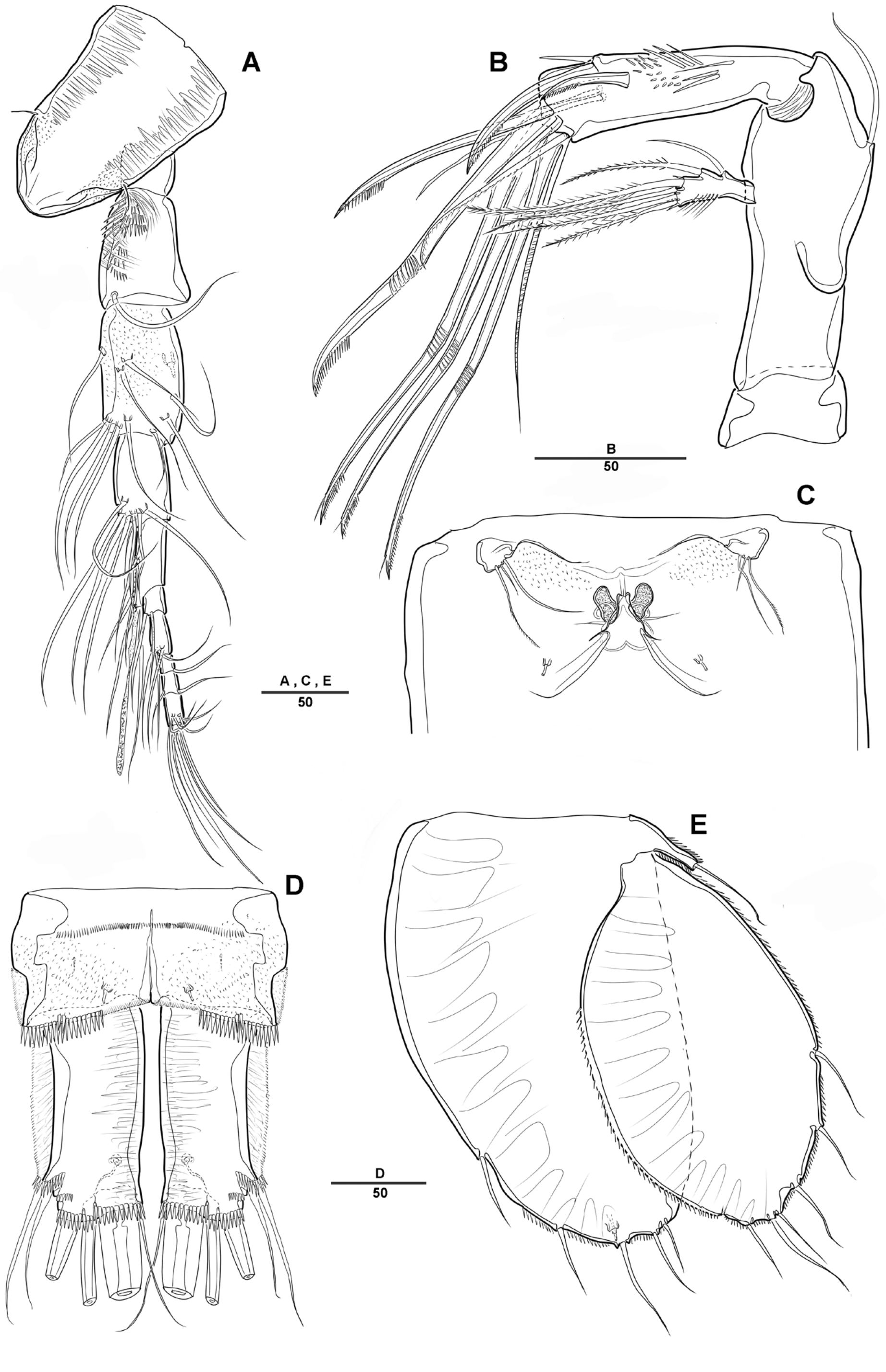

Figure 10. *Parathalestris verrucosa* Itô, 1970, female **A** rostrum and antennule **B** antenna **C** sixth thoracopod and genital field **D** caudal rami, ventral **E** fifth thoracopod. Scale bars indicate length in µm.

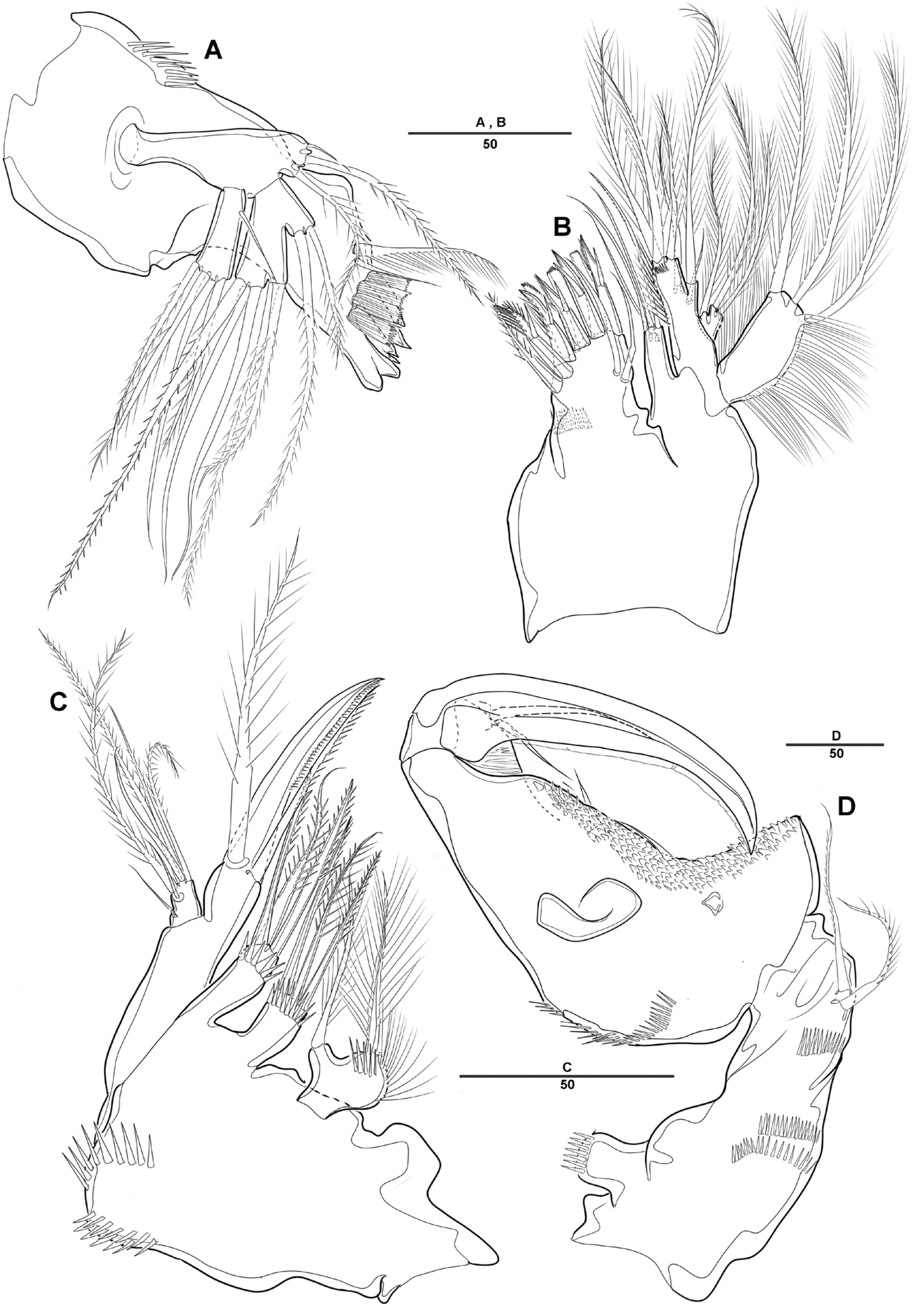

Figure 11. *Parathalestris verrucosa* Itô, 1970, female **A** mandible **B** maxillule **C** maxilla **D** maxilliped. Scale bars indicate length in µm.

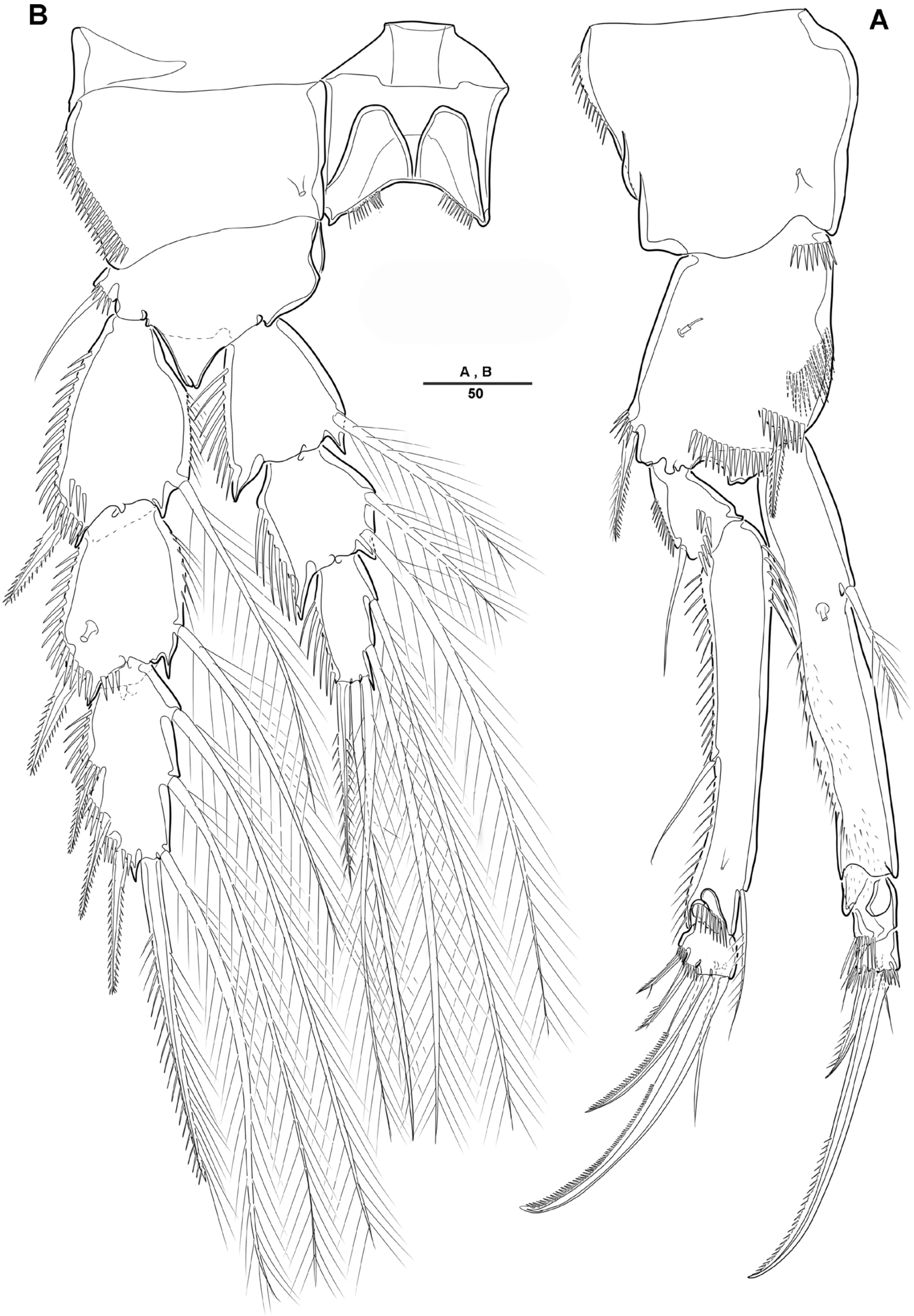

Figure 12. *Parathalestris verrucosa* Itô, 1970, female **A** first thoracopod **B** second thoracopod. Scale bars indicate length in µm.

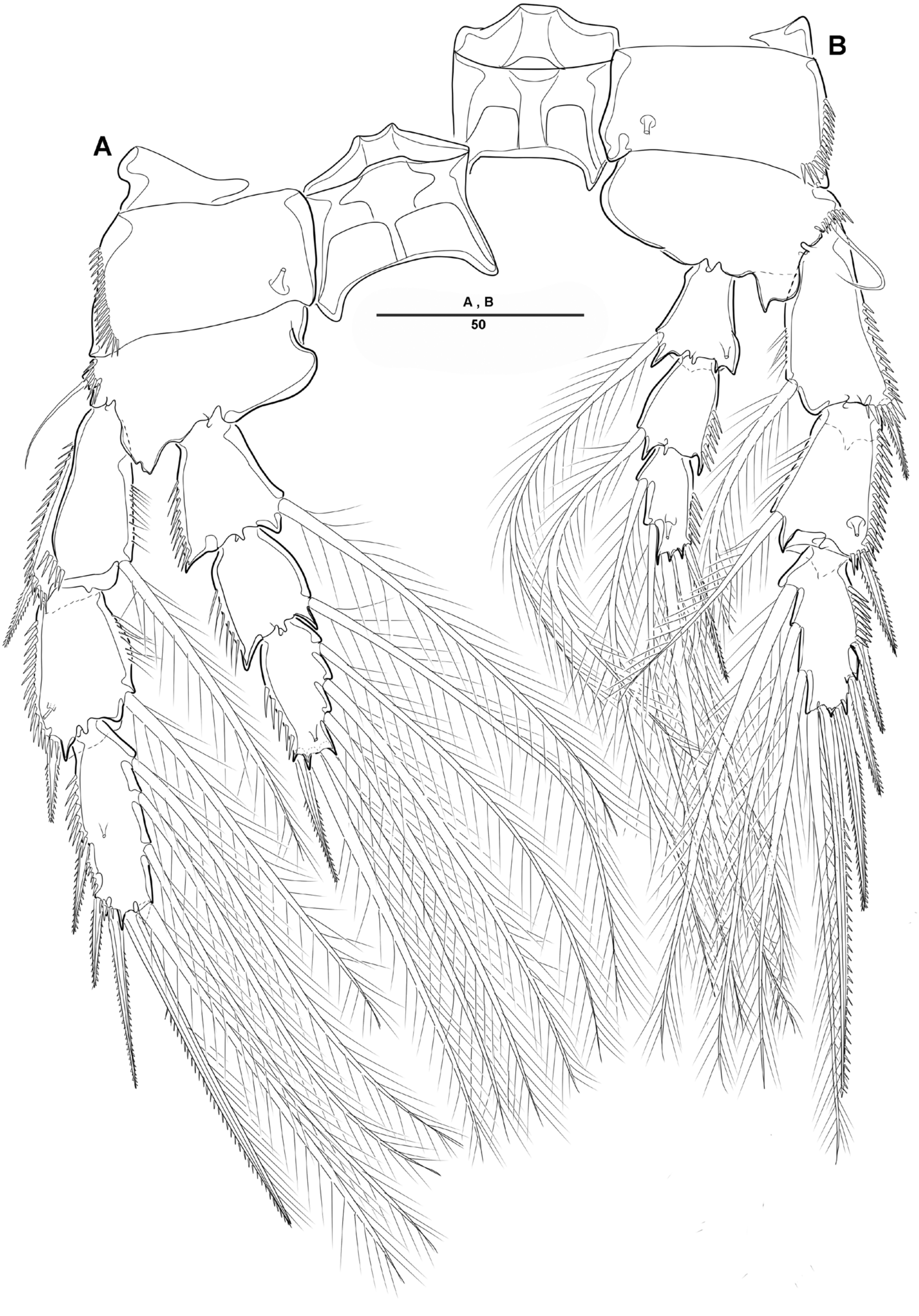

Figure 13. *Parathalestris verrucosa* Itô, 1970, female **A** third thoracopod **B** fourth thoracopod. Scale bars indicate length in µm.

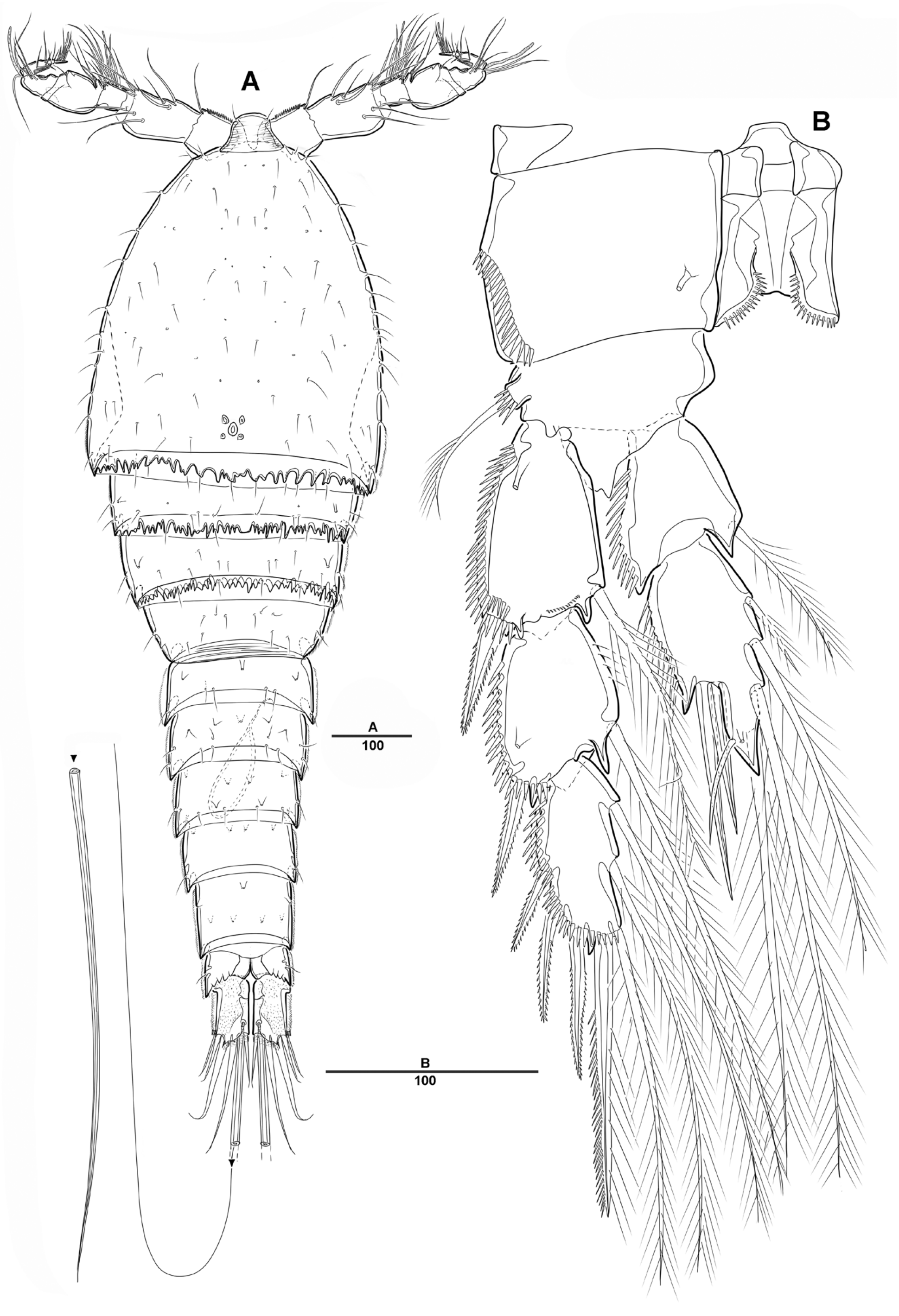

Figure 14. *Parathalestris verrucosa* Itô, 1970, male **A** habitus, dorsal **B** second. Scale bars indicate length in µm.

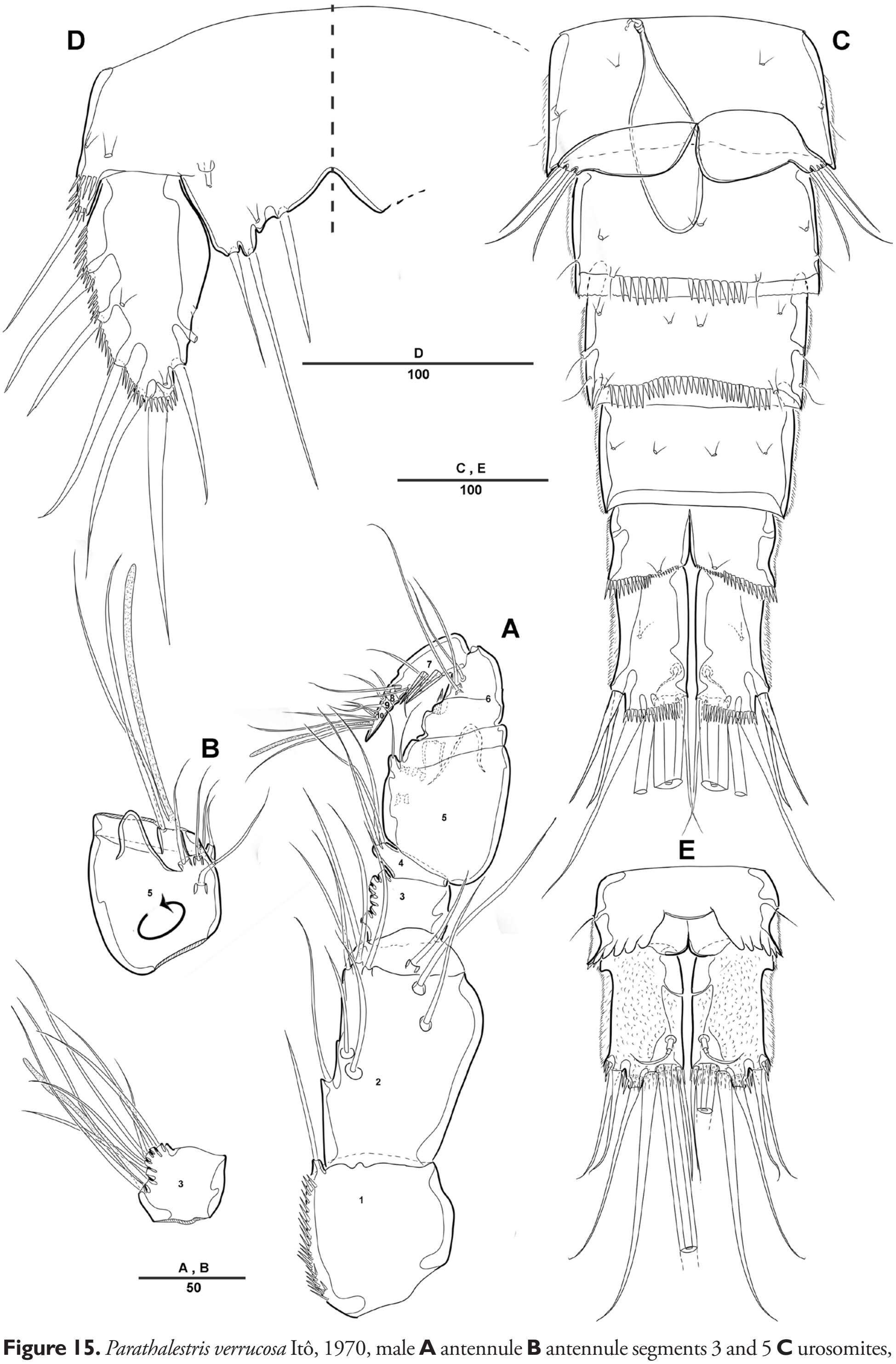

Figure 15. *Parathalestris verrucosa* Itô, 1970, male **A** antennule **B** antennule segments 3 and 5 **C** urosomites, ventral **D** fifth thoracapod **E** caudal rami, dorsal. Scale bars indicate length in µm.

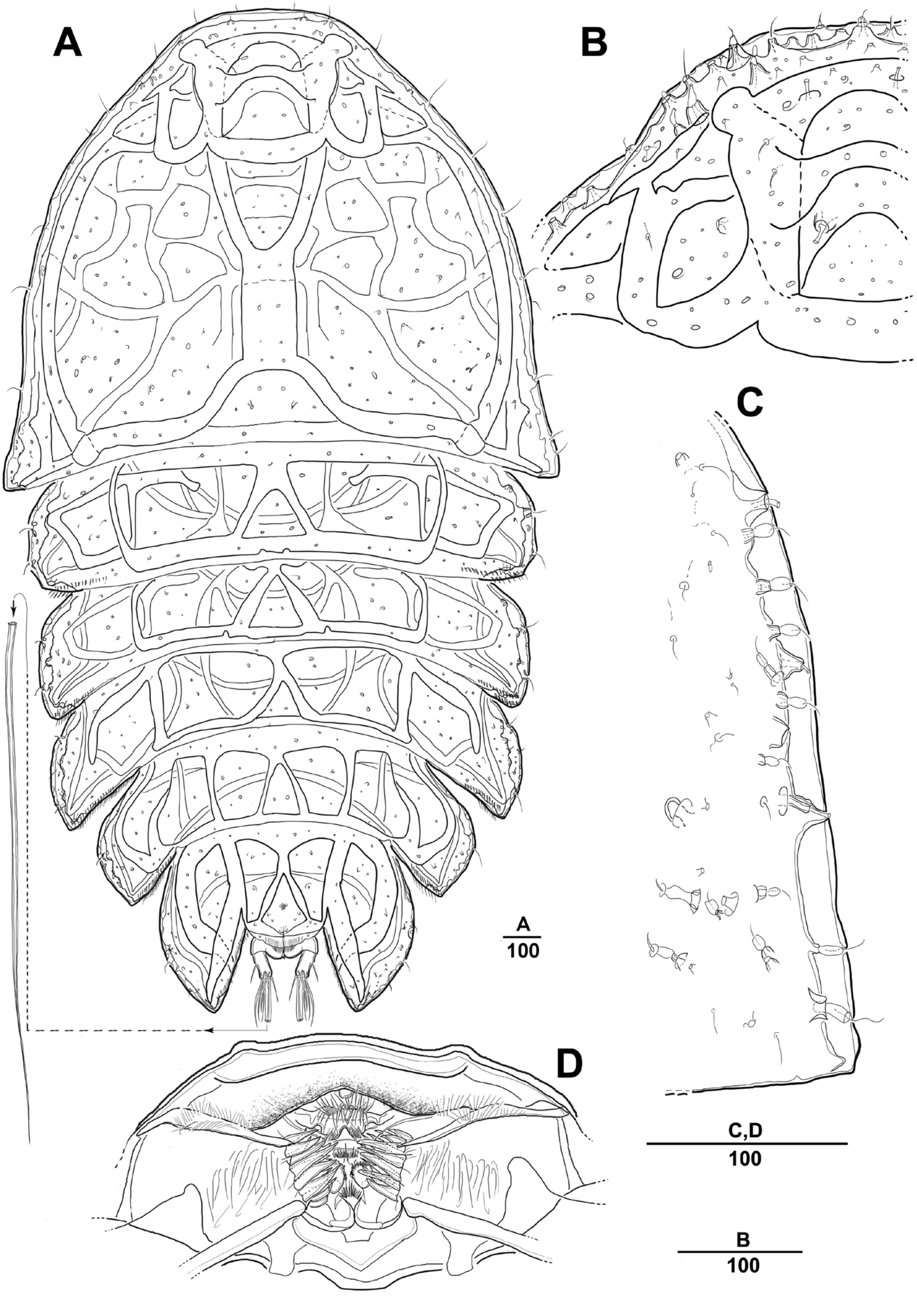

Figure 16. *Peltidium quinquesetosum* Song & Yun, 1999, female **A** habitus, dorsal **B** anterior tip of cephalic shield **C** lateral margin of cephalic shield **D** rabrum. Scale bars indicate length in μm.

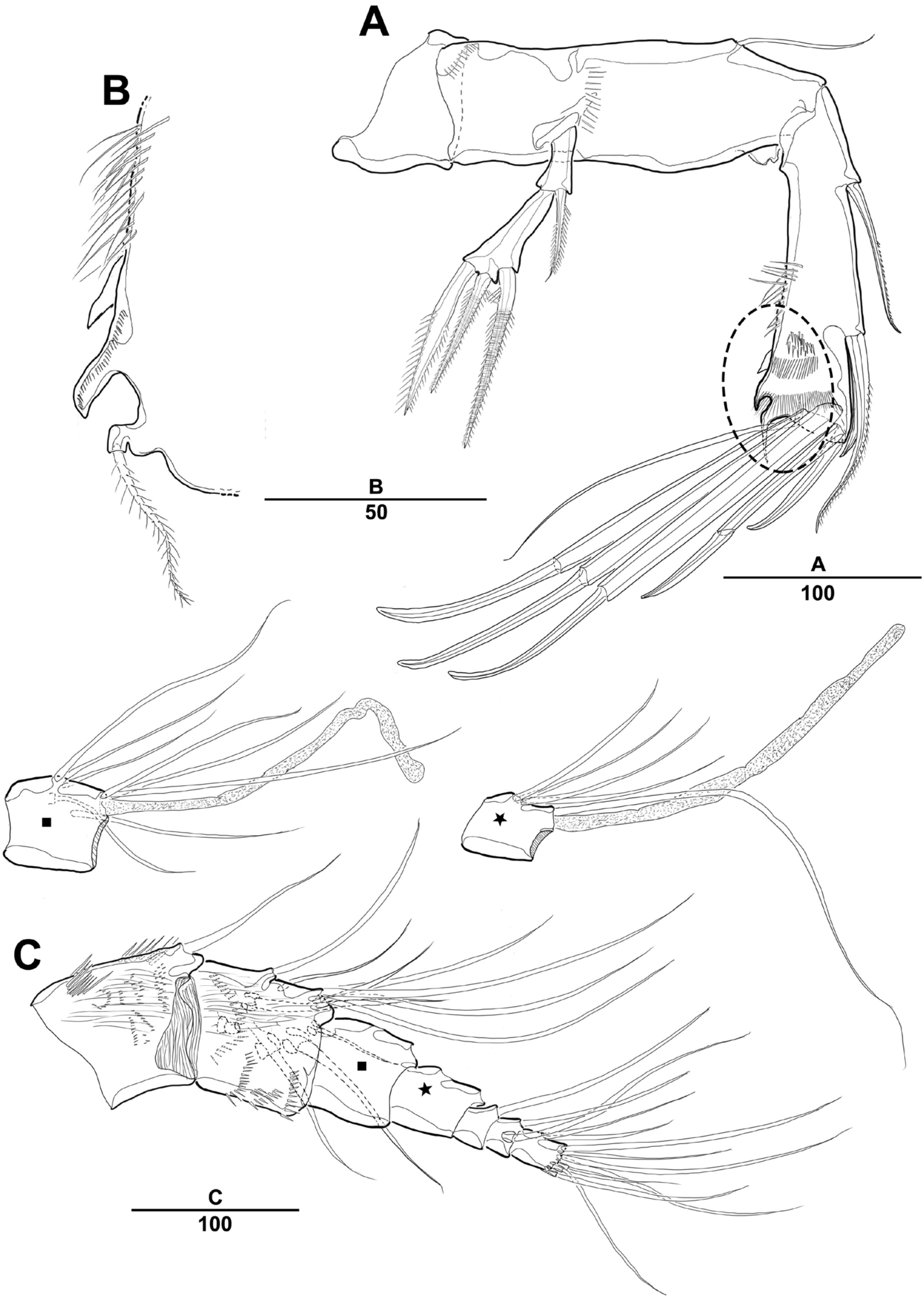

Figure 17. *Peltidium quinquesetosum* Song & Yun, 1999, female **A** antenna **B** end of antennary endopod **C** antennule. Scale bars indicate length in µm.

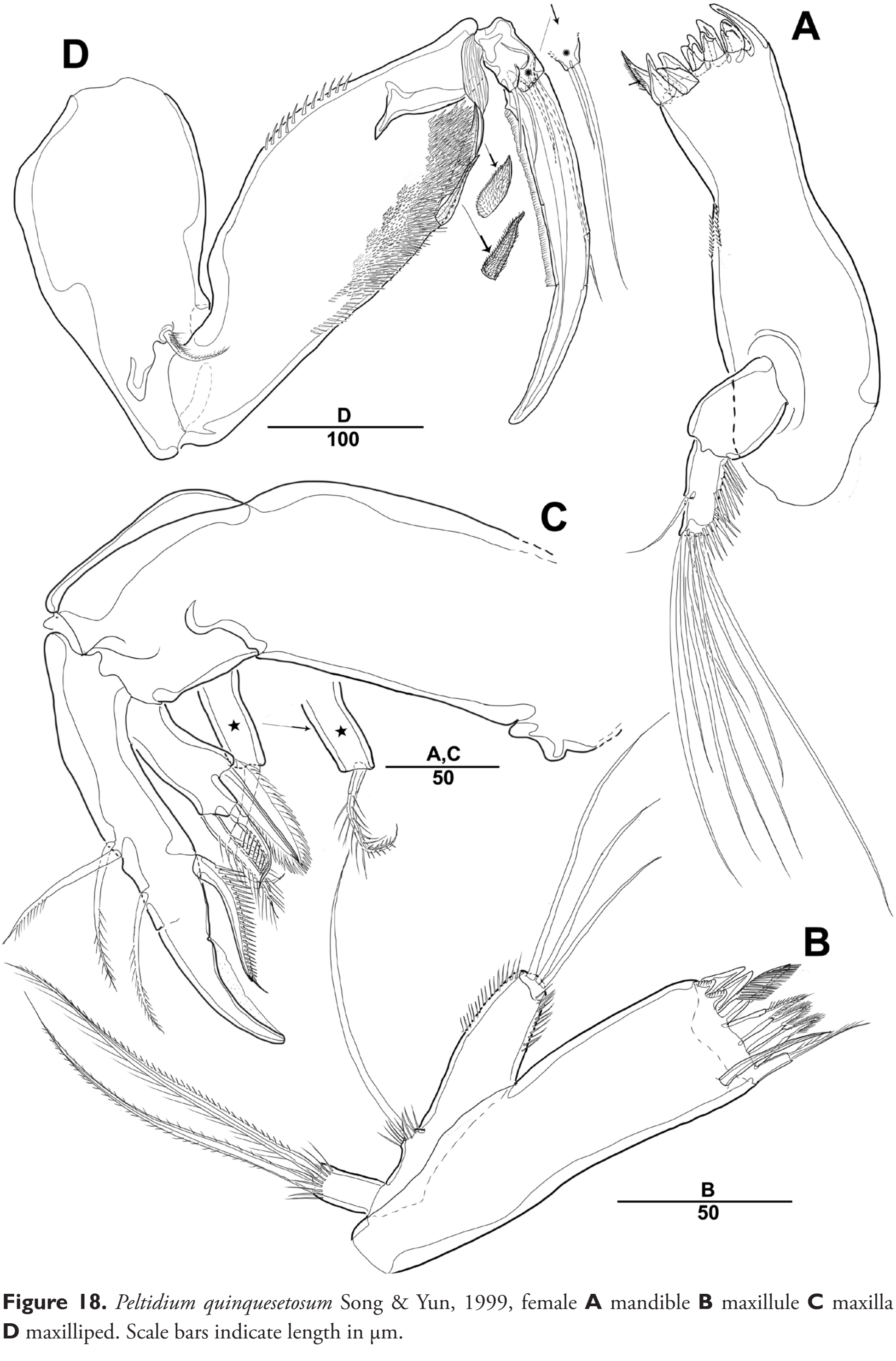

Figure 18. *Peltidium quinquesetosum* Song & Yun, 1999, female **A** mandible **B** maxillule **C** maxilla **D** maxilliped. Scale bars indicate length in µm.

Figure 19. *Peltidium quinquesetosum* Song & Yun, 1999, female **A** first thoracapod **B** shape of setae on second endopod in first thoracapod **C** second thoracapod. Scale bars indicate length in µm.

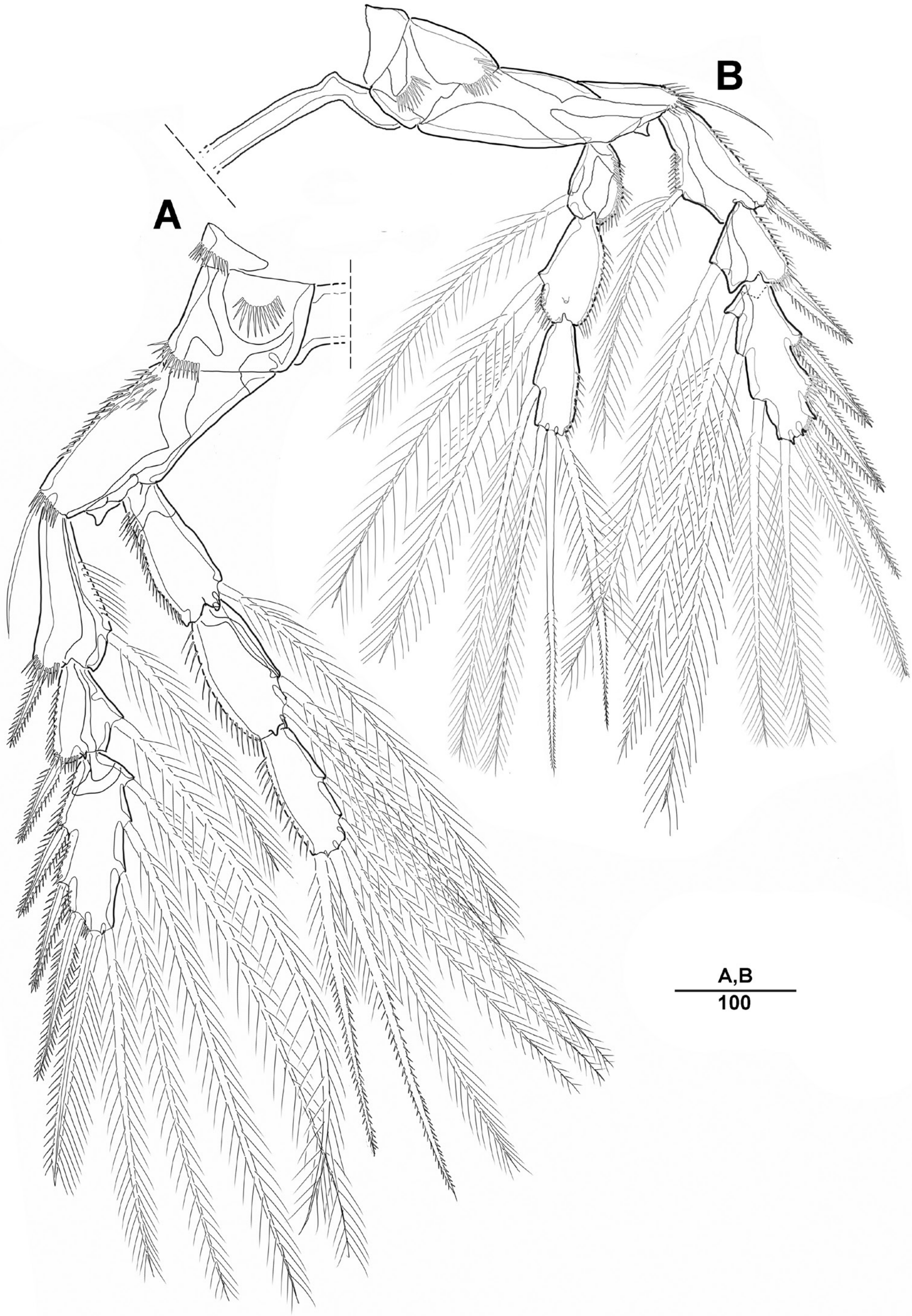

Figure 20. *Peltidium quinquesetosum* Song & Yun, 1999, female **A** third thoracapod **B** fourth thoracapod. Scale bars indicate length in μm.

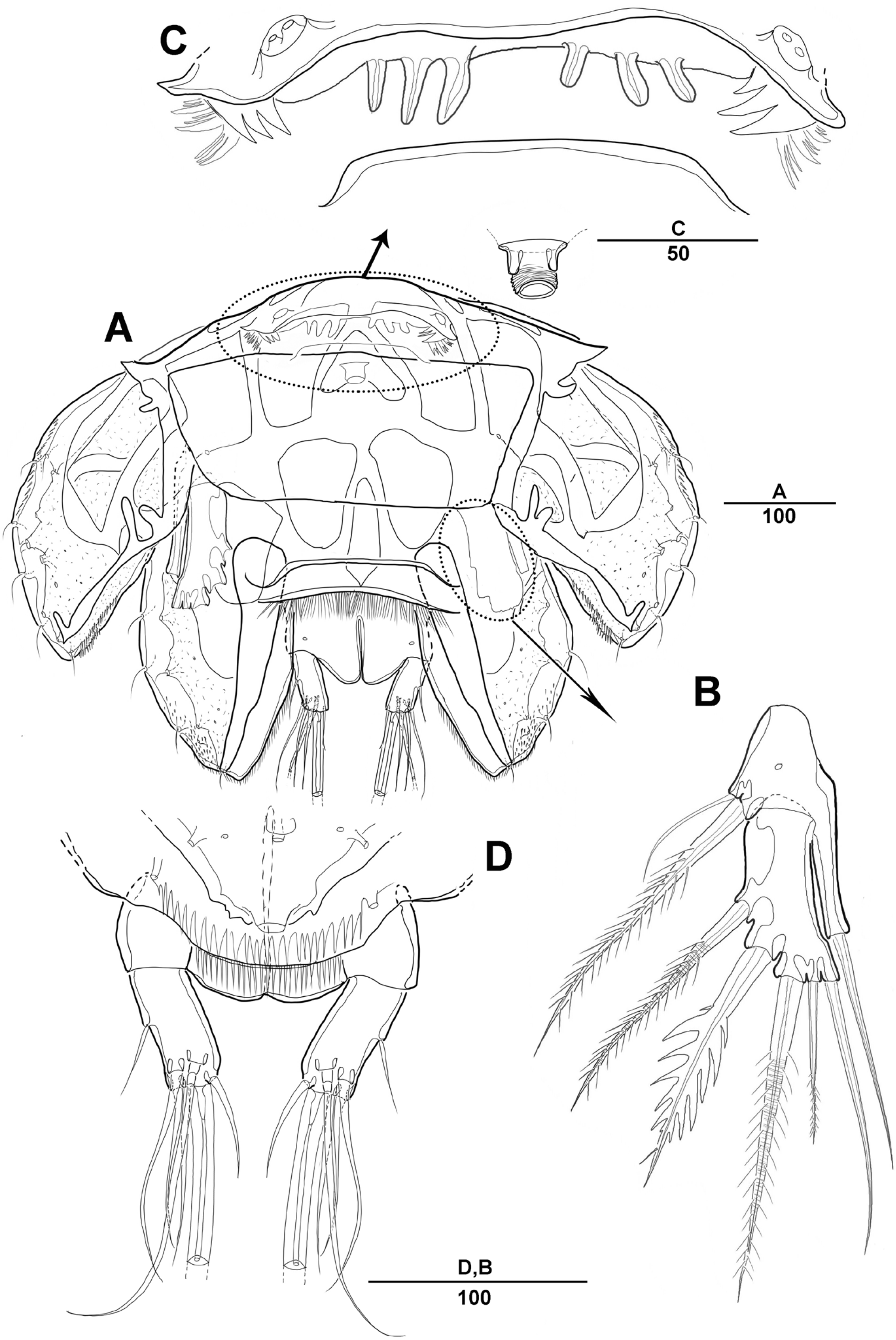

Figure 21. *Peltidium quinquesetosum* Song & Yun, 1999, female **A** urosomites, ventral **B** fifth thoracapod **C** genital field **D** caudal rami, dorsal. Scale bars indicate length in µm.

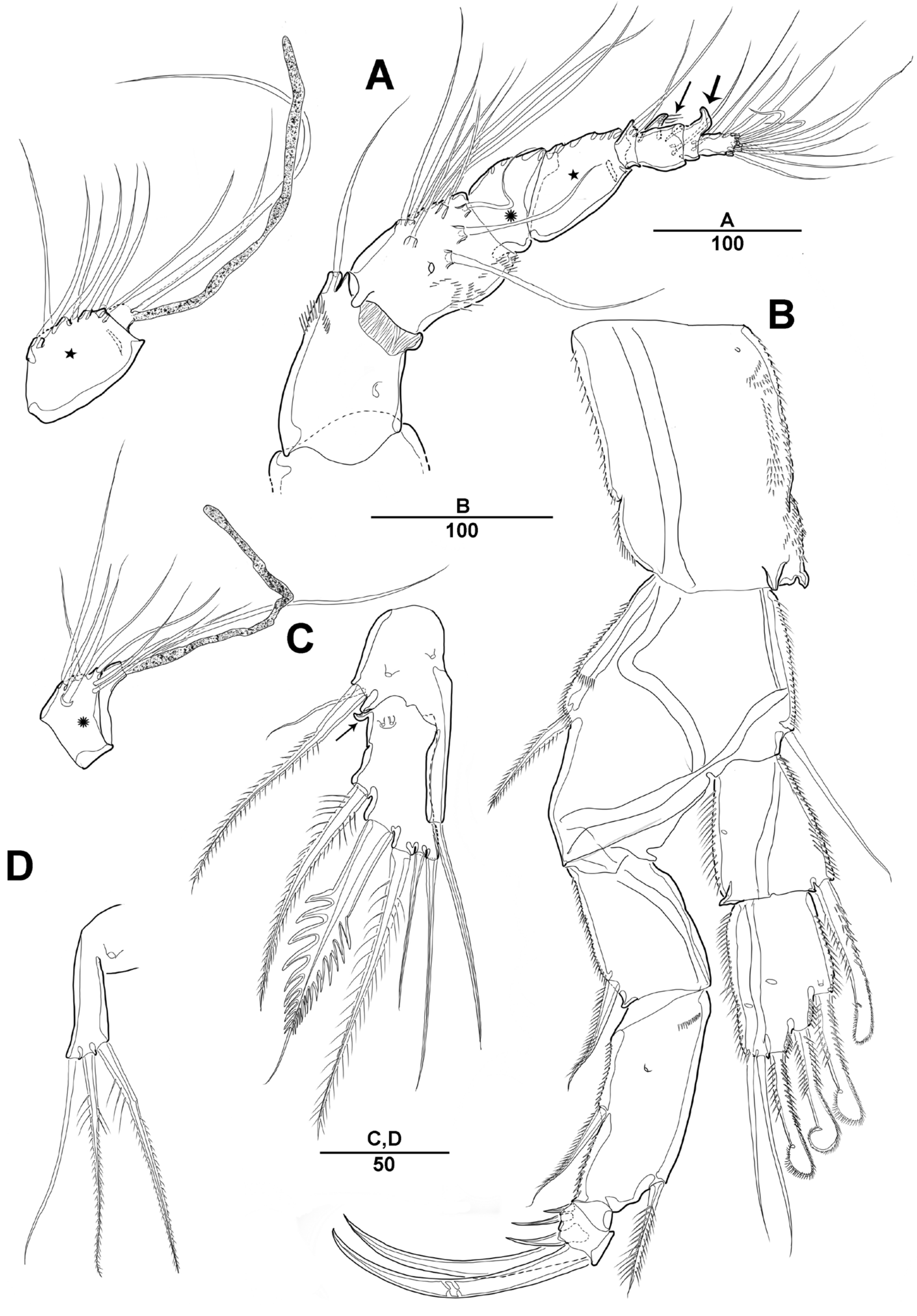

Figure 22. *Peltidium quinquesetosum* Song & Yun, 1999, male **A** antennule **B** first thoracapod **C** fifth thoracapod **D** sixth thoracapod. Scale bars indicate length in µm.

Itô, 1974, based on the setae formula of the swimming legs, mouthpart structures, and the shapes of P5 and P6. However, the new species was also clearly distinguishable from *D. ezoensis* based on the length of the second inner seta on the P5 exp (obviously longest in the female) and the presence of long setules along the outer margin of the P2 enp-3, as previously noted by Song et al. (1999). In addition, the present study found that *D. koreanus* sp. nov. could be further distinguished on the basis of caudal seta VII, which was located halfway from the rami base (vs. on anterior extremity in *D. ezoensis*), and P6 with 3 setae in the female (vs. 2 setae in *D. ezoensis*).

The genus *Diosaccus* currently contains 14 valid species (Bodin 1997; Wells 2007), one of which includes two subspecies and two of which are only placed in the genus provisionally. In addition, the latest dichotomous key (Lang 1965) for the genus used doubtful characters, like moderate length seta and the length of caudal rami, based on old manuscripts, and the tabular keys provided by Wells (2007) also include suspicious characters, such as the relative length between P1 enp-2 and enp-3, mainly owing to the lack of information about the species. Therefore, an updated key, which includes *D. koreanus* sp. nov., is presented below. Attempts were made to update the key on the basis of accurate characters. However, this was difficult because most of the original papers did not include full descriptions of the species. Because there is no apparent differentiation between *D. hamiltoni* and *D. tenuicornis* females, a single male character was added to the key. For species recorded before 1948 refer to the description of Lang (1948).

Key to *Diosaccus* species, based mainly on female specimens

1 A2 exp 3-segmented...**2**
– A2 exp 2-segmented...**4**
– A2 exp 1-segmented...**7**
2 P1 enp-2 without inner seta; basis of mxp robust....***D. rebus* (Sewell, 1940)**
– Specimen without this combination of characters **3**
3 Basis of mxp slender; P1 enp-3 longer than enp-2; P1 enp-2 with 1 inner seta ....***D. valens* (Gurney, 1927)**
– Base of mxp robust; P1 enp-3 as long as enp-2; P1 enp-2 without inner seta..... ....***D. robustus* (Thompson & Scott, 1903)**
4 Seg-3 and seg-4 with sharp dorsal teeth; P5 exp with 7 setae; benp with 5 spines, nearly equal in length ....***D. dentatus* (Thompson & Scott, 1903)**
– Specimen without this combination of characters **5**
5 P1 enp 2-segmented ....***D. varicolor biarticulatus* (Monard, 1924)**
– P2 enp 3-segmented **6**
6 P5 exp with 6 setae ....***D. varicolor varicolor* (Farran, 1913)**
– P5 exp with 5 setae ....***D. varicolor pentasetosus* (Noodt, 1955)**
7 P1 enp 2-segmented ....***D. monardi* Sewell, 1940**
– P1 enp 3-segmented **8**

8 Benp with 6 setae/spines..**9**
– Benp with 5 setae/spines..**11**
9 Caudal seta VII on proximally, P5 with 6 uniform (in length) setae, P6 with 2 setae..***D. ezoensis*** **Itô, 1974**
– Specimen without this combination of characters......................................**10**
10 Second outer seta on P5 benp longest......... ***D. borborocoetus*** **Jakobi, 1954**
– P5 benp with 6 spines..***D. koreanus*** **sp. nov.**
11 P5 benp with 5 spines...**12**
– P5 benp with 5 spines/setae..**13**
12 Second outer seta on P5 benp longest; caudal seta II slender......................... ....***D. spinatus*** **Campbell, 1929**
– First and second outer setae on P5 benp equal in length; caudal seta II strong ....***D. truncates*** **Gurney, 1927**
13 P2 exp-3 with 3 outer spines; ♂ P5 benp with 2 setae, inner seta longer than outer seta ....***D. hamiltoni*** **(Thompson & Scott, 1903)**
– P2 exp-3 with 2 outer spines; ♂ P5 benp with 2 same length setae ....***D. tenuicornis*** **(Claus, 1863)**

Non-destructive DNA extraction and identification

The classification of harpacticoids has, until now, been primarily based on adult morphology, especially that of females. Significant differences between species, such as differences in number of segments or setae, are very important and recognizable characteristic that can be used to detect new species. However, some groups require researchers to classify species by features that are difficult describe, such as the width-to-length ratio of appendages, angle of segment inclination, and seta location. In addition, most of the recently discovered cryptic species are morphologically similar to known species. Although meiofauna are difficult to describe, owing to their small, fragile bodies, which make it difficult to obtain large amounts of genomic DNA from individual wild specimens (Sands et al. 2008), DNA sequencing can help with classification. The information about DNA sequences obtained from correctly classified species allows other researchers, for example, ecologists and researchers concerned with invasive species (Garrick et al. 2004) to quickly and easily classify species, even if they lack taxonomic knowledge. The use of DNA sequencing to identify and distinguish among cryptic species also allows taxonomists to identify more accurately taxonomically informative characteristics.

Previously identified harpacticoid species were described on the basis of morphological characteristics, not molecular ones. To classify benthic harpacticoids, observation is usually necessary under a high-power microscope. In this process, DNA in the specimen is destroyed by prolonged microscopic observation and the use of toxic media. Until now, it was difficult to get the DNA sequence and morphological information using same specimen. Therefore, there may be cases of incorrect registration of genetic information for other species. As in the present study and in Cornils (2015), the use of genetic information can reduce the error of species identification. However, specimen vouchers must be preserved for both the verification of genetic sequences and for morphological studies. The present study did not use genetic information for the phylogenetic analysis because the purpose of the study was to match accurately morphological features with the genetic information for each harpacticoid species. For an accurate phylogenetic study based on molecular and morphological data more species belonging to family Miraciidae are needed.

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RESEARCH ARTICLE

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Two new pseudoscorpion species (Pseudoscorpiones, Chthoniidae, Cheiridiidae) from the Tonga Islands, Polynesia, with a redescription of the genus *Nesocheiridium*

Katarína Krajčovičová[1], Aleksandr Vladimirovich Matyukhin[2], Jana Christophoryová[1]

1 *Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina, Ilkovičova 6, SK–842 15 Bratislava, Slovakia* **2** *Institute of Ecology and Evolution, Severtsov Russian Academy of Sciences, Federal State Institution of Science, Leninsky pr. 33, 117 071 Moscow, Russia*

Corresponding author: Katarína Krajčovičová (krajcovic.katarina@gmail.com)

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Abstract

The genera *Tyrannochthonius* Chamberlin, 1929 and *Nesocheiridium* Beier, 1957 are recorded from the Tonga Islands, Polynesia, for the first time. *Tyrannochthonius eua* **sp. nov.** is described from the island of Eua. *Nesocheiridium onevai* **sp. nov.** is described from the island of Onevai. This is the first discovery of a representative of the genus *Nesocheiridium* in more than 60 years. The holotype of the type species, *Nesocheiridium stellatum* Beier, 1957, is redescribed, allowing a better understanding of this poorly known genus. The genus *Nesocheiridium* is diagnosed by the following combination of characters: integument coarsely granulate, dorsally granulo-reticulate; vestitural setae either relatively long, with a leaf-like outline, or arcuate with a small spine; cucullus short; only 10 abdominal tergites visible in dorsal view; cheliceral rallum of four blades; venom apparatus present in both chelal fingers; fixed chelal finger with granulate swelling mesally and seven trichobothria; trichobothria *ib* and *ist* located distad of granulate swelling; *eb* and *esb* situated close together at the base of the finger; moveable chelal finger with two trichobothria.

Keywords

Endemism, *Nesocheiridium*, Oceania, taxonomy, *Tyrannochthonius*

Introduction

Polynesia is a subregion of Oceania, comprising more than a thousand islands spread across the central and southern Pacific Ocean. The small size of the islands and their isolation promote strong evolutionary selection (Filin and Ziv 2004) and high endemism of the fauna (Udvardy 1965). During an expedition to collect invertebrates in Oceania in 1980, a few pseudoscorpions were collected on the Tonga Islands. The Kingdom of Tonga comprises 169 islands, stretching approximately 800 km in a north-south line in Polynesia, flanked by Fiji to the northwest and Samoa to the northeast. Tonga, like much of Polynesia, is poorly known in terms of its pseudoscorpion fauna. Except for New Zealand (Harvey 2013), only a few works have dealt with the pseudoscorpions of this region (With 1907; Kästner 1927; Beier 1932, 1940; Chamberlin 1939a, b; Muchmore 1979, 1983, 1989, 1993, 2000; Harvey 2000). Only a single species, *Geogarypus longidigitatus* (Rainbow, 1897), had been recorded from the Tonga Islands before now (Harvey 2000). Two species are added here, belonging to the genera *Tyrannochthonius* Chamberlin, 1929 and *Nesocheiridium* Beier, 1957.

The genus *Tyrannochthonius* is widely distributed in tropical and subtropical regions of the world. It is one of the largest chthoniid genera, with 130 described species. Most of these have restricted distributions, known from only a few locations. The available data indicate a tendency for short-range endemism of its species (Edward and Harvey 2008; Harvey 2013). In Polynesia, *Tyrannochthonius* species have only been recorded from Hawaii (Muchmore 1983, 1989, 1993, 2000) and New Zealand (Chamberlin 1929; Beier 1966, 1967, 1976). The Tongan specimen belongs to a new species, which is described here.

The genus *Nesocheiridium* was erected in the Cheiridiidae by Beier (1957), with *Nesocheiridium stellatum* Beier, 1957 as its only included species. Until now, the holotype of *N. stellatum*, from Saipan, Marianna Islands, Micronesia, has been the only known specimen of the genus. A re-examination of that species and the new species described here allow a better characterization of the genus.

Methods

All specimens examined for this study had been preserved in 75% ethanol. They were studied as temporary slide mounts, prepared by immersing the specimens in lactic acid for clearing. After study, they were rinsed in water and returned to 75% ethanol, with the dissected portions placed in microvials.

Morphological and morphometric analyses were performed using a Leica DM1000 compound microscope with an ICC50 Camera Module (LAS EZ application, 1.8.0). Measurements were taken from digital images using the AxioVision 40LE application. Reference points for measurements follow Chamberlin (1931), except that the pedicel was included in the measurements of the lengths of the chela and chelal hand. Drawings were made using a Leica DM1000 drawing tube. Digital photographs of new species (Figs 2, 4) were taken using a Canon EOS 5D camera attached to a Zeiss Axio Zoom.V16 stereomicroscope. Image stacks were produced manually, combined using Zerene Stacker software, and edited with Adobe Photoshop CC. Photographs of *N. stellatum* were taken at the Collaborative Invertebrate Laboratory, Field Museum, Chicago, USA (**FMNH**) using a Digital Microptics system consisting of a Nikon D5100 camera, a flash lighting system, P-51 Camlift with controller and software including Base plate, on a computer workstation.

Terminology follows Chamberlin (1931), except for the naming of the palpal and pedal segments (Harvey 1992) and the use of the terms rallum (Judson 2007) and duplex trichobothria (Judson 2018). Trichobothrial homologies follow Harvey (1992).

The types of new species are deposited in the zoological collections of the Naturhistorisches Museum Wien, Austria (**NHMW**).

Results

Chthoniidae Daday, 1889

Tyrannochthonius Chamberlin, 1929

Diagnosis. See Edward and Harvey (2008).

Tyrannochthonius eua sp. nov.

http://zoobank.org/F65825EA-F0DC-4D86-92E5-8B7D67C38383
Figs 1–3

Material examined. ***Holotype*:** Polynesia • ♂; Tonga, Eua [-21.387, -174.930]; 215 m a.s.l.; 11 Jul. 1980; Galina Fedorovna Kurcheva leg.; moss; **NHMW** 29197.

Description. ***Adult male*** (Figs 2, 3). ***Carapace*** (Fig. 3A): 0.97 × longer than broad; with four corneate eyes; epistome present, triangular; with 18 setae arranged 6: 4: 4: 2: 2; without furrows; with two pairs of small lyrifissures, first pair situated in ocular row, second pair situated lateral to setae of posterior row. ***Coxae*** (Fig. 3B): coxa I with rounded apical projection, not bearing microsetae; chaetotaxy of coxae (Fig. 3B): palpal coxae 3; pedal coxae I 3, II 4, III 5, IV 5. Coxa II with eight terminally incised spines, set in an oblique row (Fig. 3B, C). Intercoxal tubercle absent. ***Chelicera*** (Fig. 3D): 1.53 × longer than broad; five setae on hand, all acuminate; moveable finger with one medial seta; fixed finger with 11, moveable finger with nine teeth; one ventral and two dorsal lyrifissures on hand; galea absent; serrula exterior with 15 blades; rallum consisting of seven bipinnate blades. ***Pedipalp*** (Fig. 3E): all setae acuminate, femur setal formula: 5: 2: 1: 3: 5; trochanter 1.44 ×, femur 4.11 ×, patella 2.11 ×, chela 5.18 ×, hand 2.18 × longer than broad. Hand without spine-like seta, dorsal surface with a single row of five chemosensory setae between trichobothria

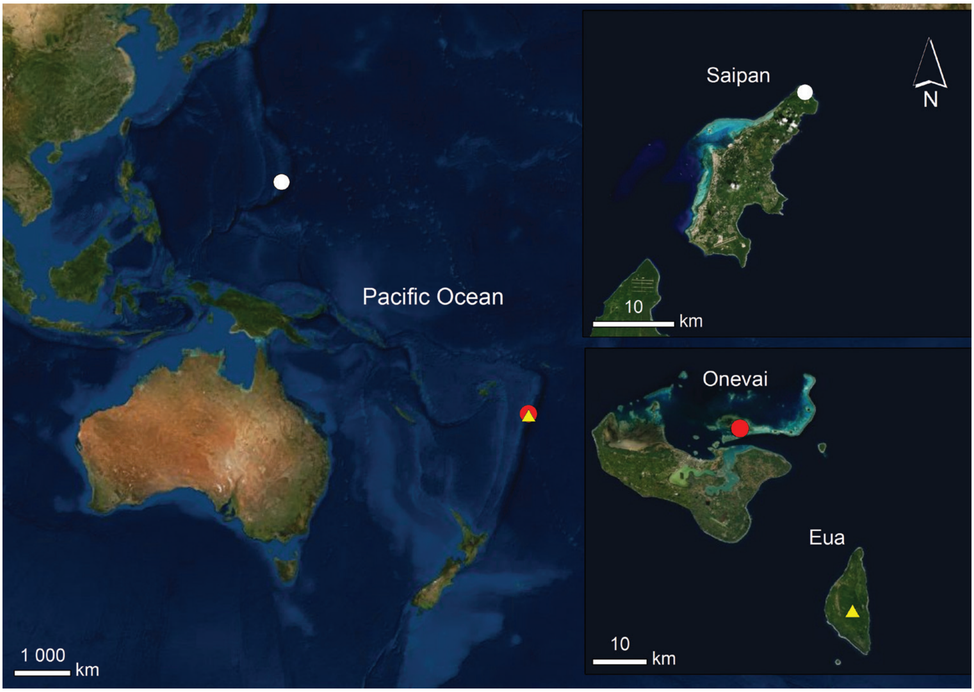

Figure 1. Distribution of the studied species: *Tyrannochthonius eua* sp. nov. (yellow triangle), *Nesocheiridium stellatum* (white circle), *N. onevai* sp. nov. (red circle).

esb and *ib*/*isb*; hand and fixed chelal finger together with eight trichobothria, moveable chelal finger with four trichobothria; *ib* and *isb* close together, submedially on dorsum of chelal hand; *eb* and *esb* close together, at base of fixed finger; *ist* distal to *eb* and *esb*; *it* and *est* less than one areolar diameter apart, *it* slightly distal to *est*; *et* near tip of finger; trichobothrium *st* of moveable finger sub-basally; *sb* slightly closer to *st* than to *b*; *b* and *t* subdistally, *t* at same level as *it*; *b* slightly basal to *est*. Chelal teeth heterodentate: fixed finger with three small teeth followed by 17 large, erect, well-spaced teeth, decreasing in size towards base, distally alternating with six small intercalary teeth; moveable finger with nine large, erect, well-spaced teeth, without intercalary teeth. ***Opisthosoma*:** tergites and sternites undivided; setae uniseriate and acuminate. Tergal chaetotaxy I–IX: 4: 4: 4: 4: 4: 5: 6: 6: 6. Sternal chaetotaxy II–IX: 10: 28: 15: 10: 10: 8: 8: 8 (Fig. 3F). Sternal lyrifissures II–IX: 2: 2: 0: 0: 0: 0: 0: 0. Genitalia: sternite III with narrow V-shaped opening. Genitalia not studied in detail. ***Leg I*:** trochanter 1.13 ×, femur 4.40 ×, patella 2.75 ×, tibia 3.25 ×, tarsus 5.67 × deeper than broad. ***Leg IV*:** trochanter 1.22 ×, femoropatella 1.88 ×, tibia 3.43 ×, metatarsus 2.00 ×, tarsus 6.67 × deeper than broad. Tactile seta present on metatarsus of leg IV; arolium slightly shorter than claws, not divided; claws simple.

Dimensions (length/width or, in the case of the legs, length/depth) in mm. Body length 1.10. Pedipalp: trochanter 0.13/0.09, femur 0.37/0.09, patella 0.19/0.09, chela 0.57/0.11, hand 0.24/0.11, fixed finger 0.31, moveable finger 0.33. Chelicera

Figure 2. *Tyrannochthonius eua* sp. nov., holotype male, dorsal. Scale bar: 1 mm.

0.26/0.17, moveable finger 0.15. Carapace 0.38/0.39. Leg I: trochanter 0.09/0.08, femur 0.22/0.05, patella 0.11/0.04, tibia 0.13/0.04, tarsus 0.17/0.03. Leg IV: trochanter 0.11/0.09, femoropatella 0.32/0.17, tibia 0.24/0.07, metatarsus 0.12/0.06, tarsus 0.20/0.03.

Etymology. The specific epithet refers to the island of Eua, on which this species occurs. It is used as a noun in apposition.

Remarks. The presence of intercalary teeth on the fixed chelal finger but not on the moveable chelal finger is unusual in *Tyrannochthonius* species. However, a few other species possess this combination: *T. convivus* Beier, 1974, *T. brasiliensis* Mahnert, 1979, *T. amazonicus* Mahnert, 1979, *T. rex* Harvey, 1989, and *T. swiftae* Muchmore, 1993 (Beier 1974; Mahnert 1979; Harvey 1989; Muchmore 2000). *Tyrannochthonius eua* sp. nov. differs from *T. convivus*, *T. amazonicus*, *T. rex*, and *T. swiftae* by the significantly shorter palpal femur length (0.37 mm, versus 0.42–0.49 mm in *T. convivus*, 0.46–0.56 mm in *T. amazonicus*, 1.24–1.34 mm in *T. rex*, and 0.53 mm in *T. swiftae*). It also differs from *T. amazonicus*, *T. rex*, and *T. swiftae* in having a lower number of teeth on fixed chelal fingers. In contrast, *T. brasiliensis* has a shorter palpal femur (length 0.28 mm) than *T. eua* sp. nov., as well as lower number of coxal spines on coxae II (4–5, versus 8 in *T. eua* sp. nov.).

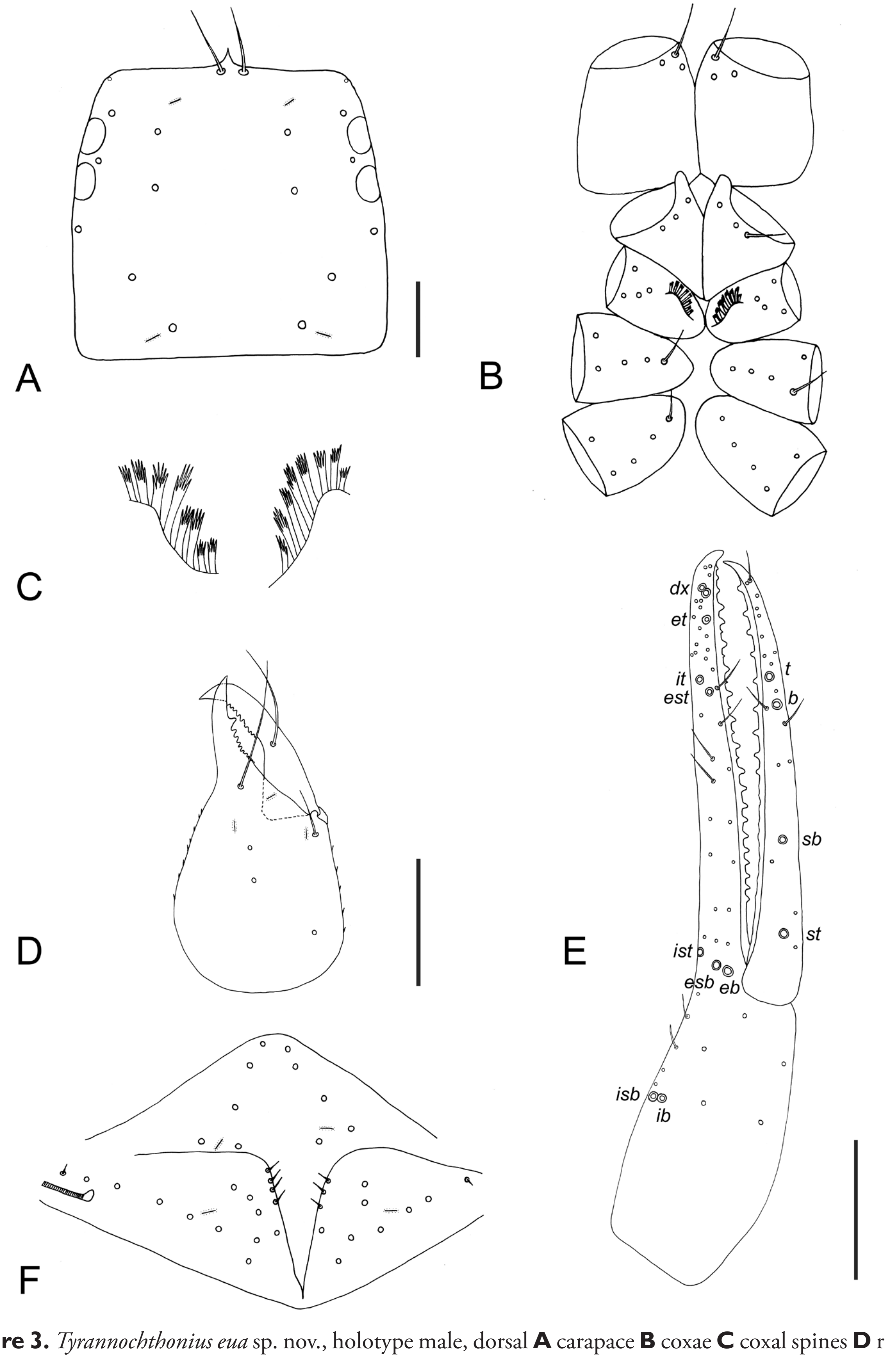

Figure 3. *Tyrannochthonius eua* sp. nov., holotype male, dorsal **A** carapace **B** coxae **C** coxal spines **D** right chelicera **E** right chela, showing trichobothrial pattern **F** chaetotaxy of genital area (sternites II–III). Abbreviations: trichobothria of moveable chelal finger: *t*–terminal, *b*–basal, *sb*–subbasal, *st*–subterminal; trichobothria of fixed chelal finger: *dx*–duplex trichobothria, *et*–exterior terminal, *it*–interior terminal, *est*–exterior subterminal, *ist*–interior subterminal, *esb*–exterior subbasal, *eb*–exterior basal, *isb*–interior subbasal, *ib*–interior basal. Scale bars: 0.1 mm.

Cheiridiidae Hansen, 1894
Cheiridiinae Hansen, 1894

Nesocheiridium Beier, 1957

Diagnosis. Small species, with adult body length ranging from 0.85 to 0.94 mm. Integument coarsely granulate, dorsally granuloreticulate. Vestitural setae relatively long, arcuate with a small spine, often covered by a fine exudate, giving them a leaf-like shape. Carapace narrowed towards anterior end, with short cucullus and a deep, submedian, transverse furrow. One pair of eyes. Cheliceral hand with four setae (seta *ls* absent), all acuminate. Galea long and slender, simple in male, with three terminal rami in female. Rallum of four blades, distal one enlarged and dentate. Ten abdominal tergites visible in dorsal view, I–IX divided. Ventral anal opening large and circular. Pedipalps densely and strongly granulate, including hand and the base of the fixed fingers, femur pedicellate. Fixed chelal finger with granulate swelling mesally, most distinct from ventro-lateral view. Chelal fingers slightly shorter than hand without pedicel. Venom apparatus present in both chelal fingers. Seven trichobothria present on fixed chelal finger, situated mainly in its basal half. Trichobothria *ib* and *ist* located distad of the granulate swelling, *eb* and *esb* situated close together subbasally. Moveable chelal finger with two trichobothria, situated in its basal half.

Remarks. *Nesocheiridium* shares a combination of characters with most genera in the subfamily Cheiridiinae: reduced number of trichobothria on fixed chelal finger (seven at most) and moveable finger (two at most), four setae present on cheliceral hand, first blade of rallum enlarged, femur and patella of legs fused, tarsus of legs as about the same length as tibia (Chamberlin 1931; Beier 1957). The present study confirms the characters mentioned by Beier (1957) to justify the genus *Nesocheiridium*, namely the short cucullus, presence of a granulate swelling on the fixed chelal finger, trichobothria *ib* and *ist* located distad of the granulate swelling, and *eb* and *esb* situated close together subbasally.

Nesocheiridium stellatum Beier, 1957
Figs 1, 4A, 5

Material examined. ***Holotype***: Northern Mariana Islands • ♂; Saipan, Mount Marpi [15.283, 145.817]; 40 m a.s.l.; 01 Mar. 1945; Henry S. Dybas leg.; under stone; **FMNH-INS** 0000 011 070.

Redescription. ***Adult male*** (Figs 4A, 5). Integument coarsely granulate, dorsally granuloreticulate (Fig. 5B, C). Vestitural setae arcuate with a small spine, often covered by a fine exudate giving them a leaf-like shape (Fig. 5A, D, E). ***Carapace*** (Figs 4A, 5A): 0.85 × longer than broad, subtriangular, distally narrowed; cucullus short; two distinct eyes with lenses; submedian transverse furrow deep; anterior disk laterally with perceptible swellings; posterior disk without a medial depression (Fig. 4A); with 43

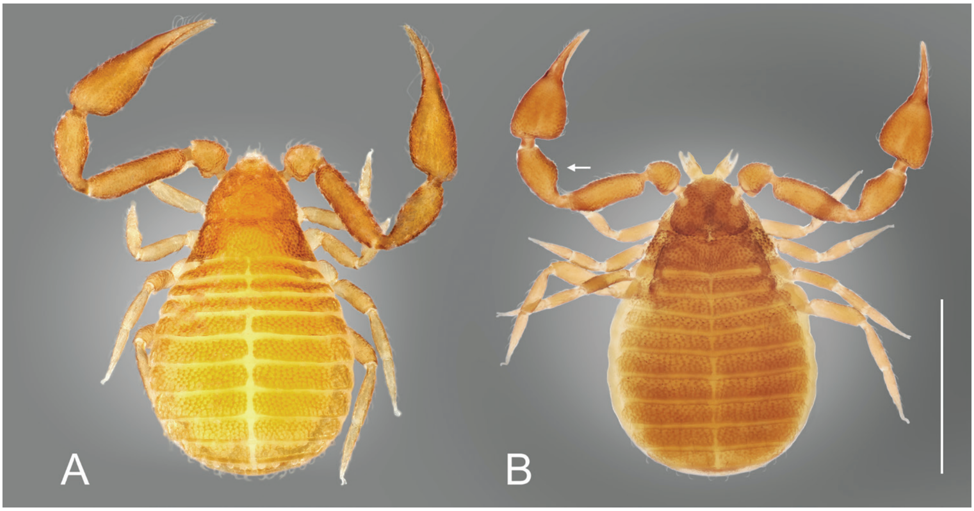

Figure 4. *Nesocheiridium* species, dorsal view **A** *N. stellatum*, holotype male **B** *N. onevai* sp. nov., holotype female. Arrow indicates widening of palpal patella. Scale bar: 0.5 mm.

leaf-like setae (24 before furrow, 19 behind); with one pair of lyrifissures in ocular area. ***Chelicera*** (Fig. 5F): 1.60 × longer than broad; four setae on hand, all setae acuminate; moveable finger with one short seta; fixed finger with three teeth near the tip; with two lyrifissures on hand; galea long, slender, stylet-like, without rami; serrula exterior with 10 blades; rallum consisting of four blades. ***Coxae*** (Fig. 5G): coarsely granulate; chaetotaxy: manducatory process three acuminate setae, rest of palpal coxa with three acuminate and five leaf-like setae in anterior half; pedal coxae I six or seven acuminate setae, II seven acuminate setae, III six or seven acuminate setae, IV 9 or 10 acuminate setae. Lyrifissures: one or two on coxa III, 1 on coxa IV; maxillary lyrifissures not visible. ***Pedipalp*** (Fig. 5H–K): coarsely granulate; trochanter with distinct dorsal hump; patella with distinct pedicel. Trochanter 1.40 ×, femur 4.13 ×, patella 2.89 ×, chela 3.62 ×, hand with pedicel 2.15 × longer than broad. Chela, including base of fixed finger, coarsely granulate (Fig. 5K). Venom apparatus present in both fingers. Fixed chelal finger with seven trichobothria, moveable finger with two. Fixed finger with 16 flat marginal teeth; moveable finger with three flat marginal teeth. Fixed finger with granulate swelling mesally (Fig. 5I), trichobothria *ib* and *ist* distad of swelling. Arrangement of trichobothria as in Figure 5J. ***Opisthosoma*:** Tergal chaetotaxy: 4+5: 6+6: 6+6: 6+7: 7+6: 7+7: 8+8: 7+7: 6+5: 5+5; I–X with leaf-like setae. Tergal lyrifissures I–X: 0+0: 1+1: 1+1: 1+1: 1+1: 1+1: 1+1: 0+0: 0+0: 0+0: 0+0. Tergal pores I–X: 0+0: 0+0: 0+0: 0+0: 2+2: 0+0: 2+1: 0+0: 0+3: 0+3. Sternal chaetotaxy (Fig. 5L): 17: 10: 5+4: 7+6: 7+8: 7+7: 5+6: 7+6: 5+6: 3+3; II–VII with acuminate setae, VIII–XI with leaf-like setae. Sternal lyrifissures II–XI: 2: 2: 1+1: 0+0: 1+1: 1+1: 1+1: 1+1: 1+1: 0+0. Sternal pores II–XI: 4: 0: 7+8: 7+7: 5+6: 1+1: 0+0: 0+0: 1+2: 3+3. Anal opercula each with two short, acuminate setae. Genitalia not studied in detail. ***Leg I*** (Fig. 5M): trochanter 0.86 ×, femoropatella 3.60 ×, tibia 3.00 ×, tarsus 4.33 × deeper than broad.

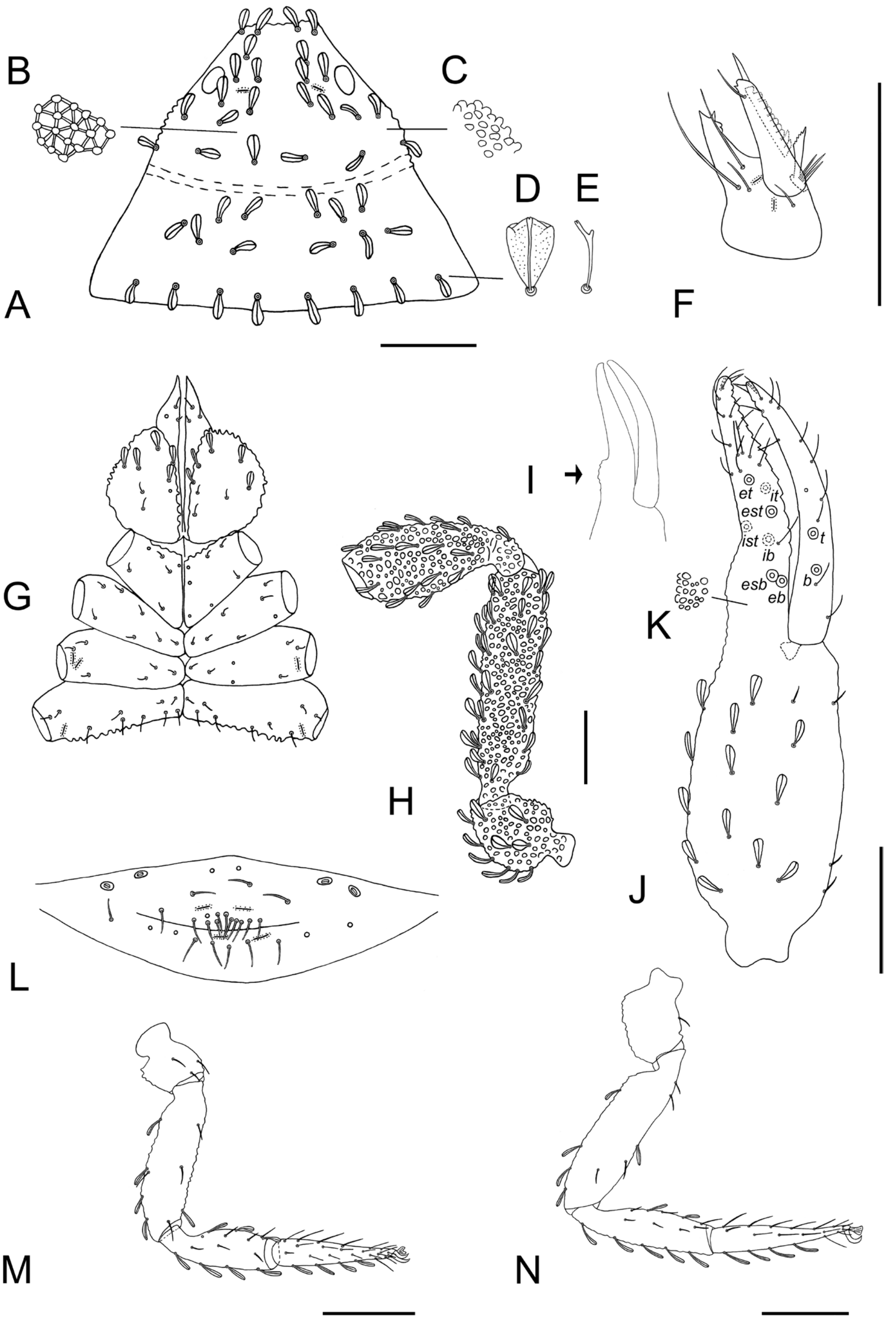

Figure 5. *Nesocheiridium stellatum*, holotype male **A** carapace **B, C** details of granulation types on carapace **D** leaf-like seta **E** arcuate seta with a small spine **F** right chelicera **G** coxae **H** right palp minus chela **I** chelal fingers, ventro-lateral view, showing swelling on fixed finger **J** right chela **K** detail of granulation on chela **L** chaetotaxy of genital area (sternites II–III) **M** right leg I **N** right leg IV. Abbreviations as for Figure 3. Scale bars: 0.1 mm.

Leg IV (Fig. 5N): trochanter 1.43 ×, femoropatella 3.83 ×, tibia 3.60 ×, tarsus 5.67 × longer than deep. No tactile setae present; claws simple; arolia shorter than claws.

Dimensions (length/width or, for legs, length/depth) in mm. Body length 0.94. Pedipalp: trochanter 0.14/0.10, femur 0.33/0.08, patella 0.26/0.09, chela 0.47/0.13, hand with pedicel 0.28/0.13, hand without pedicel 0.23, moveable finger 0.21. Chelicera: 0.08/0.05, moveable finger 0.07. Carapace 0.34/0.40. Leg I: trochanter 0.06/0.07, femoropatella 0.18/0.05, tibia 0.12/0.04, tarsus 0.13/0.03. Leg IV: trochanter 0.10/0.07, femoropatella 0.23/0.06, tibia 0.18/0.05, tarsus 0.17/0.03.

Remarks. Some of the morphometric values given here differ slightly from the original description (Beier 1957) (e.g., body size 0.94 versus 0.90 mm; length of carapace 0.34 versus 0.32 mm; width of carapace 0.40 versus 0.37 mm).

Nesocheiridium onevai **sp. nov.**

http://zoobank.org/1EBB04B6-8414-42BE-950D-BE50D4397D44
Figs 1, 4B, 6

Material examined. ***Holotype*****:** Polynesia • ♀; Tonga, Onevai [-21.087, -175.115]; 7 m a.s.l.; 10 Jun. 1980; Galina Fedorovna Kurcheva leg.; moss; **NHMW** 29188.

Description. ***Adult female*** (Figs 4B, 6). Integument coarsely granulate, dorsally granuloreticulate (Fig. 6B, C). Vestitural setae arcuate with a small spine, often covered by a fine exudate, giving them a leaf-like shape. ***Carapace*** (Fig. 6A): 0.72 × longer than broad, subtriangular; cucullus short; two distinct eyes with lenses; two lateral lighter sections at the level of the eyes (this is not due to damage); submedian transverse furrow deep (carapace slightly damaged in middle); anterior disk laterally with two protuberances, posterior disk with a shallow medial depression in its middle (Fig. 6A); with 30 leaf-like setae (20 before furrow, 10 behind); with one pair of lyrifissures in ocular area. ***Chelicera*** (Fig. 6D): 1.80 × longer than broad; four setae on hand, all acuminate; moveable finger with one short seta; fixed finger with two teeth near tip; with two lyrifissures on hand; galea long, slender, with three apical rami; serrula exterior with 10 blades; rallum consisting of four blades (Fig. 6E). ***Coxae*** (Fig. 6F): coarsely granulate; chaetotaxy: manducatory process two or three acuminate setae, rest of palpal coxa with four acuminate and two leaf-like setae, situated in anterior half; pedal coxae I 5–7 acuminate setae, II 6–8 acuminate setae, III seven or eight acuminate setae, IV eight or nine acuminate setae. Lyrifissures: one on coxa III, one on coxa IV; maxillary lyrifissures not visible.

Pedipalp (Fig. 6G–J): coarsely granulate; trochanter with distinct dorsal hump; patella markedly broadened mesally, with a distinct pedicel (Fig. 4B). Trochanter 1.56 ×, femur 3.25 ×, patella 2.10 ×, chela 2.60 ×, hand with pedicel 1.40 × longer than broad. Chela, including the base of the fixed finger, coarsely granulate (Fig. 6J). Venom apparatus present in both fingers. Fixed chelal finger with seven trichobothria, moveable chelal finger with two trichobothria. Fixed chelal finger with 10 flat marginal teeth; moveable finger with four flat marginal teeth. Fixed finger with granulate swell-

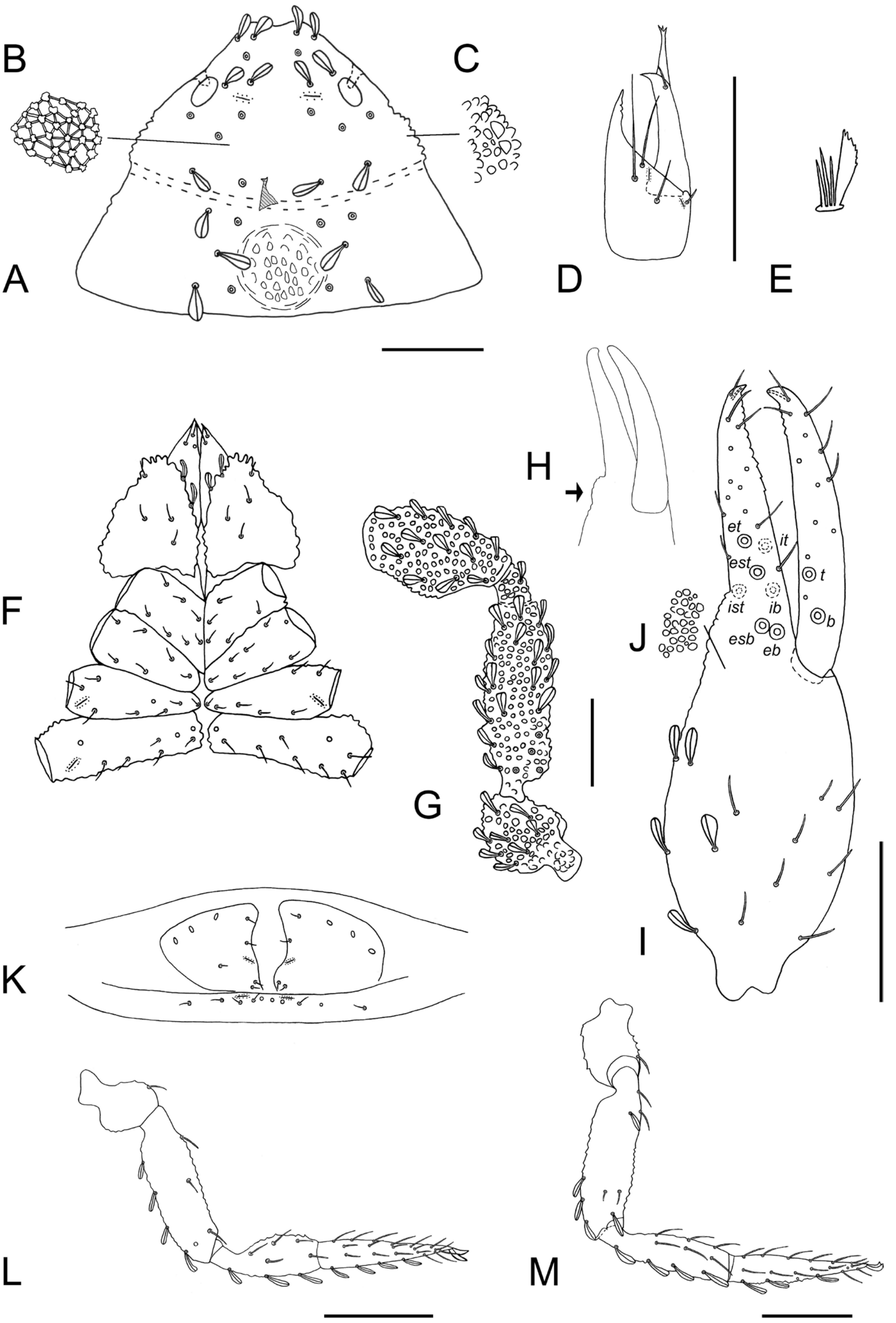

Figure 6. *Nesocheiridium onevai* sp. nov., holotype female **A** carapace (damaged part cross-hatched) **B, C** details of granulation types on carapace **D** right chelicera **E** rallum **F** coxae **G** right palp minus chela **H** chelal fingers, ventro-lateral view, showing swelling on fixed finger **I** right chela **J** detail of granulation on chela **K** chaetotaxy of genital area (sternites II–III) **L** right leg I **M** right leg IV. Abbreviations as for Figure 3. Scale bars: 0.1 mm.

ing mesally (Fig. 6H), trichobothria *ib* and *ist* distad of swelling. Trichobothrial pattern as in Figure 6I. ***Opisthosoma*:** Tergal chaetotaxy: 3+4: 4+4: 5+5: 6+7: 7+7: 7+7: 6+7: 7+6: 5+5: 4+3; I–X with leaf-like setae. Tergites without lyrifissures. Tergal pores I–X: 0+0: 1+2: 1+2: 2+2: 1+2: 2+2: 1+2: 2+2: 0+0: 0+0. Sternal chaetotaxy (Fig. 6K): 5+4 small entrance setae: 10: 4+5: 6+6: 6+7: 7+7: 7+7: 7+7: 5+4: 3+3; II–VIII with acuminate setae, IX–XI with leaf-like setae. Sternal lyrifissures II–XI: 1+1: 2: 0+0: 1+2: 1+1: 1+1: 1+1: 1+1: 1+1: 0+0. Sternal pores II–XI: 6: 0: 5+5: 5+5: 5+4: 0+1: 0+0: 0+0: 1+1: 3+4. Anal opercula: each with two short, acuminate setae. Anterior genital operculum with sternal plates divided. Genitalia not studied in detail. ***Leg I*** (Fig. 6L): trochanter 1.00 ×, femoropatella 3.00 ×, tibia 2.75 ×, tarsus 4.33 × longer than deep. ***Leg IV*** (Fig. 6M): trochanter 1.67 ×, femoropatella 3.80 ×, tibia 3.75 ×, tarsus 5.33 × longer than deep. No tactile setae present; claws simple.

Dimensions (length/width or, for legs, length/depth) in (mm). Body length 0.85. Pedipalp: trochanter 0.14/0.09, femur 0.26/0.08, patella 0.21/0.10, chela 0.39/0.15, hand with pedicel 0.21/0.15, hand without pedicel 0.19, moveable finger 0.18. Chelicera: 0.09/0.05, moveable finger 0.07. Carapace 0.28/0.39. Leg I: trochanter 0.05/0.05, femoropatella 0.15/0.05, tibia 0.11/0.04, tarsus 0.13/0.03. Leg IV: trochanter 0.10/0.06, femoropatella 0.19/0.05, tibia 0.15/0.04, tarsus 0.16/0.03.

Etymology. The species epithet refers to the island Onevai, on which this species occurs. It is used as a noun in apposition.

Remarks. The two species currently placed in the genus are easy to distinguish from each other by the form of the carapace (*N. stellatum* lacks a medial depression on the posterior disk, whereas *N. onevai* sp. nov. has a weak medial depression on the posterior disk); the shape of the palpal patella (not broadened in *N. stellatum*, versus markedly broadened mesally in *N. onevai* sp. nov.); the number of setae on the carapace (43 in *N. stellatum*, 30 in *N. onevai* sp. nov.); the number of marginal teeth on the fixed chelal finger (16 in *N. stellatum*, 10 in *N. onevai* sp. nov.); the shape of the setae on sternite VIII (leaf-like in *N. stellatum*, as opposed to acuminate in *N. onevai* sp. nov.); and the lengths of the palpal segments (femur 0.33 mm in *N. stellatum*, 0.26 mm in *N. onevai* sp. nov.; patella 0.26 mm in *N. stellatum*, 0.21 mm in *N. onevai* sp. nov.; hand with pedicel 0.28 mm in *N. stellatum*, 0.21 mm in *N. onevai* sp. nov.).

Discussion

The original description of the genus *Nesocheiridium* was based on a single male of *N. stellatum*. The discovery of a new, congeneric species affords the opportunity to clarify the diagnostic characters of this inadequately known genus. The presence of a granulate swelling on the base of the fixed chelal finger is considered to be the main diagnostic character of *Nesocheiridium*. However, it is worth noting that, although this character has not previously been mentioned in descriptions of other Cheiridiinae, some drawings, such as those published for *Neocheiridium corticum* (Balzan, 1877) by Mahnert and Aguiar (1986) and for *N. africanum* Mahnert, 1982 by Mahnert (1982), indicate its presence in *Neocheiridium*. Thus, despite a better understanding of the species of *Nesocheiridium*, doubts remain about the validity of the genus. One obstacle to clearly defining the genus within Cheiridiinae is the fact that many of the other genera of Cheiridiinae remain inadequately diagnosed. The need for revisionary work in this subfamily Cheiridiinae has previously been mentioned by other authors (e.g., Mahnert and Aguiar 1986; Judson 2000; Sammet et al. 2020).

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Study on the Pauropoda (Myriapoda) from Tibet, China – Part II: New species and new record of the genus *Samarangopus*

Yun Bu[1]

[1] *Natural History Research Center, Shanghai Natural History Museum, Shanghai Science & Technology Museum, Shanghai 200041, China*

Corresponding author: Yun Bu (buy@sstm.org.cn)

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Abstract

The pauropod family Eurypauropodidae Ryder, 1879 is recorded from Tibet, China for the first time. In this study, a new species *Samarangopus zhongi* **sp. nov.** is described and illustrated from Motuo County, southeastern Tibet of China. It is distinguished from other species in this genus by having one pair of spiniform appendages on the sternum of the last trunk segment, 28–34 marginal protuberances on tergite I, the distal quarter of bothriotricha T_3 golf-club-shaped, and the leaf-shaped seta *st* on tergum of pygidium. In addition, *Samarangopus canalis* Scheller, 2009 is newly recorded from China.

Keywords

appendages, Eurypauropodidae, Motuo County, pauropod, taxonomy

Introduction

Four species of pauropods were recognized in Tibet: *Sphaeropauropus* sp. belonging to the family Sphaeropauropodidae Silvestri, 1930 (Zhang and Chen 1988), *Decapauropus biconjugarus* Qian & Bu, 2018, *D. tibeticus* Qian & Bu, 2018, and

Hemipauropus quadrangulus Qian & Bu, 2018 (Qian et al. 2018) belonging to the family Pauropodidae Lubbock, 1867. However, the investigation of pauropod diversity in Tibet is still insufficient.

The family Eurypauropodidae Ryder, 1879 is currently comprised of more than 60 species (Scheller 2011). It is diagnosed by the following characters: 1) body flattened dorsoventrally; 2) entire tergites strongly sclerotized; 3) incapability to coil the body; 4) coarse and ornamented surface of tergites with modified setae and marginal protuberances. Only two species of Eurypauropodidae were so far reported from China: *Eurypauropus* sp. from Zhejiang Province (Zhang and Chen 1988) and *Samarangopus dilatare* Qian, 2014 from Jiangxi Province (Qian et al. 2014).

The purposes of this study are 1) to record the occurrence of family Eurypauropodidae Ryder, 1879 in Tibet; 2) to describe a new species of the genus *Samarangopus* Verhoeff, 1934; 3) to record the presence of *Samarangopus canalis* Scheller, 2009 in southeastern Tibet for the first time.

Materials and methods

All pauropods were collected using a Tullgren's funnel. The specimens were sorted under a stereomicroscope and preserved in 80% alcohol. They were mounted on slides using Hoyer's solution and dried in an oven at 50 °C. Observations were performed under a phase contrast microscope (Leica DM 2500). Photos were taken using a digital camera (Leica DMC 4500). Line drawings were made using a drawing tube. All specimens were deposited in the collection maintained by the Shanghai Natural History Museum (**SNHM**).

Abbreviations used in the descriptions follow Qian et al. (2018). Absolute lengths of all other body parts are given in mm and μm. Otherwise, the text refers relative lengths. For the description of the new species, measurements and indices of paratypes are given in brackets.

Results

Taxonomy

Family Eurypauropodidae Ryder, 1879

Genus *Samarangopus* Verhoeff, 1934

Type species. *Samarangopus jacobsoni* (Silvestri, 1930).

Diagnosis. Fourth antennal segment with 3 well developed setae; globulus of sternal antennal branch g short-stalked; all legs 5-segmented; empodia with 1 anterior accessory claw (Scheller 2011).

Distribution. Palaearctic, Ethiopian, Oriental, and Australian regions.

Samarangopus zhongi **sp. nov.**

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Figures 1−3

Material examined. ***Holotype***, male adult with 9 pairs of legs (slide no. XZ-PA2015004) (SNHM), China, Tibet, Motuo county, Dexing town, extracted from soil samples in a broad-leaf forest, alt. 1100 m, 29°40'N, 95°26'E, 3-XI-2015, coll. Y. Bu. ***Paratypes***, 5 male adults with 9 pairs of legs (slides no. XZ-PA2015001, XZ-PA2015006, XZ-PA2015052, XZ-PA2015056, XZ-PA2015057) (SNHM), 3 female adults, with 9 pairs of legs (slides no. XZ-PA2015005, XZ-PA2015024, XZ-PA2015054) (SNHM), same data as holotype. Other material, 1 juvenile, with 6 pairs of legs (slides no. XZ-PA2015051) (SNHM), same data as holotype.

Diagnosis. *Samarangopus zhongi* sp. nov. is characterized by one pair of spiniform appendage on sternum of last trunk segment, 28–34 marginal protuberances on tergite I, the distal quarter of bothriotricha T_3 golf-club-shaped, and the leaf-shaped seta *st* on tergum of pygidium.

Description. Adult body length (0.62–) 0.69 (–0.75) mm (*n* = 9); body yellow to brown (Figs 1A, 3A).

Head (Figs 1D, 3E) setae strongly reduced, dorsally with first row setae a_1 and 1 pair of lateral setae, other setae absent. Temporal organs rectangular in tergal view, length 0.9 of shortest interdistance, glabrous. Tiny pistils present laterally.

Antennae (Figs 1E, 3C). Chaetotaxy of segments 1–4: 2/2/2(g')/3. Setae thin, cylindrical, striate, length of seate on segment 4: *p* =14 (–15) µm, *p'* =14 (–17) µm, *p"* =12 (–15) µm; *u* and *r* absent. Tergal branch *t* cylindrical, (2.1–) 2.6 times as wide as greatest diameter and 1.0 (–1.1) times as long as sternal branch. Sternal branch *s* with distinct anterior indentation at level of *F2*, 1.9 (–2.3) times as long as greatest diameter, anterodistal corner distinctly truncate. Seta *q* similar to setae of segment 4, 15 (–17) µm, (0.9 of–) 1.1 times as long as the length of *s*. Globulus *g* with conical stalk, length of *g* (8–10 µm) 1.7 (–1.8) times as long as its greatest diameter; the latter 0.3 (–0.4) of greatest diameter of *t*; 10 bracts, capsule spherical, diameter = 4–5 µm; stalk length 4–5 µm. Relative lengths of flagella (base segments included): F_1 = 100, F_2 = 48 (–55), F_3 = (78–) 84 (–89). Lengths of base segments: bs_1 = (10–) 12, bs_2 = 5 (–7), bs_3 = 10 (–11) µm. F_1 (4.1–) 4.4 times as long as *t*, F_2 and F_3 (1.9–) 2.3 and (3.2–) 3.7 times as long as sternal branch *s*, respectively. Calyces of F_1 largest, conical, those of F_2 and F_3 smaller, subhemispherical.

Trunk. Setae of collum segment similar, furcate, branches tapering, pointed; main branch striate; secondary branch rudimentary, glabrous; both setae length 10 (–11) µm (Fig. 2A). Appendages barrel-shaped; caps flat (Fig. 2A). Sternite process broad and low, with anterior V-shaped incision. Tergites densely covered with protuberances (Figs 1A–C, 3D, L). Three main types of protuberances observed: large and stalked protuberances present on anterior margin of tergite I and lateral margins of I–VI; smaller fungiform protuberances with transparent hat and subcylindrical foot; small cylindrical cuticular structures with distal candle flame-like vesicle surrounded by cir-

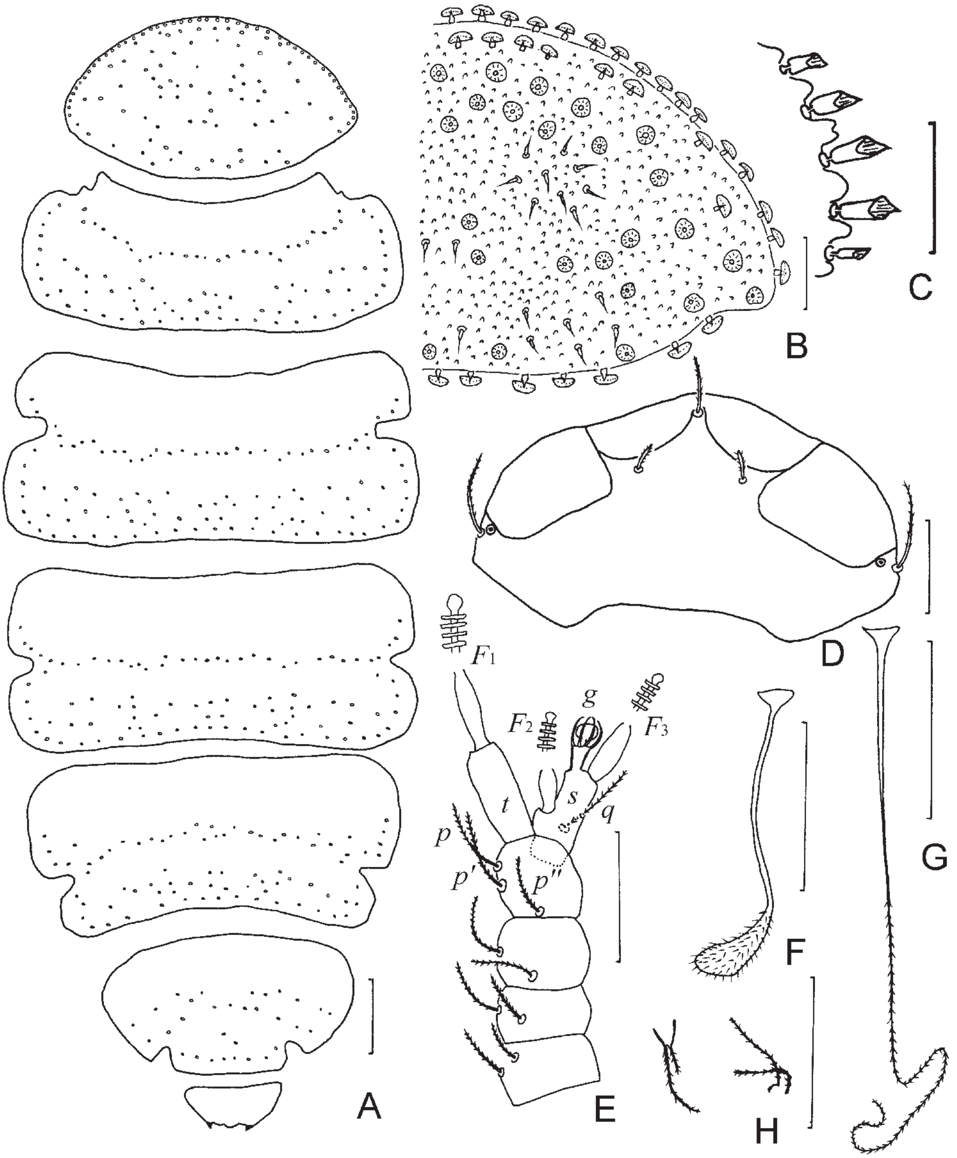

Figure 1. *Samarangopus zhongi* sp. nov. (holotype) **A** body, tergal view **B** tergite I, right side **C** protuberances on posterolateral corner of tergite I, right side **D** head, anteriodorsal view **E** left antenna, tergal view **F** T_3 **G** T_5 **H** setae on coxa (left) and trochanter (right) of leg 9. Scale bars: 20 µm.

cular collar. Cuticles between these structures coarse. Number of marginal protuberances: I, (28–) 34; II, 1 small- T_1-1 small-(9–10); III, 1 small-7- T_2-l small-7; IV, 1 small-(7–8)- T_3-l small-5; V, 1 small-(7–9)- T_4-1small-3; VI, (6–8)- T_5-l. Length/width ratio of tergites: I=0.56 (–0.6), II=0.35 (–0.37), III and IV = 0.38 (–0.43), V = 0.45 (–0.48), V = (0.54–) 0.57 (Fig. 1A). Sternum of last trunk segment with one pair of blunt, spiniform, pubescent posterior appendages (Figs 2B, 3I), 23 (–26) µm in length.

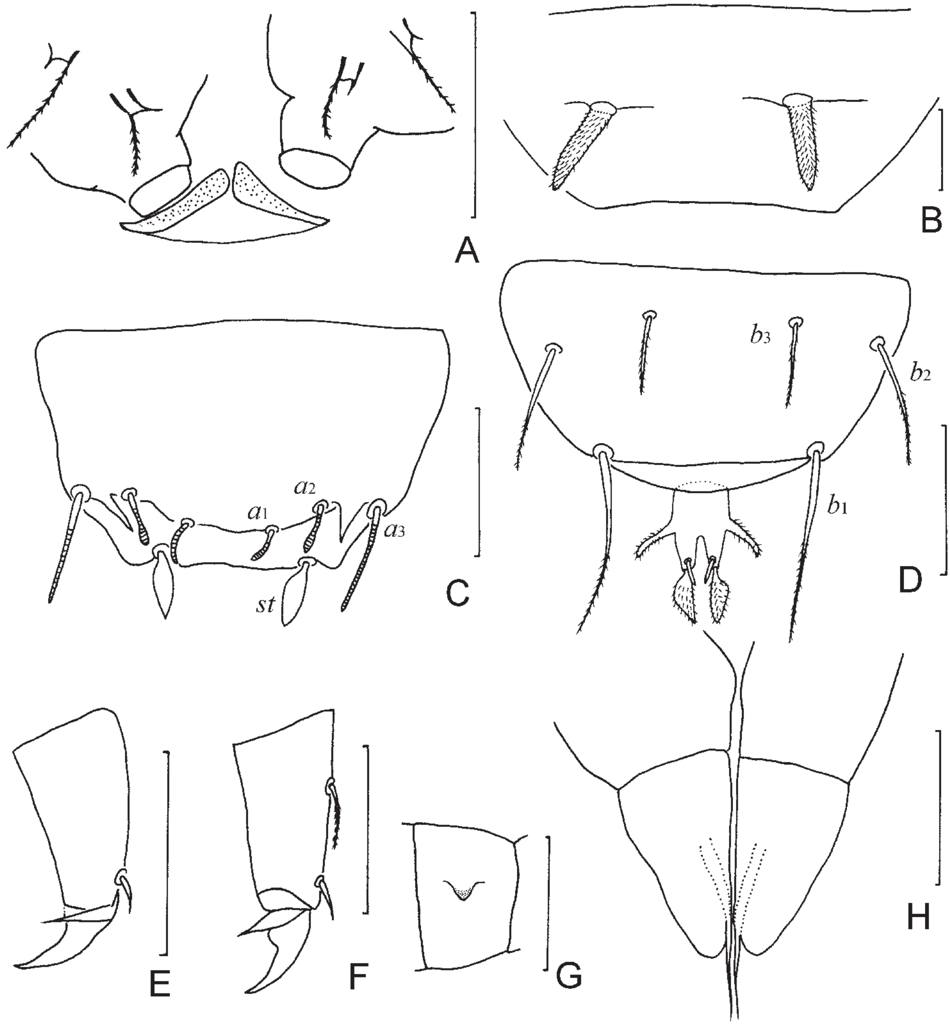

Figure 2. *Samarangopus zhongi* sp. nov. (holotype) **A** collum segment, sternal view **B** sternum of the last trunk segment, show spiniform appendages **C** tergum of pygidium **D** sternum of pygidium and anal plate **E** tarsus of leg 1 **F** tarsus of leg 9 **G** femur of leg 1 with appendage **H** male genital papillae. Scale bars: 20 µm.

Bothriotricha. All with thin axes and short almost erect pubescence, T_1, T_2, T_4, and T_5 with distal part curled (Fig. 1G), T_3 shorter than others, with thicker axis and distal quarter flat, golf-club-shaped, densely pubescent (Figs 1F, 3L). Relative lengths of bothriotricha: T_1 = 100, T_2 = (91–) 94, T_3 = (45–) 53, T_4 = (100–) 106, T_5 = 112 (–115).

Legs. All legs 5-segmented. Setae on coxa and trochanter of leg 9 similar to each other, thin, furcate, striate, with glabrous base, length of secondary branch 0.7 (–0.8) of primary one (Figs 1H, 3J). On more anterior legs these setae similar to those of collum segment (Fig. 3G); Tarsi tapering, those of leg 9 (1.9–) 2.5 times as long as greatest

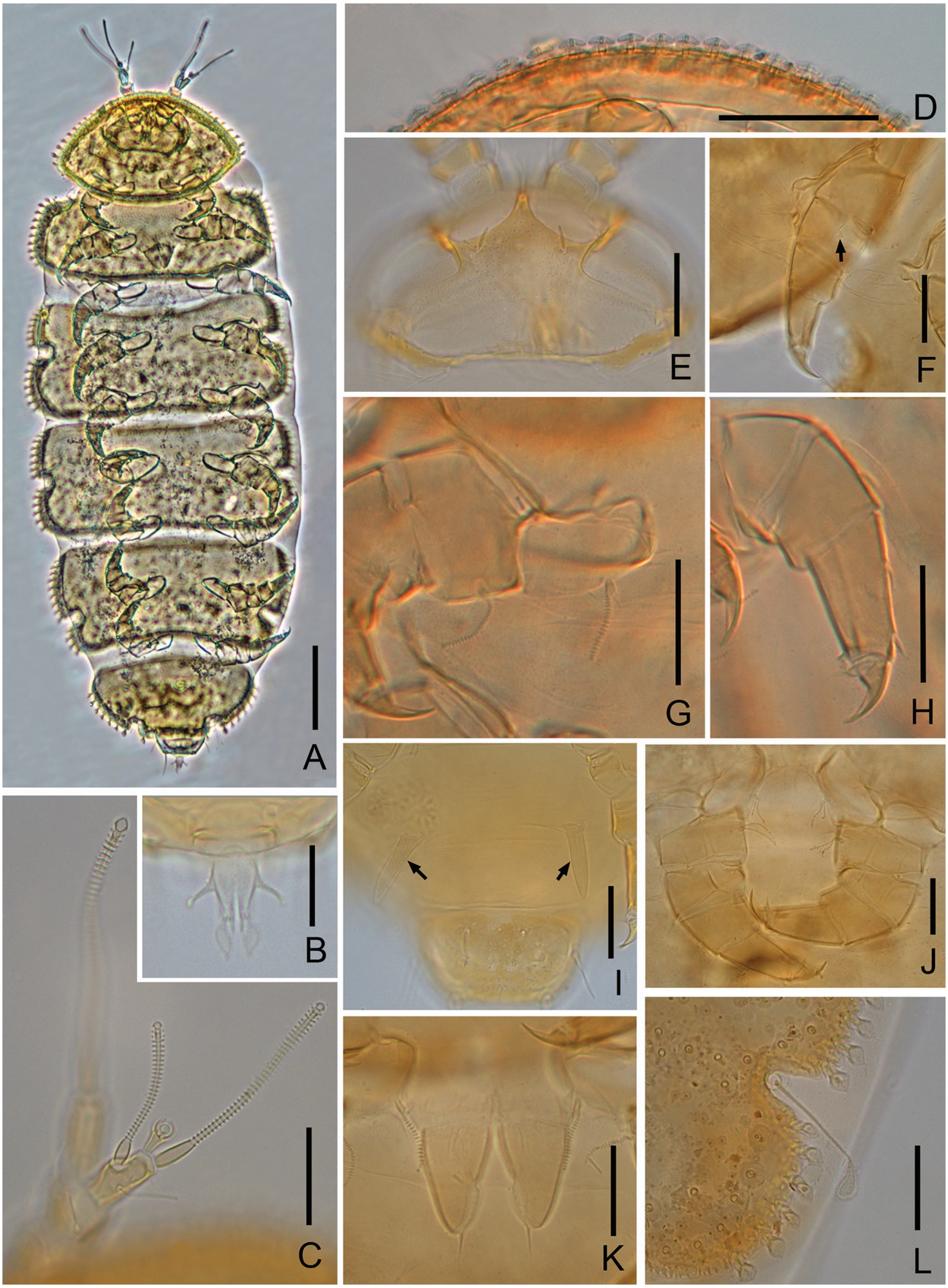

Figure 3. *Samarangopus zhongi* sp. nov. (holotype) **A** habitus, sternal view **B** anal plate **C** right antenna, sternal view **D** anterior margin of tergite I **E** head, tergal view **F** leg 1, arrow shows appendage on femur **G** setae on coxa and trochanter of leg 1 **H** tarsus of leg 9 **I** last trunk segment, arrows show one pair of appendages, sternal view **J** setae on coxa and trochanter of leg 9 **K** male genital papillae and coxa of leg 2 **L** right side margin of tergite IV and T_3. Scale bars: 100 µm (**A**); 20 µm (**B–L**).

diameter; proximal seta striate 11 µm, (0.4–) 0.5 of the length of tarsus (22–28 µm) and (1.7–) 2.0 times as long as distal glabrous seta (5–6 µm) (Figs 2F, 3H). Cuticle of tarsus glabrous (Fig. 3F–H). Tarsus of leg 1 with only glabrous distal seta (Fig. 2E). All legs with large main claw and small setose anterior secondary claw, the former on those of leg 9 0.5 of tarsi. On anterior side of femur of leg 1 with 1 blunt granulated appendage (Figs 2G, 3F).

Genital papillae (Figs 2H, 3K). Base segments cylindrical. Length of papillae = 25 (–28) µm, greatest diameter =15 (–16) µm, length of seta= 9 (–11) µm. Proximal part of genital papillae subcylindrical, distal part conical, seta 0.4 (–0.5) of length of papilla. Cuticle glabrous. Coxal seta of leg 2 same as on leg 1 (Fig. 3G, K).

Pygidium. Tergum (Fig. 2C). Posterior margin with 2 lateral triangular appendages between a_2 and a_3. Setae annulated, a_1 and a_2 somewhat clavate, the former curved inwards and the latter almost straight; a_3 straight, cylindrical, somewhat tapering, diverging; *st* leaf-shaped, glabrous. Lengths of setae: $a_1 = a_2 = 5$ µm, $a_3 = 10$ (–12) µm, *st* = 8 (–9) µm. Distances a_1–a_1=6 (–8) µm, a_1–a_2=5 (–6) µm, a_2–a_3=(4–) 5 µm, *st*–*st*=(8–) 10 µm.

Sternum (Fig. 2D). Posterior margin between b_1 straight. Setae thin, tapering, distally striate, pointed. Lengths of setae: b_1= (25–) 28 (–30) µm, b_2=17 (–20) µm, b_3= (11–) 13 µm. Distance b_1–b_1= (26–) 27 µm, b_2–b_2= 45 (–47) µm, b_1–b_2=15 (–16) µm, b_3–b_3= (18–) 20 µm. b_1 1.0 (–1.3) times as long as interdistance, b_2 (0.9 of–) 1.1 times as long as distance b_1 –b_2, b_3 (0.6–) 0.7 of interdistance.

Anal plate (Figs 2D, 3B) (2.1–) 2.2 times as long as broad, tapering posteriorly; lateral margins with a pair of thin, diverging, cylindrical, distal part faintly inflated, pubescent branches which are (0.3–) 0.4 of the length of plate; posterior 2/5 of plate divided into 2 tapering branches by a narrow V-shaped incision, each branch with 2 appendages: a submedian short, straight, tapering, glabrous one and a stalked bladder of triangular shape in sternal view. Bladder-shaped appendages (0.6–) 0.7 of length of plate. Plate glabrous, bladder-shaped appendages with short erect pubescence.

Etymology. The new species is dedicated in honor of the late Professor Zhong Yang (1964–2017) who was an eminent botanist from Fudan University and Tibet University, for his great contribution to the knowledge of flora and biodiversity of Tibet. This study is also to express my great gratitude to his help.

Distribution. Known only from the type locality.

Remarks. *Samarangopus zhongi* sp. nov. can be easily distinguished from all other congeners by the presence of the one pair of spiniform appendages on the sternal side of last trunk segment. It is most similar to *S. campanulatus* Scheller, 2004 from Vietnam in the shape of anal plate, the chaetotaxy of pygidium and the protuberances on the body. It can be distinguished from *S. campanulatus* by: the spiniform appendages on the sternum of last trunk segment (present in *S. zhongi* sp. nov. vs absent in *S. campanulatus*), shape of bothriotricha T_3 (distal quarter golf-club-shaped, densely pubescent in *S. zhongi* sp. nov. vs distal 2/5 part clavate, end-swelling in *S. campanulatus*), shape of seta *st* on tergum of pygidium (leaf-shaped in *S. zhongi* sp. nov. vs lanceolate in *S. campanulatus*), the shape of proximal seta on tarsus 9 (striated in *S. zhongi* sp. nov. vs glabrous in *S. campanulatus*), and the shape of appendage on the femur of leg 1 (broad triangular in *S. zhongi* sp. nov. vs blunt cylindrical in *S. campanulatus*). The shape of posterior appendage on anal plate of the new species is also similar to *S. tuberosus* Scheller, 2007 from Singapore and *S. cylindratus* Scheller, 2009 from Indonesia. The new species differs from *S. tuberosus* in the shape of setae b_2 on pygidium (slender and pointed in *S. zhongi* sp. nov. vs large and lanceolate in *S. tuberosus*). It differs from *S. cylindratus* in the shape of appendages of the collum segment (barrel-shaped in *S. zhongi* sp. nov. vs cylindrical and large in *S. cylindratus*).

Samarangopus canalis Scheller, 2009, new record to China

Figure 4

Material examined. 1 male adult with 9 pairs of legs (slide no. XZ-PA2015053) (SNHM), 1 female adult with 9 pairs of legs (slide no. XZ-PA2015055) (SNHM), China, Tibet, Motuo county, Dexing town, extracted from soil samples of broad-leaf forest, Alt. 1100 m, 29°40'N, 95°26'E, 3-XI-2015, coll. Y. Bu.

Diagnosis. *Samarangopus canalis* Scheller, 2009 is characterized by the peculiar shape of distal part of male genital papillae which forming an anteriorly open furrow and the ovoid posterior appendages of the anal plate.

Description of new materials. Length 0.90 mm (n = 2), yellow to brown in color (Fig. 4A). Head covered by tergite I and chaetotaxy not studied in detail.

Antennae (Fig. 4B). Chaetotaxy of segments 1–4: 2/2/2/3. Setae thin, cylindrical, striate, length of seate on segment 4: p = 16–18 µm, p'= 15–17 µm, p''= 10–12 µm; p''' rudimentary; u and r absent. Tergal branch t fusiform, 2.9–3.2 times as wide as greatest diameter and 1.2–1.3 times as long as sternal branch. Sternal branch s with distinct anterior indentation at level of F_2, 1.8–2.0 times as long as greatest diameter, anterodistal corner distinctly truncate. Seta q similar to setae of segment IV, 15–16 µm, 0.8 of the length of s. Globulus g with conical stalk, length of g (10–12 µm) 1.7–2.0 times as long as greatest diameter; the latter 0.2–0.3 of greatest diameter of t; 9–10 bracts, capsule spherical, diameter = 3 µm; stalk length 5 µm. Relative lengths of flagella (base segments included): F_1 = 100, F_2 = 35–40, F_3 = 82–84. Lengths of base segments: bs_1 = 15–18 µm, bs_2 = 7–8 µm, bs_3 = 13–14 µm. F_1 3.3–3.7 times as long as t, F_2 and F_3 1.6–1.9 and 3.6–3.9 times as long as sternal branch s. Calyces of F_1 largest, conical, those of F_2 and F_3 smaller, subhemispherical.

Trunk. Setae of collum segment similar, furcate, branches tapering, pointed; main branch cylindrical, annulated, blunt, secondary branch 0.3 of the length of primary branch, glabrous (Fig. 4C); submedian seta 0.8–0.9 of the length of sublateral seta. Sternite process broad and low, with anterior incision and rounded pubescent lobes. Appendages subcylindrical, caps flat. Process and appendages glabrous. Tergites densely covered with protuberances. Anterior and lateral margins of tergites with a single row of large protuberances (Fig. 4A, E, F). Posteriomedian margin of tergites with comb-shaped ornaments (Fig. 4G). Number of marginal protuberances: I, 26–29; II, 1

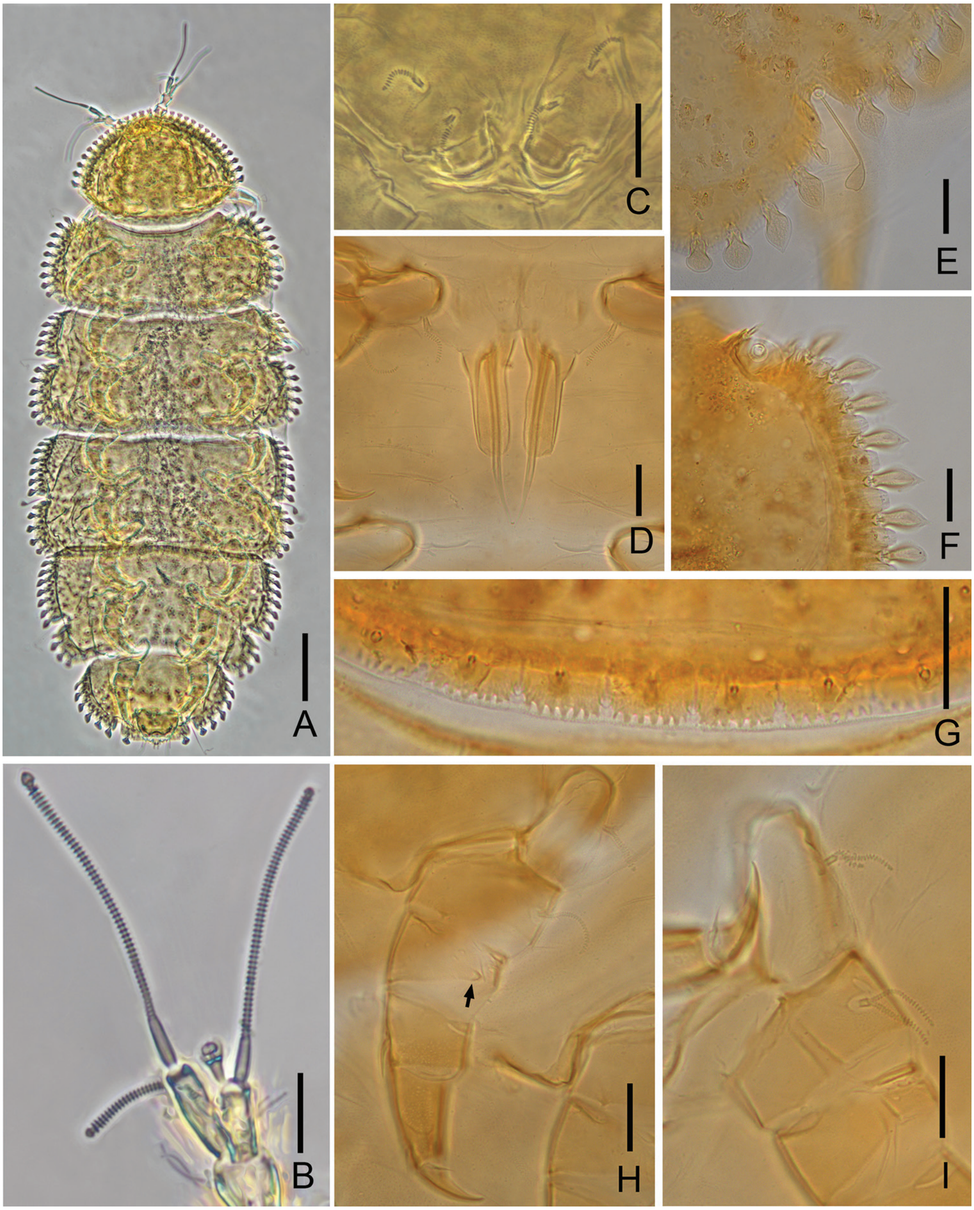

Figure 4. *Samarangopus canalis* Scheller, 2009 (Chinese specimens) **A** habitus, tergal view **B** left antenna, tergal view **C** collum segment, sternal view **D** male genital papillae **E** right margin of tergite IV, show T_3 **F** right anterolateral corner of tergite II, tergal view **G** posteriomedian margin of Tergite I **H** leg 1 **I** setae on coxa and trochanter of leg 9. Scale bars: 100 μm (**A**); 20 μm (**B–I**).

small- T_1-1 small-9; III, (5–6)- T_2-l small-6; IV, (6–7)- T_3-l small-5; V, 8- T_4-l small-3; VI, 6- T_5-l. Length/width ratio of tergites: I = 0.67–0.72, II = 0.34–0.36, III and IV = 0.42–0.45, V = 0.44–0.46, VI = 0.56–0.59.

Bothriotricha. All with thin axes, glabrous proximal parts, distally with minute pubescence, T_1, T_2, T_4 and T_5 curled distally, T_3 shorter than others, with thicker axis and terminated by an ovoid swelling (Fig. 4E). Relative lengths of bothriotricha: T_1 = 100, T_2 = 94, T_3 = 56, T_4 = 94, T_5 = 100.

Genital papillae (Fig. 4D). Base segments in the shape of a truncated cone, relatively long, length of papillae 65 μm, greatest diameter 20 μm, seta 55 μm. Proximal part of papillae strongly tapering outward, distal 3/4 forming an anteriorly open furrow. Papilla 3.3 times as long as greatest diameter, seta 0.8 of length of papilla. Cuticle glabrous. Coxal seta of leg 2 in male with long base, furcate, primary branch cylindrical, annulated, secondary branch short, tapering, pointed, glabrous.

Legs. All legs 5-segmented. Setae on coxa and trochanter of leg 9 similar to each other, thin, furcate, densely annulated, length of secondary branch 0.7–0.8 of primary one (Fig. 4I). On more anterior legs these setae with rudimentary secondary branches (Fig. 4H). Tarsi of leg 9 short and thick, tapering, 2.2 times as long as greatest diameter; tergal setae pointed, glabrous. Proximal seta length 9–10 μm, 0.3 of the length of tarsus (33 μm) and 1.2–1.3 times as long as distal seta (7–8 μm). Cuticle of tarsus with minute granules. No proximal seta on tarsus of leg 1. All legs with large main claw and small setose anterior secondary claw, the former on those of leg 9 0.5 of tarsi. Anterior side of femur of leg 1 with one blunt appendage with short pubescence, length = 4–5 μm (Fig. 4H).

Pygidium. Tergum. Posterior margin with two narrow, digitiform posterior directed processes protruding from between setae a_2 and a_3. Setae glabrous, a_1 straight, a_2 clavate, short, a_3 slender and long; *st* long and leaf-shaped, 10–11 μm. Two semicircle plates close to *st* with dense pubescence. Lengths of setae: a_1= 5 μm, a_2 =6–8 μm, a_3 = 15 μm. Distance a_1–a_1=7–9 μm, a_1–a_2=5–7 μm, a_2–a_3=4–5 μm, *st*–*st*=10 μm.

Sternum. Posterior margin between b_1 almost straight. Setae thin, tapering, pointed, distal part of b_1 annulated, b_2 and b_3 striated. Lengths of setae: b_1= 32 μm, b_2=23–25 μm, b_3= 11–13 μm. Distances b_1–b_1= 28–30 μm, b_2–b_2= 50–53 μm, b_1–b_2= 21–23 μm, b_3–b_3= 23–25 μm. b_1 1.1 times as long as interdistance, b_2 1.0–1.2 of distance b_1 –b_2, b_3 0.55 of interdistance.

Anal plate. 1.2 times as long as broad; lateral margins straight anteriorly, concave posteriorly; distal part of plate cleft by narrow U-shaped incision, depth 0.3–0.4 of the length of plate, incision forming two posterior branches with subparallel sides, each with two appendages: a submedian short, straight, glabrous one and a thin folioform stalked appendage protruding backward. Folioform appendage about 0.6 of length of plate. Plate glabrous, distal appendages with somewhat granular surface.

Distribution. China (Tibet), Indonesia (Sulawesi).

Remarks. *Samarangopus canalis* was originally described and only known from Sulawesi Island, Indonesia (Scheller 2009). The anal plate, the male genital papillae as well as the protuberances on the body of Chinese specimens are nearly the same with *S. canalis* which proved the species identity. The main difference is that the posterior branches of anal plate of Chinese specimens each have two appendages, with a submedian, short, straight, glabrous appendage present, but absent in the types from Sulawesi. Other minor differences are the body size, numbers of protuberances on the body and the lengths of setae, bothriotricha, and flagella, which might belong to the variances between populations of different localities. In addition, the anal plate of *S. canalis* and *S. zhongi* sp. nov. both having two appendages, but the shape of posterior one is different: bladder is triangular in *S. zhongi* sp. nov. but folioform in *S. canalis*.

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RESEARCH ARTICLE

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After 157 years, a second specimen and species of the phylogenetically enigmatic and previously monobasic genus *Nototylus* Gemminger & Harold, 1868 (Coleoptera, Carabidae, Nototylini)

Terry L. Erwin[1], David H. Kavanaugh[2], David R. Maddison[3]

1 *Hyper-diversity Group, Department of Entomology, MRC-187, National Museum of Natural History, Smithsonian Institution, Washington, P.O. Box 37012, DC 20013-7012, USA* **2** *Curator Emeritus, Department of Entomology, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA* **3** *Department of Integrative Biology, 3029 Cordley Hall, Oregon State University, Corvallis, OR 97331, USA*

Corresponding author: Terry L. Erwin (erwint@si.edu)

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Abstract

The enigmatic beetle tribe Nototylini (Carabidae) is revised and a key to species is provided. Two species from South America are included in the genus. One species, *Nototylus fryi* (Schaum), is reviewed and a second, *Nototylus balli* Erwin & Kavanaugh, **sp. nov.**, is described as new. Each species is known from a single specimen, neither of which is in good condition. The possible function of what appears to be a unique antennal grooming structure on the front femur is discussed.

Resumen

La tribu enigmática de escarabajos Nototylini (Carabidae) se revisa y se provee una clave para las especies. Dos especies de América del Sur están incluidas bajo este género. Se revisa una especie, *Nototylus fryi* (Schaum), y una segunda, *Nototylus balli* **sp. nov.**, se describe como nueva. Ambas especies se conocen de un solo espécimen, ninguno de los cuales está en buenas condiciones. Se discute la posible función de lo que aparece ser una estructura para acicalamiento antenal el fémur anterior.

Résumé

Une révision taxonomique de la tribu énigmatique de coléoptères Nototylini (Carabidae) est présentée et une clé pour l'identification d'espèces est fournie. Deux espèces d'Amérique du Sud sont incluses dans le genre. Une espèce est examinée (*Nototylus fryi* (Schaum)) et, une deuxième est décrite comme nouvelle espèce (*Nototylus balli* **sp. nov.**). Chaque espèce est connue à partir d'un seul spécimen dont aucun est en bon état. La possible fonction de ce qui semble être une structure de toilette antennaire sur le pro-fémur est discutée.

Resumo

Uma enigmática tribo de besouros, Nototylini (Carabidae), é revisada, e uma chave para as espécies de *Nototylus* é fornecida. O gênero agora inclui duas espécies: *Nototylus fryi* (Schaum), aqui redescrita, e *Nototylus balli* **sp. nov.**, descrita como nova. Ambas são conhecidas por somente um espécimen em mal estado de conservação. Discute-se a função de uma estrutura singular do femur anterior, possivelmente usada na escovagem da antena.

Keywords

Brazil, French Guiana, rainforest, antennal comb

Palabras clave

Brasil, Guyana Francesa, bosque lluvioso, peine antenal

Mots clés

Brésil, Guyane, forêt tropicale, structure de toilette antennaire

Palavras chaves

Brasil, Guiana Francêsa, floresta tropical, pente antenal

Introduction

The affinities of the taxon *Nototylus* as described by Schaum in 1863 (under the preoccupied name *Tylonotus*) based on a single specimen have posed a conundrum for carabidologists since Schaum's time. Complicating the interpretation of the form and structure of this unique specimen is the fact that its poor initial preservation has led to its almost complete disarticulation during subsequent studies (see Deuve 1994).

It has been reported that, unlike all other carabid beetles except highly evolved Paussini adults, adults of *Nototylus fryi* (Schaum), the type species of the genus, have no antennal cleaner on the anterior tibia, hence the origin of a long-standing debate about whether or not it belongs in the family Carabidae (Deuve 1994). Although Schaum's original description made no mention of an antennal cleaner, nor was one shown in his illustrations, and Deuve (1994) made no mention of an antennal cleaner in his fine overall redescription, Erwin (1979, 2011) noted that the overall shape of the beetle is very ozaenine-like. Throughout their evolutionary history, carabid beetles have made sure that their antennae are kept clean, mainly through the development of combing structures ("antennal cleaners") on the front legs, typically on the front tibiae. The selection pressure of ants on the Paussinae (including Ozaenini) and termites on taxa such as the Orthogonini has resulted in incredible transformations in carabid adult structures, so if *Nototylus* is another ant- or termite-associated group, then the "loss" of an antennal comb from the protibia would not be surprising (Fig. 1; Deuve 1994: fig. 11).

We here report on a second *Nototylus* specimen, one in somewhat better condition and representing a second species. This specimen, together with a re-examination of Schaum's original specimen, permits us to report that there does indeed appear to be an antennal grooming structure present in *Nototylus* adults, but one in a different place and perhaps having a different function than is typical for a carabid. The purpose of this paper is to describe this new species and thereby confirm that the tribe Nototylini is still extant, at least in undecimated tropical forests in French Guiana. *Nototylus fryi* was described from the Brazilian State of Espíritu Santo, which is now mostly sugar cane fields, cacao plantations, and cattle ranches; and it has been considered that this species is likely now extinct (but see notes below).

Materials and methods

This study is based on the examination of the only two *Nototylus* specimens known. Codens used in the text for institutions in which data or specimens are deposited (with names of curators in parentheses) are as follows:

NHMUK	The Natural History Museum, London, United Kingdom (Beulah Garner);
NMNH	National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (Terry L. Erwin).

Methods and species concepts follow Erwin and Kavanaugh (1981) and Kavanaugh and Erwin (1991). The diagnosis and description format follow as closely as possible that suggested in Erwin and Johnson (2000). Measurements of length (ABL, SBL) and width (TW) follow those suggested by Ball (1972) and Kavanaugh (1979): ABL (apparent body length), measured from apex of labrum to apex of longer elytron; SBL (standardized body length), equals the sum of the lengths of the head (measured from apex of clypeus to a point on midline at level of the posterior edge of compound eyes), pronotum (measured from apical to basal margin along midline), and elytron length (measured from apex of scutellum to apex of the longer elytron); and TW, (total width), measured across both elytra at their widest point.

The images provided of the adult beetles described herein show most of the character states referred to in the description. The habitus images of the adult were made with a Visionary DigitalTM high resolution imaging system. Figures are all of the holotypes. The ADP number, which is a unique identification number for the specimen, links the specimen and associated illustrations and/or image to additional information in electronic databases at the National Museum of Natural History, Smithsonian Institution in Washington, DC (NMNH).

The photograph of a mesotibia and its setae were taken with a Leica Z6Apo lens and DMC4500 camera, and the close-up photograph of the setal apex with a Leica DM5500B compound microscope and DMC425C camera. Leica Application Suite v4.9 software was used to capture each image, and stacks of images from different focal positions were merged using the PMax procedure in Zerene Systems' Zerene Stacker.

Geographical data of the new species were provided by the collector. A map (Fig. 9) indicates where the exact locale is in French Guiana. An English vernacular name also is proposed here because common names are becoming increasingly needed in conservation and/or agricultural and forestry applications.

Taxonomic account

Genus *Nototylus* Gemminger & Harold, 1868

Strange-combed carabid beetles

Tylonotus Schaum, 1863: 74 (preoccupied by *Tylonotus* Haldeman, 1847, a genus in the beetle family Cerambycidae, and *Tylonotus* Fieber, 1858 (Hemiptera).
Nototylus Gemminger & Harold, 1868: 161, new name.

Diagnostic combination. Head domed, sub-hypognathus, with a partial sulcus (Fig. 5) under the anterior part of eye. Profemur with a subapicoventral concavity (Fig. 6) containing slender, elongate and apically ovospatulate setae (Fig. 8). This structure, unique within Carabidae, is presumed to be used for grooming the antenna (we will refer to it as a "grooming structure" below). Protibial antennal cleaner absent. Tibiae flattened as in carabids known to live with ants and lined with sparse apically spatulate setae. Deuve (1994) provided the following additional characteristics: procoxal cavities (*sensu* LeConte 1853) closed with pleural lobe fitted into the prosternal process; the harpalidian-type post abdomen (*sensu* Deuve 1988). Tergite IX differentiated as a thin transverse arch, laterotergites IX reduced and very lateral in location. The combination of character states in this enigmatic genus is unique within Carabidae.

Included species currently recognized.
Nototylus fryi (Schaum), 1863
Nototylus balli Erwin & Kavanaugh, new species

Key to adult females of *Nototylus* Gemminger & Harold, 1868

1 Elytron (Fig. 1) only slightly swollen posterior to humerus; pronotum (Fig. 1) elongate, more strongly narrowed posteriorly, with lateral margins slightly sinuate, not rounded; brachypterous, hindwing without venation distal to stigma .... ***Nototylus fryi* (Schaum)**

– Elytron (Fig. 2) more distinctly swollen posterior to humerus; pronotum (Fig. 2) subquadrate, with sides slightly rounded; macropterous .... ***Nototylus balli* Erwin & Kavanaugh, sp. nov.**

Nototylus fryi **(Schaum), 1863**

Fry's strange-combed beetle
Figures 1, 9

Tylonotus fryi Schaum, 1863: 75.
Nototylus fryi (Schaum): Gemminger & Harold, 1868: 161.

Type material. ***Holotype*** female deposited in NHMUK. Detailed description and illustrations in Deuve (1994). See also Schaum (1863), Bänninger (1927), and Erwin (2011).

Geographical distribution (Fig. 9). Known only from Brazil, Espíritu Santo, without precise locality.

Dispersal potential. Brachypterous (wing truncated without distal venation), probably not capable of flight.

Way of life. Unknown, except that they live in Southern Atlantic Forest (Mata Atlântica).

Note. This taxon is known from a single disarticulated specimen in the NHMUK, which one of us (TLE) has re-examined twice. Its habitat, somewhere in the state of Espíritu Santo, Brazil, likely has suffered forest conversion to sugar cane, cacao plantations, or cattle ranches. The Bahia Coastal Forests ecoregion, which includes the state of Espíritu Santo, has less than 5% of the original forest vegetation remaining. See the web site http://www.worldwildlife.org/ecoregions/nt0103 (last accessed on 19 December 2019) for a very good description of what the area where this unique species lived was like previously and is like now (not good). Perhaps *N. fryi* is still extant in remaining protected areas such as Sooretama Biological Reserve and/or Linhares Forest Reserve. A major effort needs to be made to seek more specimens, particularly males, which remain unknown.

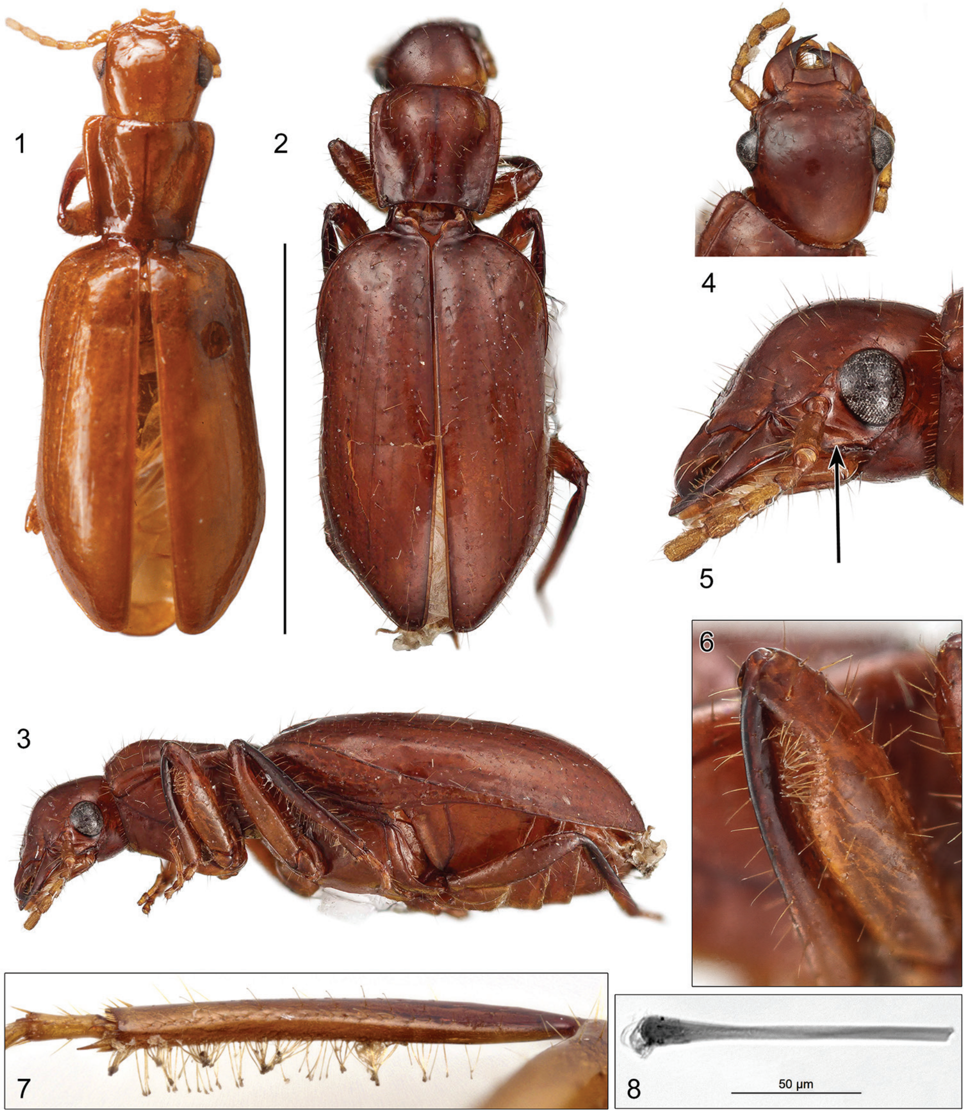
Nototylus balli **Erwin & Kavanaugh, sp. nov.**

http://zoobank.org/2E9A04F6-AF58-43E2-8C79-7765690039A2
Ball's strange-combed beetle
Figures 2–9

Type material. ***Holotype*****:** A female, deposited in NMNH, labeled: French Guiana, Cayenne, track Bélizon, pk 4.5, 90 m (4.3704N, 52.3216W), July 2015 (JL Giuglaris) (NMNH: ADP143591, female).

Derivation of specific epithet. The epithet, *balli*, is a Latinized eponym based on the family name of George E Ball, carabidologist and academic leader of a host of younger carabidologists, including all three coauthors, in celebration of his 90th birthday, 25 September 2016. This species was introduced to George and many other carabidophiles at Athens, GA, during the Fourth International Symposium of Carabidologists in September 2016.

Diagnosis. With the attributes of the genus as described above and slightly larger-sized than the *N. fryi* specimen. Adult with pale brown integument; only the mandibu-



Figures 1–8. *Nototylus* **1** *Nototylus fryi* (Schaum), habitus, dorsal aspect, apparent body length (ABL) = 8.2 mm **2–6** *Nototylus balli* sp. nov.: **2** habitus, dorsal aspect, ABL = 9.1 mm **3** habitus, left lateral aspect **4** head, dorsal aspect **5** head, left lateral aspect; arrow indicates location of sulcus beneath eye **6** left foreleg, lateral aspect; femur with antennal cleaner present subapicoventrally **7** left mesotibia, ventral aspect **8** closeup of a middle leg spatulate seta. Scale bars: 0.5 mm (**1–8**).

lar apices and dorsal margins of tibiae infuscated. Head slightly broader and less narrowed posteriorly and with eyes more convex and hemispheric than in *N. fryi*. Frons and occiput moderately domed, aspect sub-hypognathus; smooth with fine, scattered setigerous punctures, perhaps with one slightly longer superorbital seta. Pronotum markedly domed, subquadrate, grossly explanate basolaterally, with lateral margins

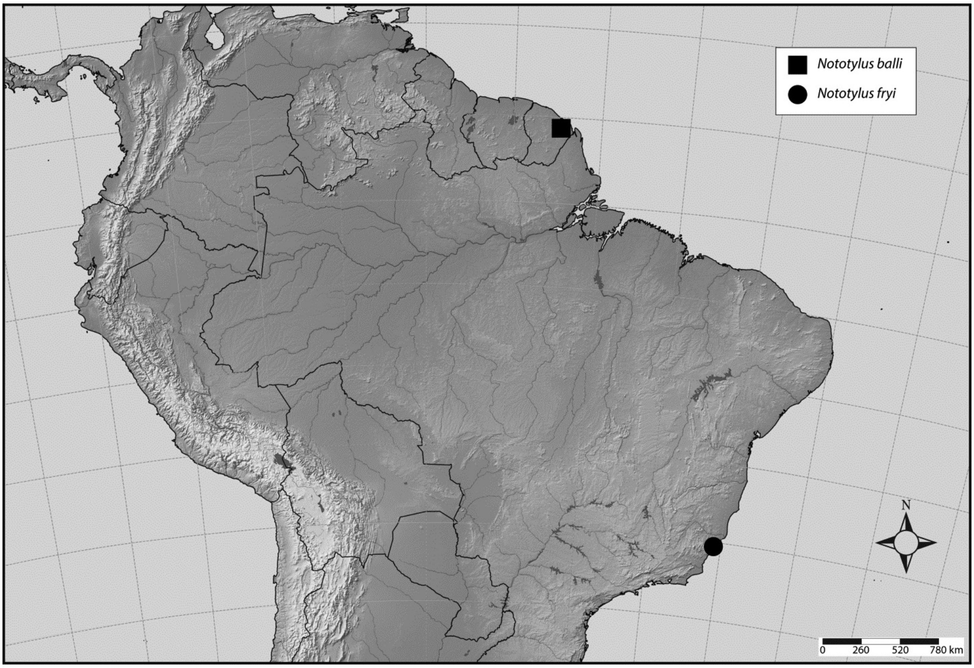


Figure 9. Map illustrating known distributions of species of *Nototylus*. Key: ■ = precise locality for *N. balli* sp. nov. (see text); ● = generalized locality for *N. fryi* (Schaum) in Brazil.

very slightly and evenly convex between front and hind angles (in *N. fryi*, lateral margins straighter and slightly sinuate anterior to hind angles); dorsum, margins and proepipleura sparsely setiferous. Elytron with humerus perfectly rounded, elytral silhouette more distinctly swollen posterior to humerus than in *N. fryi*, lateral margin markedly sinuate, disc markedly convex, apex at level of tucked post-femoral apex obliquely angulate, narrowly rounded apically to suture, not dentate, not plicate; lateral margin and epipleuron markedly setiferous. Interneurs with rounded or slightly elongate punctures, with uneven spacing between punctures. Hindwings macropterous.

Description. (Fig. 2). ***Size*:** ABL = 9.1 mm, SBL = 9.00 mm, TW = 4.6 mm. ***Color*:** As described above. ***Luster*:** Shiny. ***Head*:** As described above. Antennae moderately short, filiform; antennal scape and flagellar antennomeres about twice as long as wide, length of pedicel slightly less than twice its width; all antennomeres with pubescence in addition to multiple fixed setae, with only sparse pubescence on scape and pedicel and denser pubescence on flagellar antennomeres. ***Prothorax*:** Subquadrate, slightly constricted near base, hind angles produced posteriorly, anterior margin broader than neck; surface of disc as described above. ***Pterothorax*:** Elytron markedly convex, slightly broader in anterior third with small epipleural flange, moderately flared from middle to apical third and rounded to hind angle; intervals flat, intervals 1, 3, and 5 with setigerous pores throughout length, interneurs striate. ***Legs*:** Profemur with antennal grooming structure as described for genus (Fig. 6) and with protibia ventrally and mesotibia ventromedially (Fig. 7) with fringes of slender, elongate and apically ovospatulate setae (Fig. 8) like those in the profemoral grooming structure. ***Abdomen*****:** As described above. ***Male genitalia*****:** Unknown for this species. ***Female ovipositor*****:** see Deuve (1994) for *N. fryi*.

Note. Based on unpublished scanning electron microscope images of the foreleg of the female holotype of *N. fryi* from George Ball and shared with us by Wendy Moore, we can now report that *N. fryi* also has the strange ovospatulate setae in the same locations as we have observed in the holotype of *N. balli*.

Geographic distribution. (Fig. 9). This species is currently known only from the type locality in French Guiana.

Dispersal potential. Macropterous and capable of flight. The holotype was collected with a glass pane flight intercept trap (FIT) (JL Giuglaris, pers. comm.).

Way of life. Unknown, except that these beetles live in lowland rainforests and are extremely rarely collected. Only one specimen has been found so far despite the ongoing beetle inventory by the Société entomologique Antilles-Guyane (SEAG) since 2014 in French Guiana (see Notes, below).

Notes. The rainy season in French Guiana consists of heavy rainfall between December and July while the remaining months are comparatively dryer. Annual precipitation reaches 9.652 cm in and around Cayenne. Temperatures reach 25 to 27 °C as an average high at Cayenne. Thus, the specimen described herein was collected in the late warm rainy season a mere 57.4 km south of Cayenne.

From materials thus far collected by the SEAG inventory program, 19,272 carabid specimens have been sent to the first author, TLE. These specimens were collected from 30 different localities in French Guiana, mainly by FITs (flight intercept traps of both the glass pane and net types), but also at lights of various wave lengths (blue, pink), GEM lights, and SLAM traps (a small 4-sided malaise called the Sea, Land, and Air Malaise (SLAM) Trap that floats on water, stands on the ground, or hangs in the sky) (Erwin et al. 2012). None of those was a *Nototylus* specimen. An independent collector (not part of SEAG) came upon the single specimen described here, also collected in a glass pane FIT. It is unclear just why no adults of this species have been collected in any of the many SEAG FITs.

Discussion

The setae in the profemoral grooming structure of the two specimens of *Nototylus* are unlike those seen in the protibial antennal cleaners of all other carabids. Here, they are long, slender, flexible, and apically ovospatulate (Fig. 8), whereas those of antennal cleaners of other carabids are stout, not or barely flexible, and sharply pointed apically. This suggests that the profemoral grooming structure seen here may have a somewhat different function than the protibial antennal cleaner of other carabids, the function of which is clearly seen when watching carabids groom themselves. The location of the profemoral structure in *Nototylus* is certainly one suitable for grooming the antenna; but the form and flexibility of these setae appear more suited to painting or coating the antenna than to scraping or cleaning it. Coupled with the occurrence of similar ovospatulate setae on the ventral surface of the protibia and the ventromedial surface of the mesotibia, it seems more likely that these setae function in applying or at least spreading some substance over the antennae and other parts of the body. The source of such a hypothetical substance is unknown, and we observed no structures, such as the variously located secretory trichomes seen in most if not all other carabids (and other beetles) that live with ants or termites.

These other colony "guests" use such substances to gain acceptance within the host colony. If our hypothesis that *Nototylus* live with ants or termites is correct, then the observed grooming structure and unusual setae may help to facilitate this symbiotic relationship. Clearly, we need to find and observe a living *Nototylus* adult to see how these structures are used.

Conclusions

We had hoped, with this new specimen, to gather both molecular data and morphological data for nototyline male genitalia for the first time. Each of these data types could have led us to a better understanding of tribal relationships. Unfortunately, upon dissection the new specimen also turned out to be a female, as is the single known specimen of *N. fryi* (well-illustrated by Deuve 1994). Even more disappointing was the failure of our attempts to extract and sequence DNA from the specimen; these attempts will be reported in a separate publication. Consequently, we are no closer to understanding nototyline phylogenetic relationships than we have been for the last century and a half. At least we now know that this enigmatic group is still extant and that finding additional, fresh specimens, ideally even live specimens, is a real and most desirable possibility.

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RESEARCH ARTICLE

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Revision of the genus *Hoplodrina* Boursin, 1937 (Lepidoptera, Noctuidae, Xyleninae). I. *Hoplodrina octogenaria* (Goeze, 1781) and its sister species *H. alsinides* (Costantini, 1922) sp. rev. in Europe

Peter Huemer[1], Jean Haxaire[2], Kyung Min Lee[3], Marko Mutanen[3], Oleg Pekarsky[4], Stefano Scalercio[5], László Ronkay[6]

1 Tiroler Landesmuseen Betriebsges.m.b.H., Naturwissenschaftliche Sammlungen, Krajnc-Str. 1, A-6060 Hall, Austria **2** *Le Roc, 47310 LaPlume, France* **3** *Department of Ecology and Genetics, University of Oulu, PO Box 3000, FI-90014, Oulu, Finland* **4** *Felsőerdősor u. 16-18, H-1068, Budapest, Hungary* **5** *Council for Agriculture Research and Economics, Research Centre for Forestry and Wood, Rende, Italy* **6** *Hungarian Natural History Museum, Budapest, Hungary*

Corresponding author: Peter Huemer (p.huemer@tiroler-landesmuseen.at)

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Abstract

The taxonomic status of the European *Hoplodrina octogenaria* (Goeze, 1781) is discussed and its partly sympatric sister species, *Hoplodrina alsinides* (Costantini, 1922) **sp. rev.**, is separated and re-described based on morphological and molecular taxonomic evidence. The adults and their genitalia are illustrated and DNA barcodes, as well as genome-wide single nucleotide polymorphism data collected by fractional genome sequencing (ddRAD), of the two species are provided.

Keywords

cryptic species, ddRAD sequencing, DNA barcoding, morphology, owlet moths

Introduction

The genus *Hoplodrina* was separated from the giant clade of 'caradrines' (actually the tribe Caradrinini) by Boursin (1937) who first recognised that this group is rather remote taxonomically from the other large groups of the tribe (*Caradrina* Ochsenheimer, 1816; *Athetis* Hübner, [1821]; *Spodoptera* Guenée, 1852; *Stenodrina* Boursin, 1937; and *Stygiodrina* Boursin, 1937). Boursin stated that *Hoplodrina* possesses a number of shared features ("synapomorphies") such as the similar habitus with less striking brown or grey coloration and uniform forewing pattern with well-developed and clearly outlined orbicular and reniform stigmata, and very characteristic male clasping apparatus. The similarly distinctive features of the everted vesica and the female antrum and appendix bursae were at that time unknown to him.

The genus *Caradrina* (s.l.) has been revised by Hacker (2004); the other large clades are still unrevised though a number of new *Athetis* species were described in the last two decades. For *Hoplodrina*, the last and only comprehensive checklist was published by Fibiger and Hacker (2007); it comprised 17 species and three subspecies. Subsequently, two new eastern Asiatic species of *Hoplodrina* were described from the *H. conspicua* (Leech, 1900) species-group by Ronkay et al. (2013; *H. persequora* Ronkay, Ronkay, Fu & Wu, 2013, and *H. vestigiosa* Ronkay, Ronkay, Fu & Wu, 2013) and four new species from the *H. implacata* (Wileman & West, 1929) species-group from Taiwan by Wu and Owada (2018; *H. cienensis* Wu & Owada, 2018, *H. obscura* Wu & Owada, 2018, *H. bunun* Wu & Owada, 2018, and *H. kononenkoi* Wu & Owada, 2018). The genus *Hoplodrina* has, therefore, the largest diversity in the western Palaearctic (as stated by Fibiger and Hacker, op. cit.) but there has also been a remarkable increase in the eastern Asiatic fauna.

The European *Hoplodrina* fauna is generally considered to be well-known as all but one species was described before the description of the genus. The only exception is *H. hesperica* Dufay & Boursin, 1960, which was recognised and described only following more intensive studies of the genitalia of the Noctuidae (s.l.). The six European species are characterised and illustrated in detail by Fibiger and Hacker in the *Noctuidae Europaeae* series (2007).

This view, however, seems to be erroneous. The molecular taxonomic (barcoding) studies of the Alpine Noctuidae fauna showed, rather surprisingly, a clear splitting of "*octogenaria*" (Huemer 2013, Huemer and Hebert 2016, Huemer et al. 2019). These investigations were made independently and almost at the same time by the research groups of Peter Huemer and Jean Haxaire, providing the same results. The subsequent morphological survey was continued by Oleg Pekarsky and László Ronkay which proved the existence of clearly recognisable differences in the genitalia of both sexes and also in the forewing shape and pattern, although the external morphological features show a certain overlap. The validity of the two distinct species was finally proven true by fractional genome sequencing (ddRAD) data.

The results inspired further studies of the entire genus and the morphological results were both surprising and convincing at the same time. The study of the *octogenaria*-like populations from the entire known range demonstrates that not only the south-western Mediterranean (Spanish, Portuguese and north-west African) "*octogenaria*" taxa are partly misinterpreted but that there is an undescribed *octogenaria*-like species in the Canary Islands (Tenerife) and that the Alpine twin species of *octogenaria* occurs not only in the Alps (France, Italy, Austria and Slovenia) but also in the eastern Carpathians, the Balkans, the southern Apennines, Crete and Cyprus. Moreover, there are unsolved taxonomic problems with practically all western Palaearctic species, therefore, a full, integrative revision of the genus is required.

The present paper is the first part of this revisional work which contains the redescription of the sister species of *H. octogenaria*, including the detailed comparison of *H. alsinides* (Costantini, 1922) sp. rev. with *H. octogenaria* and the historical information on the descriptions by Costantini in 1921 and 1922.

Material and methods

Morphology and material

Our study is based on more than 3000 specimens of the *Hoplodrina octogenaria* species-group. Most of the material was traditionally set and dried, although a few specimens are pinned but remain unset. Genitalia preparations followed standard techniques for Noctuoidea, including everting the vesica.

Molecular analysis

DNA barcode sequencing and analysis

DNA barcode sequences of the mitochondrial COI gene – a 658 base-pair long segment of the 5' terminus of the mitochondrial COI gene (*cytochrome c oxidase 1*) – were obtained from 269 specimens belonging to seven species of European *Hoplodrina*, including publicly available specimens from the Barcode of Life Data Systems (BOLD). DNA samples from dried legs were prepared according to prescribed standards using the high-throughput protocol of deWaard et al. (2008). Samples were processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph). Details of successfully sequenced voucher specimens, including complete geographic data and images, can be accessed in BOLD (Ratnasingham and Hebert 2007) in the public dataset "Lepidoptera of the Europe - *Hoplodrina*" dx.doi.org/10.5883/DS- DS-LEEUHOPL. GenBank accession numbers can be retrieved from the dataset.

Degrees of intra- and interspecific variation in the DNA barcode fragments were calculated under the Kimura 2 parameter (K2P) model of nucleotide substitution using analytical tools in BOLD systems v. 4.0 (http://www.boldsystems.org).

ddRAD library preparation and bioinformatics

We used genomic DNA (gDNA) aliquots that were extracted at the CCDB following laboratory protocols used routinely in CCDB as explained in deWaard et al. (2008). The quantity of gDNA extracts was checked using PicoGreen Kit (Molecular Probes). In order to reach sufficient gDNA quantity and quality, whole genome amplification was performed using REPLI-g Mini Kit (Qiagen) due to its low concentrations of gDNA in the original extracts. The ddRAD library was implemented following protocols described in Lee et al. (2018) with two exceptions: gDNA was digested with *Pst*I and *Msp*I and the size distribution was measured with Bioanalyzer (Agilen Technologies). The de-multiplexed fastq data are archived in the NCBI SRA: SRP155300.

Raw paired-end reads were de-multiplexed with no mismatches tolerated using their unique barcode and adapter sequences using *ipyrad* v.0.7.23 (Eaton and Overcast 2016). All *ipyrad* defaults were used, with the following exceptions: the minimum depth at which majority rule base calls are made was set to 3, the cluster threshold was set to 0.85, and the minimum number of samples with data for a given locus to be retained in the final data set was set to 2–4, and 13.

We then applied a number of filters to identify candidate diagnostic single nucleotid polymorphisms (SNPs) for detecting fixed allelic differences between two species. We focused on loci genotyped for all individuals assayed (0% missing data) and on ddRAD tags containing only one bi-allelic.

Phylogenetic analysis

To study the phylogenetic relationships among taxa and to test the validity of prevailing species hypotheses, we conducted maximum likelihood (ML) trees. Phylogenetic trees were constructed for the concatenated ddRAD data. ML tree was inferred in RAxML v.8.2.0 (Stamatakis 2014) with bootstrap support estimated by a 1000 replicates rapid-bootstrap analysis from the un-partitioned GTR+CAT model. We visualized the resulting phylogeny and assessed bootstrap support using FigTree v.1.4.2 (Rambaut 2015).

Examination of *Wolbachia* infection

In order to exclude the presence of the bacterial parasite *Wolbachia*, we sequenced two markers of *Wolbachia*, FstZ and Wsp using primers and laboratory procedures of Ivanov et al. (2018). None of the samples was *Wolbachia* infected.

Abbreviations of private and institutional collections

CJHL	Collection Jean Haxaire, Laplume, France
CREA-FL	Centro di ricerca Foreste e Legno (Research Centre for Forestry and Wood), Rende, Italy

HNHM	Hungarian Natural History Museum, Budapest, Hungary
LMK	Landesmuseum Kärnten, Klagenfurt, Austria
MCSN	Museo Civico di Storia Naturale, Milano, Italy
MNHU	Museum für Naturkunde, Humboldt-Universität, Berlin, Germany
NHM	The Natural History Museum (formerly British Museum, Natural History), London, United Kingdom
NHMW	Naturhistorisches Museum Wien, Austria
RNS	Royal Natural History Museum (Naturhistoriska Riksmuseet), Stockholm, Sweden
TLMF	Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
ZMHU	Museum für Naturkunde – Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin, Germany
ZSM	Zoologische Staatssammlung, Munich, Germany

Historical interpretations of taxa in the *Hoplodrina octogenaria* (Goeze, 1781) species-group

According to the published checklist of Fibiger and Hacker (2007), the *octogenaria* species-group includes the following species:

– *octogenaria* (Goeze, 1781)
  = *alsines* (Brahm, 1791) (*Phalaena Noctua*)
  = *sordida* (Haworth, 1809) (praeocc.) (*Noctua*)
  = *sericea* (Speyer, 1867) (*Caradrina*)
  = *alsinides* (Costantini, 1922) (*Caradrina*)
  = *melendezi* Agenjo, 1941;
– *octogenaria* ssp. *amurensis* (Staudinger, 1892) (*Caradrina*)
– *pfeifferi* (Boursin, 1932) (*Athetis*)
– *blanda* ([Denis & Schiffermüller], 1775) (*Noctua*)
– *blanda* ssp. *robusta* Boursin, 1940 (*Hoplodrina*)
– *hesperica* Dufay & Boursin, 1960
– *levis* (Staudinger, 1888) (*Caradrina*)
– *straminea* (Zerny, 1934) (*Athetis*)

They noted that “The priority of *octogenaria* (Goeze, 1781) over *alsines* Brahm, 1791 was introduced by Koçak (1983). The taxon *amurensis* (Staudinger, 1892) was originally described as subspecies of “*Caradrina alsines*”, later on treated by Boursin as “ ‘bona species’ (cf. Boursin’s never published systematic lists of Palaearctic trifine Noctuidae). Today in both standard publications on the eastern Palaearctic fauna: Kononenko et al. (1998), and Kononenko (2003, 2005) *amurensis* is suggested to be the eastern subspecies of *octogenaria*”.

Most of the information presented in "Noctuidae Europaeae" (Volume 9) (Fibiger and Hacker 2007) seems to be correct, except the following statements:

- *melendezi* is not synonymous with *octogenaria* but a distinct taxon. Further investigations of the Iberian "*octogenaria*" populations are needed to clarify whether *melendezi* represents a distinct species comprising different subspecies or are there numerous subspecies of *octogenaria* that occur in Spain and Portugal;
- *pfeifferi* includes more than one closely related species;
- *blanda*, *hesperica* and *levis* represent a distinct species-group while *straminea* is a member of the *octogenaria* species-complex;
- the species occurring in Asia Minor and often called "*levis*" represents another, still undescribed, species; and
- *amurensis* is a species distinct from *octogenaria*.

The *Caradrina alsinides* problem

After the determination of the sister-species relationship of the two '*octogenaria*' species, the next major problem was to clarify the identity of the described taxa formerly considered as synonymous with *octogenaria*. The problem was rather difficult as 1) the type material of the historical taxa is generally inaccessible; 2) the type localities of *octogenaria* and *alsines* are not stated.

Our concept was that although the types of the taxa described by Goeze and Brahm are not available and their type localities are not stated, their descriptions by inference refer to taxa that occur in Germany. Checking a considerable amount of material of *octogenaria* from different parts of Europe resulted in no specimens of the second species being found from Germany and north or north-west of the Alps. Thus, we treat *octogenaria* as a widespread European species occurring also in northern and north-western Europe. The neotype of *H. octogenaria* is designated below and illustrated in Fig. 11.

As the type-locality of *sordida* (a preoccupied name) is England, and that of *sericea* is Amsterdam, only *alsinides* (Costantini, 1921/1922) remained as a possible candidate for the sister species of *octogenaria*. This species was described from northern Italy (Prov. Modena: Mutina, Sestola), the region where the two species may occur sympatrically.

The case of *alsinides* therefore seemed rather difficult, especially on the assumption that the types were missing. The species was described twice by Alessandro Costantini, first in 1921, providing only the name without any additional information; therefore, the name can be considered as nomen nudum. A year later, he published a Latin description of the species mentioned first in 1921; therefore, the date of the valid description is in reality 1922 (ICZN 1999). The description is brief, without illustrations but includes mention of external features which help towards determination: "*Caradrina alsinides* m., n. species: (*) *C. alsinii* affinis, sed alis vero amplioribus, alar. ant. mac. cellularib. majoribus, orbiculari perfecter rotunda, ambabus fusco-repletis et valde appariscentibus; caterum haec species tam similis est ad alsinem Brahm, quam sp. *taraxaci* Hb. (*blanda* Tr., nec Hb.) ad superstem Tr. similiat!" The translation of the Latin text is as follows: "*Caradrina alsinides* m[ihi]., n. species: (*) similar to *C*(*aradrina*) *alsines*, but with broader wings, larger cellular maculae, perfectly rounded orbicular stigma, conspicuous and uniformly dark filling of stigmata; this species is as similar to *alsines* Brahm as to *taraxaci* Hb. (*blanda* Tr., nec Hb.) and *superstes* Tr.!"

The description expressly states that *alsinides* differs from *alsines* by its broader wings, larger and darker filled stigmata and perfectly rounded orbicular stigma. These characters are typical of *octogenaria* while its sister species has somewhat narrower forewings, less conspicuous and usually smaller or significantly smaller stigmata and the orbicular stigma is often somewhat flattened. However, based solely on this description the identity of the species, remained doubtful. We therefore tried to obtain syntype material and finally succeeded with a request to the Museo Civico di Storia Naturale, Milano. The male syntype, illustrated in Fig. 16, is designated below as the lectotype. With support from Axel Hausmann (ZSM) this specimen was successfully sequenced with NGS protocols. The sequencing provided, however, a surprising result as the DNA barcode of the lectotype specimen is identical with that of the sister species of *H. octogenaria*, despite the different external characters of the lectotype specimen and the differently stated features provided by the original description. This fact emphasizes the need of the genitalia and molecular investigations during the identification of the southern European "*octogenaria*-like" specimens.

The consequence of our investigations is that, surprisingly, *Hoplodrina alsinides* is a cryptic species in central and southern Europe. The re-description, and the detailed comparison with its sister species, *H. octogenaria*, is provided below.

Systematic results

Hoplodrina octogenaria (Goeze, 1781)

Figs 11–20, 23, 24, 27, 28

Phalaena Noctua octogenaria Goeze, 1781, Entomologische Beyträge zu des Ritter Linné zwölften Ausgabe des Natursystems 3(3): 227. Type-locality: Germany, Bayern, Landshut. Neotype: male, in coll. TLMF, here designated.

Phalaena Noctua alsines Brahm, 1791, Handbuch der Ökonomischen Insektengeschichte in Form eines Kalenders bearbeitet 2: 114. Type-locality: no locality given [Germany];

Noctua sordida Haworth, 1809, Lepidoptera Britannica; sistens Digestionem novam Insectorum Lepidopterorum quae in Magna Britannia Reperiuntur, Larvarum Pabulo, Temporeque Pascendi; Expansione Alarum; Mensibusque Volandi; Synonymis atque Locis Observationibusque Variis 2: 207. Type-locality: Great Britain;

Caradrina sericea Speyer, 1867, Entomologische Zeitung herausgegeben von dem Entomologischen Vereine zu Stettin 28: 73. Type-locality: [Netherlands] Amsterdam;

Neotype designation. Neotype of *Phalaena Noctua alsines* Brahm, 1791 (Fig. 11): Male, "Landshut/Bay. | Roßberg | 1.7.1972 | coll.-Nr. 960C | Reiser", "TLMF | Innsbruck | H.Kolbeck | 2014-032" (coll. TLMF).

Hoplodrina alsinides (Costantini, 1922) sp. rev.

Figs 1–10, 21, 22, 25, 26

Caradrina alsinides Costantini, 1922, Neue Beiträge zur systematischen Insektenkunde 2: 98. Type-locality: Italy, Prov. Modena (Emilia Romagna), Sestola. Lectotype: male, here designated.

Lectotype designation. Lectotype of *Caradrina alsinides* Costantini, 1922 (Fig. 16): Male, "Emilia | Sestola | 21.VII.905 | A. Fiori", "♂" "BC ZSM Lep 106561" (coll. MCSN).

Additional material examined. Spain. 1 female, Aragon, Canfranc-Estacion, 1320 m, 42°45.73'N, 0°30.48'W, 13.VII.2012, leg. P. Huemer, TLMF 2013-013 (TLMF).

France. 1 female, Dep. Alpes-Maritimes, St. Martin Vesubie, 22.VII.1925, leg. A. Schmidt (HNHM); 1 female, Dep. Alpes-Maritimes, Col de la Cayolle, 2000 m, 6–13. VIII.1972, leg. R. Schütz, TLMF Innsbruck H. Kolbeck 2014-032 (TLMF); 1 male, Alpes-Maritimes, Col de la Couillole, 1600 m, 13.VII.1972, leg. M. Tarrier (TLMF); 1 female, Pyrénées Orientales, Road from Py to Mantet, 1704 m, 10.VII.1999, 42°29'03.68"N, 2°18'55.73"E, leg. J. Haxaire & O. Paquit [CJHL]; 1 male, from the same site, 21.VII.2001, leg. J. Haxaire & O. Paquit, BC-HAXNoctu0522 (barcode) [CJHL]; 2 males, from the same site, 29.VIII.2011, leg. J. Haxaire & O. Paquit [CJHL]; 4 males, 1 female, from the same site, 10.VII.2018, leg. J. Haxaire & M. Colin [CJHL]; 2 females, Pyrénées Orientales, Road from Py to Mantet, col de Mantet, 1764 m, 13.VII.2018, 42°28'52.15"N, 2°18'47.61"E, leg. J. Haxaire & O. Paquit [CJHL]; 3 males, Pyrénées Orientales, « refuge de Mariailles », trail to the Pla Guillem, 1752 m, 9.VIII.1997, 49°29'52.63"N, 2°24'33.47"E, leg. J. Haxaire & P. Beguin [CJHL]; 1 female, from the same site, 12.IX.1999, 49°29'52.63"N, 2°24'33.47"E, leg. J. Haxaire & O. Paquit [CJHL]; 1 male, from the same site, 23.VIII.2000, 49°29'52.63"N, 2°24'33.47"E, leg. J. Haxaire & O. Paquit, BC-HAXNoctu0520 (barcode) [CJHL].

Switzerland. 1 male, 6 females, Wallis, Simplon, Gabi, 7–10.VII.1968, leg. J. Wettstein (HNHM); 1 male, Wallis, Zermatt, 13.VII.1968, leg. J. Wettstein (HNHM); 1 female, Ticino, Mergoscia, 10.VIII.1971, leg. R. Müller (TLMF).

Italy. 2 males, Prov. South Tyrol, 7–10.VII.2004, leg. L. Ronkay & A. Kun (coll. HNHM); 2 males, 3 females, Prov. South Tyrol, Sesvenna Mts, above Prämajur, Watles, 1850 m, 18.VII.2006, leg. L. Ronkay & M. Tóth-Ronkay (HNHM and coll. G. Ronkay); 3 females, Prov. South Tyrol, Sesvenna Mts., Prämajur, above Lutaschg, 1700 m, 17.VII.2006, leg. L. Ronkay & M. Tóth-Ronkay (HNHM and coll. G. Ronkay); 1 male, Prov. South Tyrol, Vinschgau, Schleis, Schleiser Leiten, 1350 m, 46°41'517"N, 10°30'59"E, 5.VII.2013, leg. P. Huemer, TLMF 2013-013 (TLMF); 1 female, Prov. South Tyrol, Schnals, Fuchsberg, 1080 m, 46°40'27"N, 10°56'42"E, 7.VII.2014, leg. P. Huemer (TLMF); 1 male, South Tyrol, Ritten, Obergrünwald, 1750 m, 19.VII.2010, leg. P. Huemer, slide No. RL10288m (TLMF); 1 male, South Tyrol, Ritten, Obergrünwald, 1750 m, 19.VII.2010, leg. Peter Huemer, slide No. RL10289m; DNA Barcode ID TLMF Lep 02472 (TLMF); 2 males, Prov. South Tyrol, St. Felix, Warmesbrunn, 1500 m, 46°29'20"N, 11°09'27"E, 27.VI.2014, leg. S. Erlebach, TLMF 2014-001 (TLMF); 3 males, 5 females, South Tyrol, Mendel,


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Figures 1–10. *Hoplodrina alsinides* (Costantini, 1922), adults in dorsal view. **1** Male, Lectotype of *Caradrina alsinides*, Italy, Emilia-Romagna, Sestola **2** male, Italy, South Tyrol, Sesvenna Mts. **3** male, Romania, Transylvania, slide No.: RL12119 **4** male, Italy, South Tyrol, Sesvenna Mts. **5** male, Austria **6** male, Austria, Carinthia, slide No.: OP1415, BC TLMF Lep 02471 **7** male, Italy, South Tyrol, slide No.: RL10288, BC TLMF Lep 04569 **8** male, Austria, Carinthia **9** female, BC TLMF Lep 04568 **10** emale, Italy, South Tyrol, Sesvenna Mts.

Umg. Penegal, 1690 m, 46°26'13"N, 11°12'58"E, 22.VII.2019, leg. Huemer, TLMF Lep 27814, 27815 (barcodes) (TLMF); 1 female, South Tyrol, St. Ulrich, Ende Juli 1911, No. 7804, coll. J. Sterneck, slide No. RL12123f (NHMW); 2 males, Prov. Trento, Travignolo valley, Paneveggio, 1500 m, 31.VII., leg. F. Daniel (HNHM); 1 female, Prov. Trento, Madonna di Campiglio, campo Colf, 1650 m, 19.VII.1939, leg. A. Schmidt (coll. HNHM); 1 male, 1 female, Prov. Cuneo, Entracque S, Vallone di Moncolomb, 1430 m, 44°09'30"N, 7°24'0"E, 23.VII.2008, leg. M. Kahlen, TLMF 2009-027, slide Nos OP1413m, OP1414f (TLMF); 1 male, Prov. Cuneo, Demonte NW, Colle Valcavera NE, 2420 m, 44°23'04"N, 7°06'23"E, 17.VIII.2012, leg. P. Huemer, TLMF 2013-013 (TLMF); 1 male, Prov. Cuneo, N Colle della Lombarda, 1750 m, 44°15'08"N, 7°06'32"E, 17.VII.2012, leg. P. Huemer, TLMF 2013-013 (TLMF); 2 males, 4 females, Prov. Torino, PN Orsiera – Rocciavrè, Fenestrelle, ca. 1 km WNW Pequerel, 1840 m, 45°2'59"N, 7°3'5"E, 28.VI.2019, leg. Huemer (TLMF); 2 males, 2 females, Prov. Torino, PN Orsiera – Rocciavrè, Fenestrelle, ca. 0.7 km NE Pequerel, 1820 m, 45°3'6"N, 7°4'16"E, 23.VII.2019, leg. Huemer TLMF, Lep 27809 (barcode) (TLMF); 3 males, 6 females, Prov. Torino, PN Orsiera – Rocciavrè, Usseaux, Colle delle Finestre N, 2180 m, 45°4'21"N, 7°3'11"E, 24.VII.2019, TLMF Lep 27808, 27810 (barcodes) leg. Huemer (TLMF); 1 male, Prov. Chieti, PN della Majella, vic. of Blockhaus, ca. 2100 m, 42°08'48"N, 14°16'43"E, 22.VII.2011, leg. P. Huemer, TLMF 2012-010, slide No. OP1417m, BC TLMF Lep 05904 (barcode) (TLMF); 1 female, Prov. Chieti, Taranta Peligna, Pian di Valle, 1400 m, 42°02'19"N, 14°09'11"E, 21.VII.2011, leg. P. Huemer, TLMF 2012-010, slide No. OP1418f, BC TLMF Lep 06042 (barcode) (TLMF); 1 male, Calabria, Sila, Vivaio Sbanditi (CS), 1355 m, 39°23'30"N, 16°36'08"E, 17.VII.2014, leg. S. Scalercio; 1 male, from the same site, 29.VII.2014, leg. Scalercio & Infusino; 1 female, Calabria, Sila, Vivaio Sbanditi (CS), 1350 m, 39.3889N, 16.6022E, 6.VII.2015, leg. Scalercio & Infusino; 2 males, Calabria, SL_C2, Colle Macchie, Pedace (CS), 1450 m, 39.2597N, 16.5308E, 17.VII.2015, leg. Scalercio & Infusino; 2 males, Calabria, SPSE, Loc. Spinarva-Taverna (CZ), 1570 m, 39.0900N, 16.6800E, 27.VII.2017, leg. Scalercio & Infusino; 1 male, Calabria, SL_B1, Sila, Torre Scarda (CS), 1340 m, 39.2384N, 16.5131E, 17.VII.2015, leg. Scalercio & Infusino, LEP-SS-00439 (barcode) (CREA-FL); 1 ex., Calabria, Sila grd., La Fossita, 1305 m, 13.VII.2013, leg. Hausmann, BC ZSM lep 92607 (barcode) (ZSM).

Austria. 1 male, Styria, Prebichl, Reichenstein, 20.VII.1938, leg. Dr Szabó (HNHM); 1 male, Kals, 26.VII.1937, leg. Dr Szabó (HNHM); 1 female, Styria, Dürriegel, 21.VII.1917, coll. Dr Galvagni, slide No. RL12127f (NHMW); 1 female, Styria, Sausalgebirge, Kitzeck, 300–500 m, 3–9.VIII.1954, leg. F. Daniel (HNHM); 1 female, Styria, NP Gesäuse, Wagriedschlag SW Hieflau, 1450 m, 18.VII.2015, leg. H. Habeler, TLMF Innsbruck Slg. H. Habeler 2017-010 (TLMF); 1 male, Carinthia, Naggl, 13.VII.1934, coll. Dr Galvagni, slide No. RL12122m (NHMW); 1 male, Styria, Dfstr., Aflenz, 1882, slide No. RL12126m (NHMW); 1 male, Carinthia, Petzen N, Obere Krischa, 46.506N, 14.757E, 1900 m, 13.VII.2009, leg. P. Huemer, TLMF 2009-138 (TLMF); 1 female, Carinthia, Karawanken, 28.VII.1971, leg. Wettstein J. (HNHM); 1 male, Carinthia, Emberger Alm, Nassfeldriegel, 1920 m, 26.VII.2013.

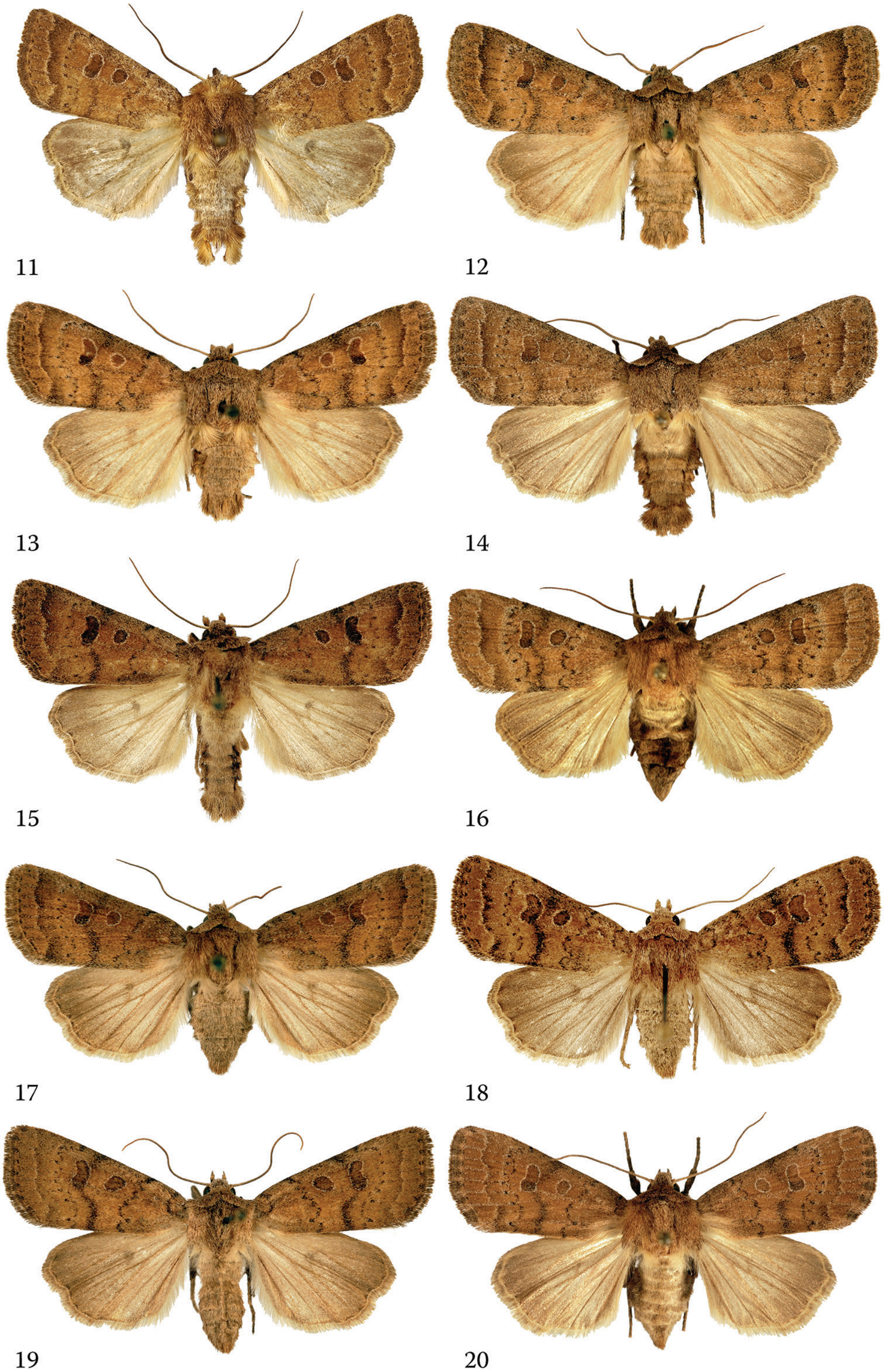


Figure 11–20. *Hoplodrina octogenaria* (Goeze, 1781), adults in dorsal view. **11** Neotype male, Germany, Bayern **12** male, Austria, Carinthia **13** male, Italy, South Tyrol, Sesvenna Mts. **14** male, Italy, South Tyrol, Sesvenna Mts. **15** male, Hungary, Vas County **16** female, Hungary, Pest County **17** female, Austria, Wien **18** female, Hungary, Pest County **19** female, Austria, Burgenland **20** female, Austria, Wien.

leg. C. Wieser (LMK); 1 male, Carinthia, Lesachtal, St. Jakob, Mussen, ca 1800 m, 46°42'42"N, 12°55'55"E, 24–25.VII.2000, leg. P. Huemer & S. Erlebach, TLMF 2000-01, slide No. OP1415m, BC TLMF Lep 04569 (barcode) (TLMF); 1 female, same data, but BC TLMF Lep 04569 (barcode) (TLMF); 1 male, Carinthia, Obervellach, 10.VII.1967, TLMF Innsbruck H. Kolbeck 2014-032 (TLMF); 1 female, Tyrol, Brennergebiet, Vennatal, 27.VII.1900, coll. Dr Galvagni, slide No. RL12124f (NHMW); 1 female, Tyrol, Venediger Mts., Dorfertal, Wiesenkreuz, 1520 m, 8.VII.1993, leg. P. Huemer, TLMF 2000-01, slide No. OP1416f, BC TLMF Lep 04568 (barcode) (TLMF).

Slovenia. 1 female, Nova Gorica, 20.VI.1979, leg. Reiser, TLMF 2014-032 (TLMF).

Hungary. 1 male, "Hungaria", coll. E. Frivaldszky, No. 1383 (HNHM).

Romania. 2 males, Transylvania, Borszék [Borsec], 16.VII.1942, leg. Dr Vargha Gyula, slide Nos RL12118m, RL12120m (HNHM); 1 male, Transylvania, Borszék [Borsec], 13.VII.1942, leg. Dr Vargha Gyula, slide No. RL12119m (HNHM).

Montenegro. 1 male, Durmitor N, Velika Stuoc N, 1940–1950 m, 43°11'25"N, 19°03'26"E, 25.VII.2011, leg. G. Tarmann, TLMF 2012-002 (TLMF); 1 male, Durmitor N, Velika Stuoc W, 1730 m, 43°11'42"N, 19°02'38"E, 24.VII.2011, leg. G. Tarmann, TLMF 2012-002 (TLMF).

North Macedonia. 1 male, NP Mavrovo, Radika valley, near bridge, 10 km NNW Sveta Voda, 41°47'20"N, 20°32'48"E, 1290–1340 m, 1–3.VIII.2011, leg. P. Huemer & G. Tarmann, slide No. OP1419m, BC TLMF Lep 05418 (barcode) (TLMF); 1 male, NP Mavrovo, Korab, Korabska jezero, Koblino pole, 2080–2180 m, 41°46'42"N, 20°34'55"E, 28.VII–1.VIII.2011, leg. P. Huemer & G. Tarmann, slide No. OP1420m, BC TLMF Lep 05528 (barcode) (TLMF); 2 males, ditto, but 28.VII.2011 (TLMF);1 male, ditto, but 2115 m, 30+31.VII.2011 (TLMF).

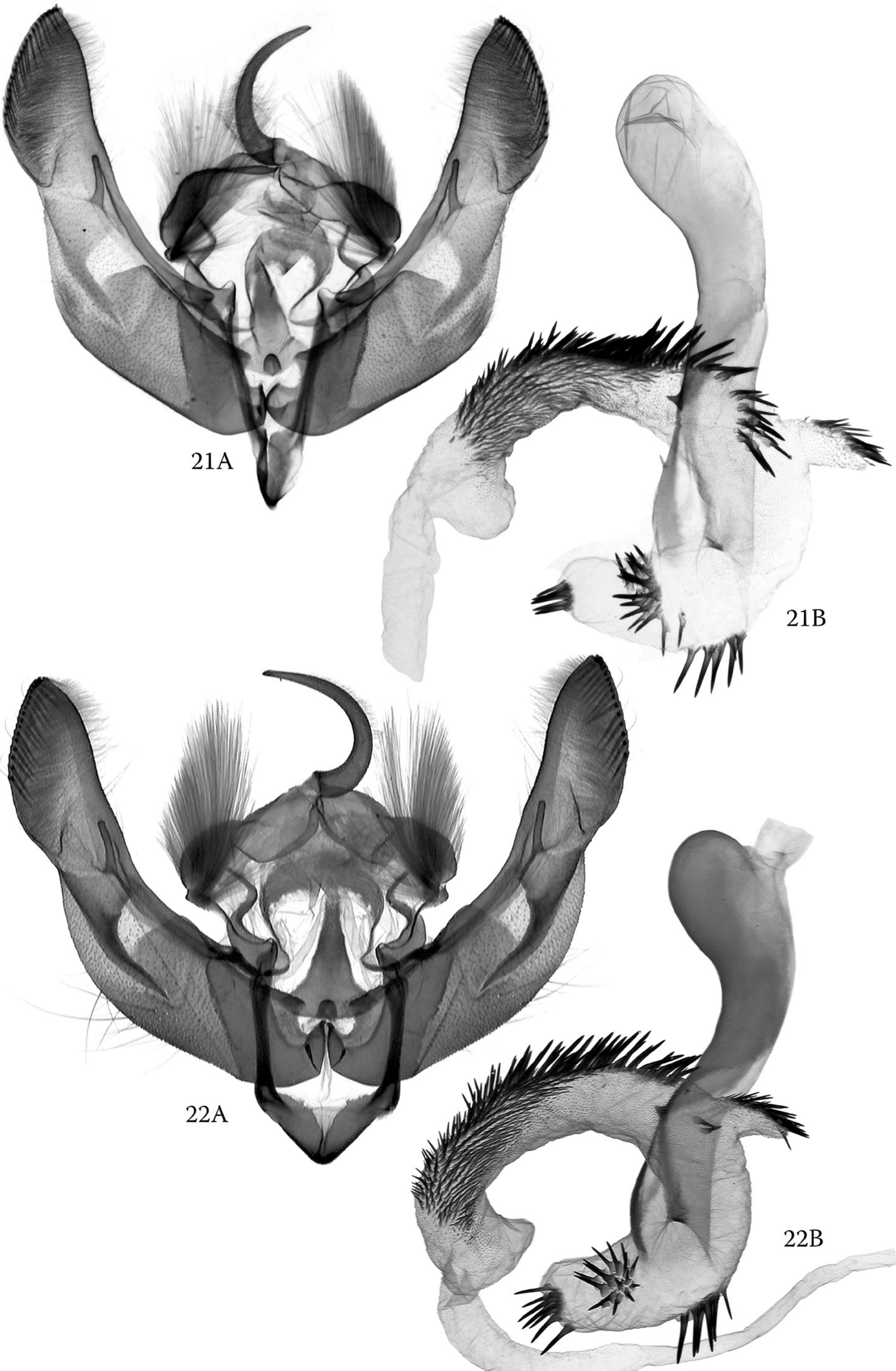
Bulgaria. 1 male, Pirin Mts, 1700 m, 15–25.VII.1933, leg. J. Thurner (HNHM).

Greece. 1 male, Crete, Palaeochora Umg.1–13.V.1999, leg. J. Wimmer, TLMF Innsbruck Slg. J. Wimmer 2016-019 (TLMF).

Diagnosis. The two sister species are often confusingly similar externally which has led to the late recognition of their distinctness. There are, however, certain differences in the forewing pattern and the coloration (see Figs 1–20) which help in the separation of the two species, although the specific identity of specimens is more safely determined by examination of the genitalia and/or consideration of the barcodes.

Hoplodrina alsinides can be characterised, compared with *H. octogenaria*, by its somewhat narrower and slightly more pointed forewings with smoother scaling and a finer sheen; less sinuous crenate antemedial line usually followed by a fine olive-grey or olive-brown suffusion which often extends to the entire basal area; more diffuse and weaker median fascia and less sharply marked, usually less crenate postmedial line. It is worth noting that certain *H. alsinides* specimens have darkened basal and marginal areas and a paler median field, this "trizonal" forewing coloration is typical only of the new species.

The male genitalia of *H. alsinides* (Figs 21, 22) can be best distinguished from those of *H. octogenaria* (Figs 23, 24) by features of the vesica though the clasping apparatus also show diagnostic characteristics. The subbasal ventral diverticulum in the vesica of the new species is elongate-subconical, more elongate and narrower than in *H. octogenaria*



Figures 21, 22. *Hoplodrina alsinides* (Costantini, 1922), male genitalia. **21A, B** Austria, Carinthia, slide No.: RL10289 **22A, B** North Macedonia, slide No.: OP1419.

and provided with three distinctly arranged groups of spiniform cornuti. This part of the vesica is shorter and broader and rather globular in *H. octogenaria* and provided with two longer groups of cornuti arranged in an oblique T-shaped structure. In the clasping apparatus, *H. alsinides* has, in comparison with *H. octogenaria*, proportionally shorter valvae with a stronger constriction below the cucullus, shorter and somewhat straighter (usually less arched) ampulla and a distinctly narrower subdeltoid juxta with a narrower basal section and more evenly tapering medial and distal parts.

In the female genitalia, the antrum of *H. alsinides* (Figs 25, 26) is more quadrangular than in *H. octogenaria* (Figs 27, 28) with rather straight lateral sides and a less dilate anterior (proximal) part, the anterior (proximal) two-thirds of the ductus bursae and the lateral appendage of the corpus bursae located opposite the appendix bursae is narrower and the corpus bursae is smaller than in its sister species. The sclerotized distal half of the last sternite is distinctly rounded triangular in the new species, being distinctly narrower, more triangular than in *H. octogenaria* which has a broader, more trapezoidal sclerotization.

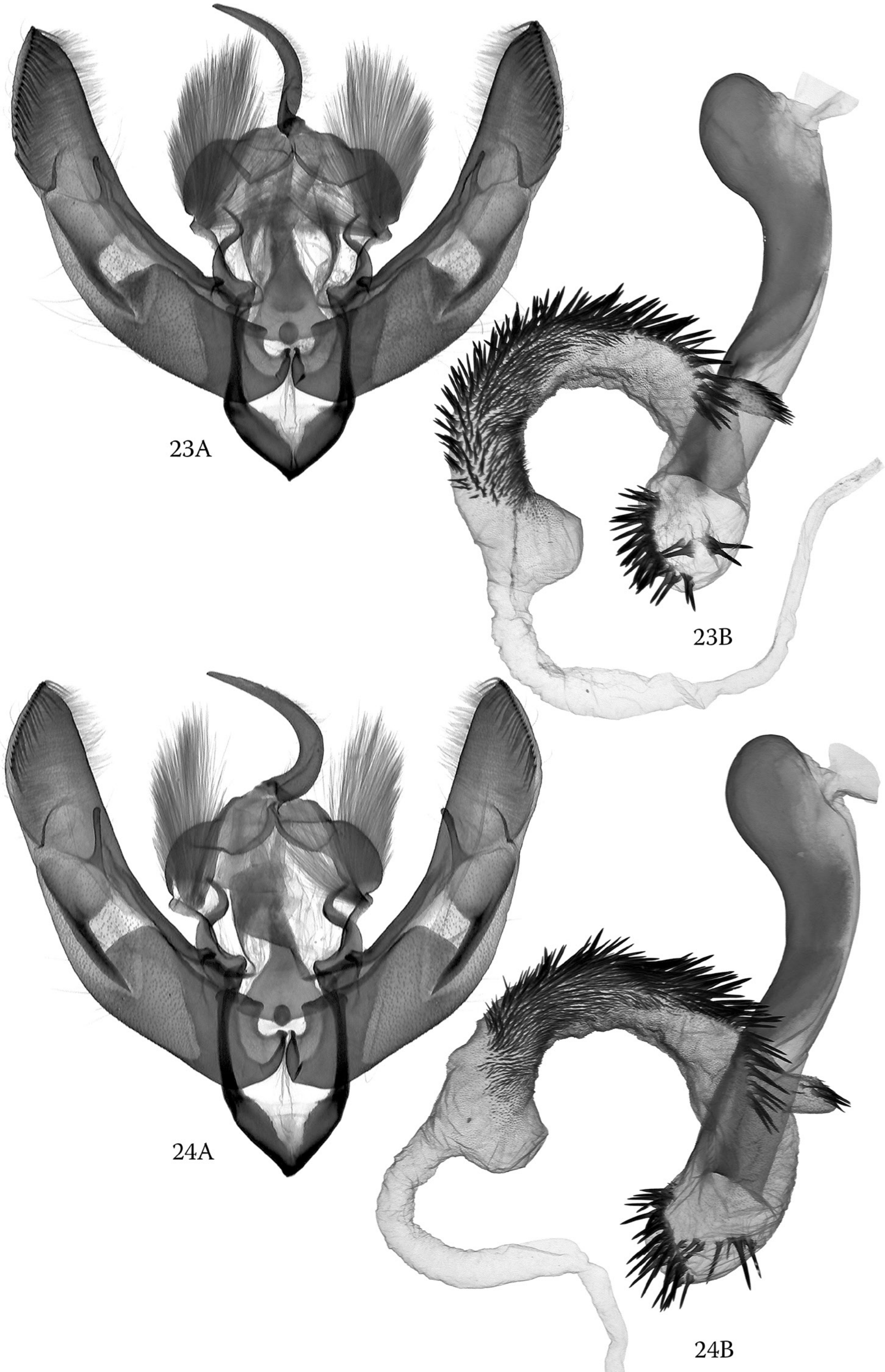
Re-description. Wingspan 27–34 mm. Sexes generally similar though the females are somewhat smaller and more narrow-winged than the males and there are slight differences also in the thickness of the antennae.

Head. Unicolorous brown; palpi short, upturned with short third segment, sides darker brown, often greyish; frons smooth, slightly prominent, covered with long hair-scales; antennae of both sexes filiform, those of males somewhat thicker, with longer fasciculate cilia.

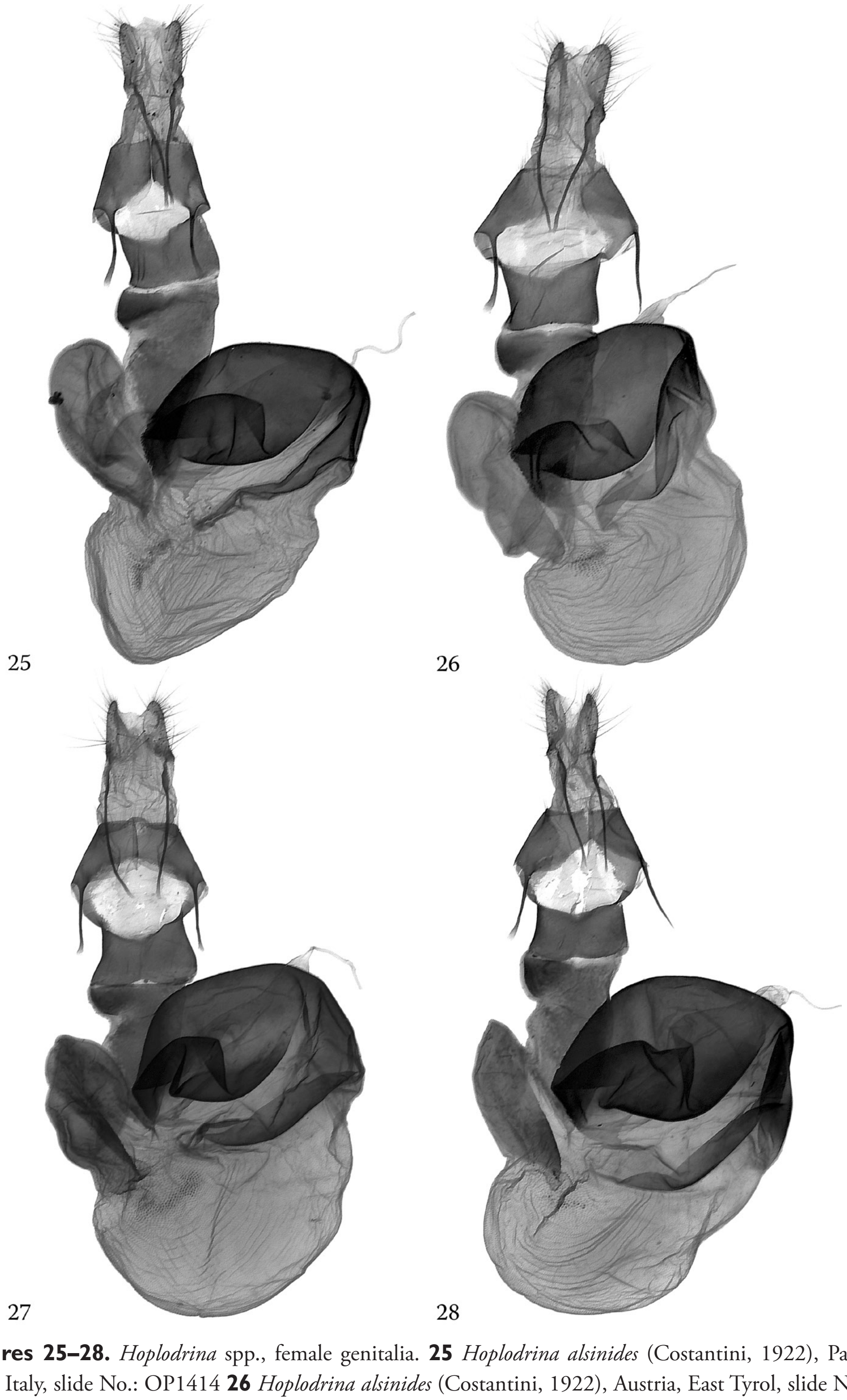
Thorax. Usually various shades of unicolorous brown, from pale ochreous brown to deep hazel-brown mixed with whitish hair-scales; collar large, unicolorous; tegulae rather narrow; pro- and metathoracic tufts large. Legs with long brown femoral fringes.

Forewing. Elongate-triangular, with finely pointed apex; ground colour matching the thorax, variable from pale ochreous brown to hazel-brown, basal and marginal areas often somewhat (or more) darker than median area; subbasal line rudimentary, dark grey; antemedial line oblique, slightly sinuous, dark grey, edged with a variably broad darker brownish or brown-grey zone on inner side; median fascia darker grey-brown, often diffuse or indistinct; postmedial line also less sharply marked, sinuous, with fine dark grey spots and streaks on veins; subterminal line pale ochreous brown, more or less straight, edged darker brown on inner side; terminal line narrow, ochreous white, marked by variably strong blackish grey dots or triangles between veins; fringes as ground colour, usually with poorly visible ochreous brown streaks at veins; orbicular stigma small, rounded or flattened, reniform stigma usually narrowly bean-shaped, both stigmata darker brown than ground colour, outlined ochreous brown; claviform stigma absent.

Hindwing. Evenly rounded, apex and tornus with minute peaks only; ground colour whitish ochre, strongly suffused dark ochreous brown to grey-brown; marginal area relatively wide, darker than other parts of wing, widest at apex and tapering towards tornal angle; transverse line absent; discal spot clearly visible but diffuse, darker brown, rounded or slightly streak-like; veins and terminal line darker brown; fringes ochreous brown with darker inner line.



Figures 23, 24. *Hoplodrina octogenaria* (Goeze, 1781), male genitalia. **23A, B** Austria, North Tyrol, slide No.: OP1407 **24A, B** Italy, South Tyrol, slide No.: OP1412.



Figures 25–28. *Hoplodrina* spp., female genitalia. **25** *Hoplodrina alsinides* (Costantini, 1922), Paratype, Italy, slide No.: OP1414 **26** *Hoplodrina alsinides* (Costantini, 1922), Austria, East Tyrol, slide No.: OP1416 **27** *Hoplodrina octogenaria* (Goeze, 1781), Austria, North Tyrol, slide No.: OP1409 **28** *Hoplodrina octogenaria* (Goeze, 1781), North Macedonia, slide No.: OP1410.

Abdomen. Male abdomen long and slender, similar in colour to that of thorax, dorsum sometimes slightly paler, especially on segments A_1 and A_2; dorsal crest absent; lateral fringes and anal tuft well-developed. Female abdomen shorter and thicker, with shorter and smoother pubescence on dorsal surface; lateral fringes reduced or very short; final segment elongate, darker in colour.

Male genitalia. Clasping apparatus sclerotized, relatively large. Uncus strong, curved and apically acute; tegumen broad and rather short, with well-developed, rounded and densely hairy penicular lobes; juxta narrow, subdeltoid with evenly tapering dorsal and moderately wide basal (ventral) parts; vinculum sclerotized, broadly V-shaped. Valvae symmetrical, elongate and almost evenly wide, slightly constricted below cucullus; sacculus sclerotized, long, clavus with stronger sclerotized and wrinkled setose surface; harpe (clasper) flattened, its basal part bar-like, more or less straight, apical (distal) part dilated, flattened; ampulla relatively short, thin, digitiform, straight or slightly curved; cucullus long, more or less helmet-like with acute apex, well-developed, long corona and small, triangular antero-ventral lobe. Phallus medium-long, strong, thick and arcuate, with broader, proximally evenly rounded coecum; ventral carinal plate sclerotized, long, beak-shaped, with eversible, long carinal extension. Vesica broadly tubular, everted forward, producing large, subconical subbasal diverticulum provided with three distinct groups of long spiniform cornuti; main tube of vesica bent ventrad from base of subbasal diverticulum and somewhat recurved dorsally; most of main tube densely covered with short and medium-long, strong, spiniform cornuti, its basal section with short, tubular diverticulum terminating in a bundle of fine spinules; terminal diverticulum large, subconical, membranous, without cornuti.

Female genitalia. Ovipositor medium-long, conical, papillae anales elongate, apically finely rounded, sparsely setose with long sensory setae; both pairs of gonapophyses narrow, long; antrum quadrangular, flattened and sclerotized, its lateral edges more or less straight; posterior margin with shallow, arcuate cleft; ductus bursae medium-long, its posterior (distal) part somewhat broader than antrum, with rounded and sclerotized lateral lobe at right side; anterior (proximal) section of ductus bursae distinctly narrower, flattened and partly sclerotized; appendix bursae large, elliptical, partly twisted and heavily sclerotized; corpus bursae discoid-globular, membranous, wrinkled, with subconical gelatinous appendage at junction of ductus bursae and with a diffuse, irregularly rounded signum patch.

Last abdominal segment. Tergite VIII very broad, quadrangular, with rounded trapezoidal, homogeneous sclerotization; sternite VIII much smaller, rounded triangular with subconical sclerotized posterior half with heavily sclerotized apical section.

Bionomics. The species inhabits dry and warm, open or lightly wooded mixed forests, rocky slopes, also in open upper forest regions or even in the timberline; between altitudes of ca 1000–2200 m, according to the confirmed records. Univoltine summer species, the moths are on the wing in July and August. The early stages and the foodplant are considered to be unknown due to the uncertainty of the identification of the reared adults. As the two sister species can be found at the same site, their bionomics is presumed to be at least partly similar.

Distribution. From our proved molecular data (Fig. 29) and morphologically verified specimens, *H. alsinides* is widely distributed in southern Central Europe and the

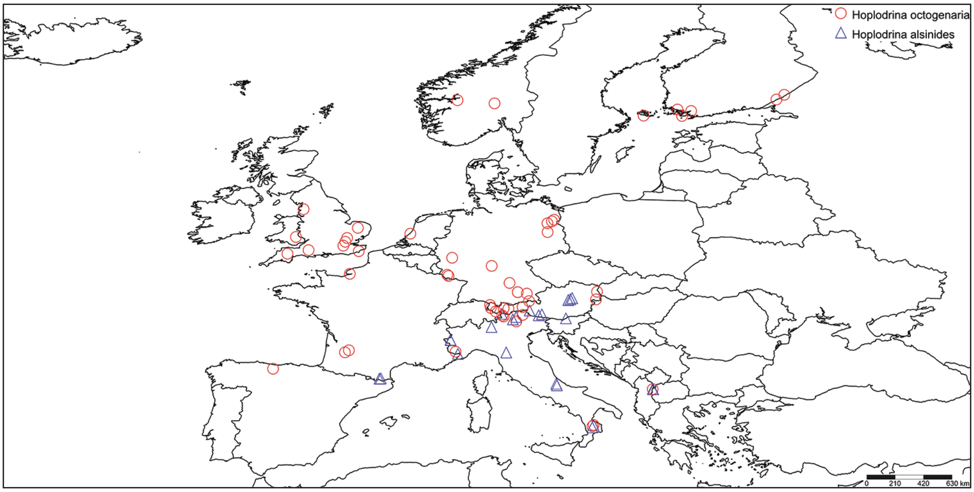


Figure 29. The distribution of *H. alsinides* (Costantini, 1922) and *H. octogenaria* (Goeze, 1781) from successfully sequenced specimens. Map created with SimpleMappr (http://www.simplemappr.net).

Mediterranean, ranging from southern Greece (Crete) across the Balkan Peninsula to the southern part of the Alps and the north-eastern part of the Iberian Peninsula (Spain: Aragon). It furthermore occurs on the Italian Peninsula, ranging from South Tyrol in the North to Calabria in the South and probably in most of the country. Further records are from the French Alps and Pyrenees, and from southern and eastern Austria. On some occasions the species has been found to be sympatric with *H. octogenaria*. The latter species generally has a much wider distribution with numerous confirmed records in large parts of central and northern Europe, extending to northern Spain and the British Isles in the West and Finland in the North-East, but also present in the mountain parts of the Mediterranean, e.g., southern Italy (Calabria) and Macedonia.

Molecular results

DNA barcode sequences

We obtained and eventually analysed 235 full length barcode sequences of 658 bp and 33 sequences ranging between 571 and 657 bp and covering all of the currently reported seven European species of *Hoplodrina*.

Nearest Neighbour distance analysis of *Hoplodrina* under the K2P model resulted in a minimum distance of 3.14% (mean 3.65%, maximum 5.25%, SE = 0.1) (Table 1) whereas the mean intraspecific distance was only 0.22%.

Hoplodrina alsinides clusters in a single BIN: BOLD: AAB4765 (Ratnasingham and Hebert 2013). The intraspecific distances of the barcode region are very low with 0.11% on average and a maximum of 0.35% (p-dist) ($N = 29$) whereas the minimum distance to the nearest neighbour *H. octogenaria* is 3.53%.

Table 1. KP2 intra- and maximum interspecific distances (in %) in European species of *Hoplodrina*.

Species	Mean Intra-Sp	Max Intra-Sp	Nearest Species	Distance to NN
Hoplodrina ambigua	0.1	0.66	*Hoplodrina octogenaria*	5.25
Hoplodrina blanda	0.38	0.96	*Hoplodrina octogenaria*	3.24
Hoplodrina hesperica	N/A	0	*Hoplodrina octogenaria*	3.14
Hoplodrina octogenaria	0.17	1.4	*Hoplodrina hesperica*	3.14
Hoplodrina respersa	0.24	0.62	*Hoplodrina octogenaria*	3.46
Hoplodrina alsinides	0.11	0.35	*Hoplodrina octogenaria*	3.82
Hoplodrina superstes	0.55	1.24	*Hoplodrina octogenaria*	3.46

Table 2. Summary of ddRAD data.

Species	Sample ID	Total reads (× 10^6)	Reads passed filter (× 10^6)	Clusters at 85%	Clusters coverage	Heterozygosity	Retained loci	Loci in assembly
H. octogenaria	BC ZSM Lep 82261	3.28	2.80	13514	81.4	0.001977	4711	2835
	MM07463	2.43	2.09	11050	73.3	0.001355	4499	2634
	TLMF Lep 08140	1.58	1.33	17082	33.1	0.002595	6399	3869
	TLMF Lep 10517	2.04	1.73	16433	48.9	0.002743	6347	3859
	TLMF Lep 10569	2.43	2.01	7293	57.5	0.002009	1720	918
	TLMF Lep 10642	1.54	1.18	7764	72.4	0.001191	2131	1233
	TLMF Lep 10690	5.47	0.46	12914	172.2	0.002002	4138	2459
	TLMF Lep 10804	2.30	1.90	22038	43.2	0.002218	8718	4023
H. alsinides	TLMF Lep 02472	2.54	2.13	20286	34.9	0.001155	6018	1525
	TLMF Lep 05418	0.76	0.64	6784	42.1	0.001085	1634	907
	TLMF Lep 05904	1.92	1.62	3381	230.8	0.000865	723	302
	TLMF Lep 05905	0.93	0.81	3636	59.8	0.000753	715	344
	TLMF Lep 13128	0.72	0.62	7438	30.8	0.000480	1018	304
	Average	**2.15**	**1.49**	**11509**	**75.4**	**0.001571**	**3752**	**1939**

Data exploration, phylogenetic analyses, and SNP identification based on genome-wide SNP data

We generated a genome-wide SNP data set from 13 individuals of *Hoplodrina octogenaria* and *H. alsinides* using ddRAD sequencing, and used this data set to perform phylogenetic analyses. We obtained 2.15 million reads per individual on average, of which 1.49 million reads per individual (78.1%) were retained after quality filtering steps (Table 2). After filtering and clustering at 85% sequence similarity, we recovered 7307 putative orthologues shared across more than two samples, for a total length of 1363,146 bp. This data includes 47,676 SNPs, of which 7106 are parsimoniously informative (PIS).

Phylogenetic analysis using the concatenated ddRAD dataset produced robust support for the relationship between the individuals (Fig. 30). In the ML tree, the two revealed lineages correspond to *H. octogenaria* and *H. alsinides* that were supported by 100% bootstrap support values.

A total of 66 putative RAD loci had exactly one bi-allelic putative SNP and were genotyped in all 13 individuals of two species. The data includes a total of 143 SNPs, of which 61 are PIS. The SNPs occurs at 2.17 SNP/locus on average. Of these, we

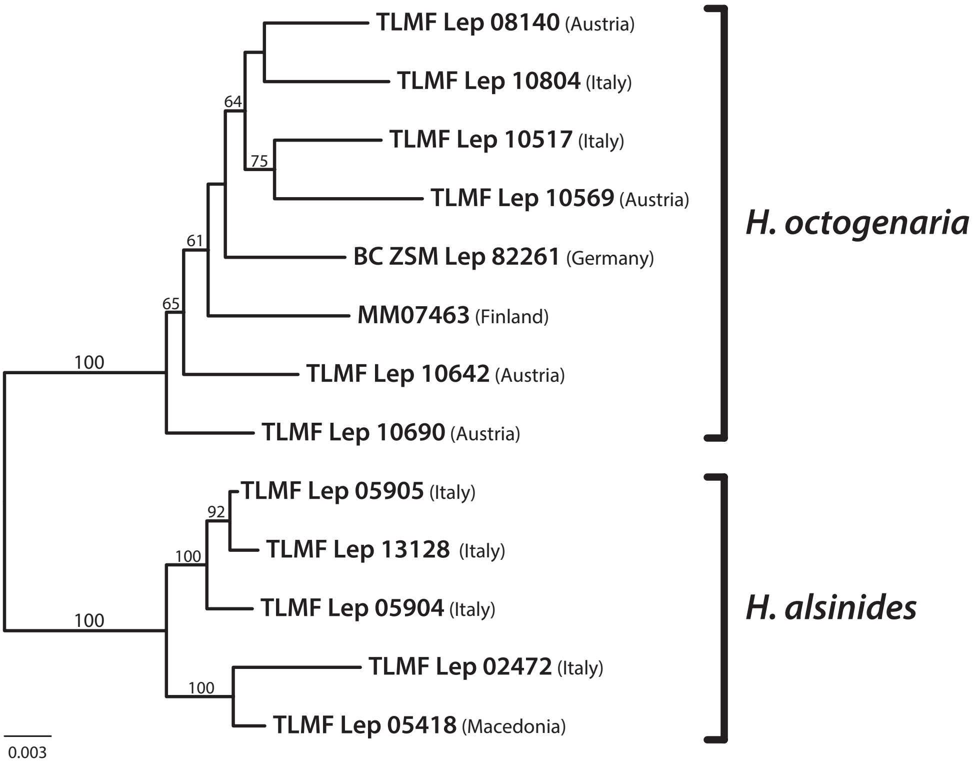
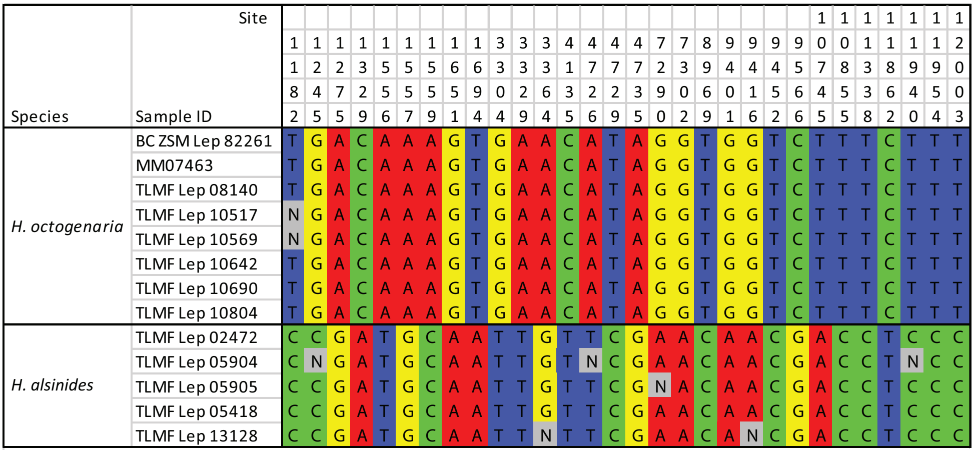


Figure 30. Maximum likelihood phylogeny inferred from the concatenated RAD data. The data matrix consisted of 47,676 SNPs in 1,363,146 bp. The phylogenetic tree was inferred with RAxML with 1,000 bootstrap replicates. Bootstrap support values are indicated above the branch, values of only > 50% are shown.



	Site																							1	1	1	1	1	1	1
		1	1	1	1	1	1	1	1	1	3	3	3	4	4	4	4	7	7	8	9	9	9	9	0	0	1	1	1	2
		1	2	2	3	5	5	5	6	6	3	3	3	1	7	7	7	2	3	9	4	4	4	5	7	8	3	8	9	9	0
		8	4	7	2	5	5	5	5	9	0	2	6	3	2	2	3	9	0	6	0	1	5	6	4	5	3	6	4	5	0
Species	Sample ID	2	5	5	9	6	7	9	1	4	4	9	4	5	6	9	5	0	2	9	1	6	2	6	5	5	8	2	0	4	3
H. octogenaria	BC ZSM Lep 82261	T	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
	MM07463	T	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
	TLMF Lep 08140	T	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
	TLMF Lep 10517	N	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
	TLMF Lep 10569	N	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
	TLMF Lep 10642	T	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
	TLMF Lep 10690	T	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
	TLMF Lep 10804	T	G	A	C	A	A	A	G	T	G	A	A	C	A	T	A	G	G	T	G	G	T	C	T	T	T	C	T	T	T
H. alsinides	TLMF Lep 02472	C	C	G	A	T	G	C	A	A	T	T	G	T	T	C	G	A	A	C	A	A	C	G	A	C	C	T	C	C	C
	TLMF Lep 05904	C	N	G	A	T	G	C	A	A	T	T	G	T	N	C	G	A	A	C	A	A	C	G	A	C	C	T	N	C	C
	TLMF Lep 05905	C	C	G	A	T	G	C	A	A	T	T	G	T	T	C	G	N	A	C	A	A	C	G	A	C	C	T	C	C	C
	TLMF Lep 05418	C	C	G	A	T	G	C	A	A	T	T	G	T	T	C	G	A	A	C	A	A	C	G	A	C	C	T	C	C	C
	TLMF Lep 13128	C	C	G	A	T	G	C	A	A	T	T	N	T	T	C	G	A	A	C	A	N	C	G	A	C	C	T	C	C	C

Figure 31. The SNPs showing a fixed difference between *H. octogenaria* and *H. alsinides*.

identified 30 fixed differences between *H. octogenaria* and *H. alsinides* sp. rev. providing candidate species-specific SNPs (Fig. 31).

Overall, the massive genomic ddRAD sequencing data provided very strong evidence that the two partially sympatric species of *Hoplodrina* are biologically distinct species.

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The amphibians and reptiles of Colima, Mexico, with a summary of their conservation status

Julio A. Lemos-Espinal[1], Geoffrey R. Smith[2], Leland J.S. Pierce[3],
Charles W. Painter[3,†]

1 *Laboratorio de Ecología UBIPRO, FES Iztacala UNAM, Avenida Los Barrios 1, Los Reyes Iztacala, Tlalnepantla Estado de México, 54090, Mexico* **2** *Department of Biology, Denison University, Granville, Ohio, USA* **3** *New Mexico Department of Game and Fish, P.O. Box 25112, Santa Fe, New Mexico 87504, USA*

Corresponding author: Julio A. Lemos-Espinal (lemos@unam.mx)

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Abstract

Colima is the fourth smallest Mexican state, covering only 0.3% of the surface area of Mexico, but due to the remarkable diversity of physiographic and environmental conditions present in Colima it contains a high biological diversity. We generated an up-to-date herpetofaunal checklist for Colima, with a summary of the conservation status of Colima's amphibians and reptiles. Our checklist contains a total of 153 species of amphibians and reptiles (three introduced). Thirty-nine are amphibians and 114 are reptiles. More than half of Colima's herpetofauna are Mexican endemics (66.7% of amphibians, 67.5% of reptiles). Less than 25% of the amphibian and reptile species in Colima are in protected categories according to the IUCN Red List and SEMARNAT. The reptiles in the Marine and Revillagigedo Archipelago regions are the most threatened taxa of the Colima herpetofauna. Colima shares > 80% of its herpetofauna with its neighboring states, Jalisco and Michoacán.

Keywords

checklist, crocodilians, frogs, herpetofauna, lizards, salamanders, snakes, turtles

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† Deceased

Introduction

A number of Mexican states still lack comprehensive species lists of amphibians and reptiles. One such state is Colima, which despite being the fourth smallest Mexican state, covering only 0.3% of the surface area of Mexico, has, as reported here, a rich herpetofauna represented by 150 native species (38 amphibians and 112 reptiles), in part due to the remarkable diversity of physiographic and environmental conditions present in Colima.

The interest in the study of amphibians and reptiles of the state of Colima dates from 1700, the year in which the first official record of a herpetological specimen collected in Colima (*Rana pustulosa* – MVZ-A20941). More than 200 years later, Oliver (1937) reported 61 species from Colima. Duellman (1958) subsequently listed 82 amphibian and reptile species from the lowlands of Colima, and Painter (1976) studied the distribution of amphibians and reptiles in Colima. More recently, Reyes-Velasco et al. (2009) reported new state records for 21 species of amphibians and reptiles from Colima. In addition, there have been several new species recently described or elevated to species status from Colima. Bryson et al. (2014) described *Crotalus campbelli* from the Sierra de Manantlán of southwestern Jalisco and northern Colima and elevated *Crotalus triseriatus armstrongi* to a full species status (*C. armstrongi*). Reyes-Velasco et al. (2015) described *Eleutherodactylus grunwaldi* from the state of Colima. Grünwald et al. (2018) described two new species of *Eleutherodactylus* from Colima, *E. colimotl* and *E. manantlanensis*, the last one endemic to Colima.

Given these recent additions and changes in the known species of amphibians and reptiles of Colima, we have conducted a comprehensive review of the specimens and documented species of amphibians and reptiles from Colima to provide an up-to-date herpetofaunal checklist from Colima. In addition, we review and summarize the conservation status of these amphibians and reptiles as a potential guide to future conservation and management efforts focused on the amphibians and reptiles of Colima.

Physiography of Colima

Colima is one of the smallest states in Mexico, covering 5,627 km^2 between 19°30'45"N and 18°41'03"N, and -103°29'11"W and -104°41'26"W. Colima is located in central-western Mexico, in the middle of the Pacific Coast of Mexico (Fig. 1). Colima is bordered by Jalisco to the north and east, Michoacán to the southeast, and the Pacific Ocean to the west and south. The Revillagigedo Archipelago is part of the state of Colima and includes the islands of Socorro, San Benedicto, Clarion, and Roca Partida (INEGI 2017), lies approximately 390 km southwest of Cabo San Lucas, the southern tip of the Baja California Peninsula, and 720 to 970 km west of Manzanillo, northwestern Colima (https://en.wikipedia.org/wiki/Revillagigedo_Islands – accessed 10 October 2019).

Almost three quarters of the surface area of Colima is covered by mountains and hills, producing a heterogeneous topography in the state (Fig. 2). This complex to-

Figure 1. Map of Mexico with the state of Colima shown in red (modified from INEGI 2018).

pography is represented by two physiographic provinces that are included within the geographic limits of the state: the Volcanic Axis, represented in the state by the subprovince of Volcanes de Colima; and the Sierra Madre del Sur, represented in the state by two subprovinces "Sierras de la Costa de Jalisco y Colima" and "Cordillera Costera del Sur" (Fig. 3). The subprovince of Volcanes de Colima is found in the northern corner of the state in the region known as Valle de Colima, and occupies 16.03% of the state's surface area. The Colima Volcanos (Nevado de Colima, which actually lies in the state of Jalisco, and Volcán de Colima which lies in the states of Jalisco and Colima) are found in this subprovince. All the northern and northeastern slopes and most of the eastern slopes of these two volcanoes lie in the state of Jalisco. The Valley of Colima, formed from the slopes of the Volcán de Colima, is also found in this region. In Colima the subprovince of Sierras de la Costa de Jalisco and Colima occupies most of the state (62.51% of the surface area). It includes the western mountains, the Marabasco River Basin, the Armeria Valley and the entire Colima coast. In the west-central and southern part of the state that parallels the coast the land is flat, and the Valle de Armeria or Llanuras de Tecomán is found here. Northwestern Colima has mountain ranges intermixed with small valleys. The subprovince of the Cordillera Costera del Sur is also part of the province of the Sierra Madre del Sur and occupies 16.03% of the surface area in extreme eastern Colima. Approximately half of this subprovince is represented by mountain ranges that do not reach 2,000 m elevation, and the other half by valleys, hills, and plains (www.inegi.gob.mx accessed 10 October 2019).

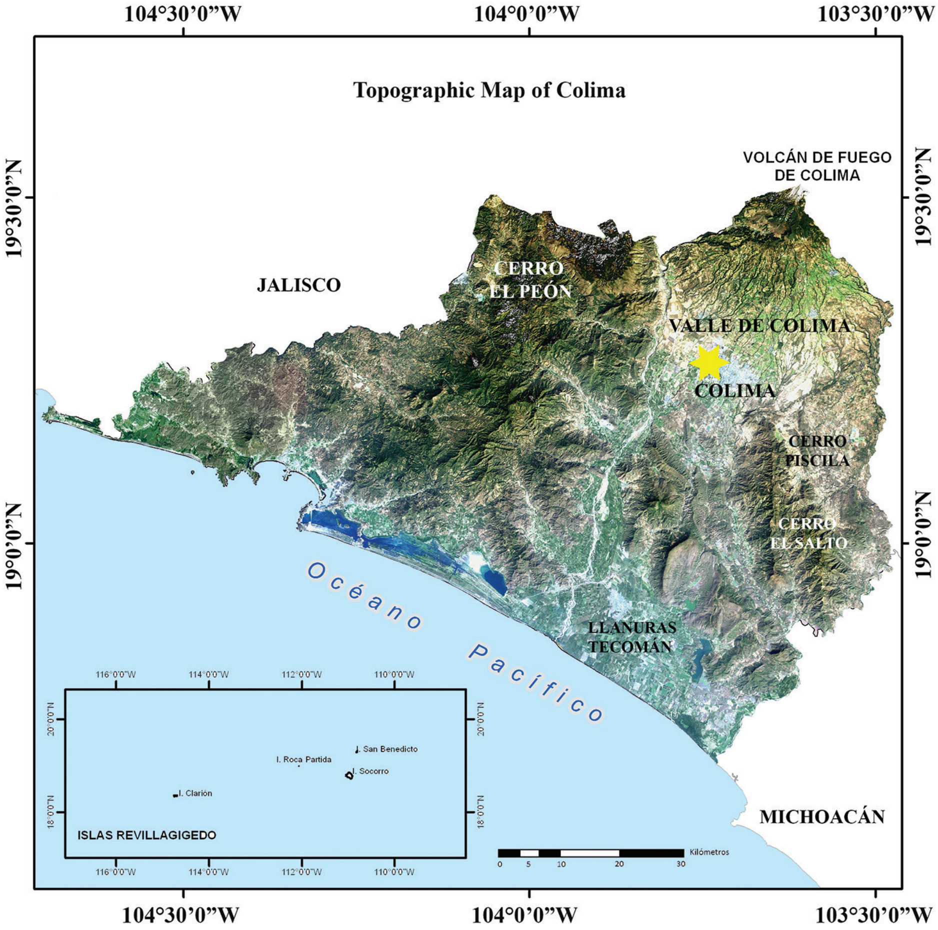

Figure 2. Satellite map showing the topographic features of Colima (from Comisión Nacional para el Conocimiento y Uso de la Biodiversidad 2008).

In Colima, the Sierra Madre del Sur consists of four mountain systems. The first system, and the most important, is located in northern Colima and includes Cerro Grande and several hills (Jurípichi or Juluapan, Los Juanillos, La Astilla, El Ocote, El Peón, El Barrigón, San Diego and La Media Luna). The second system runs from the northwestern end of the state southeast to central Colima, and is formed by mountain ranges (the Espinazo del Diablo, El Escorpión , El Tigre, El Aguacate, El Centinela, El Toro and La Vaca) that parallel to the coast between the Marabasco and Armería rivers. The third system is located in central Colima, and consists of hills (Alcomún and Partida, San Miguel and Comala, and San Gabriel or Callejones) that extend to the south between the Armería and Salado rivers. The fourth system is found in southeastern Colima between the Salado and Naranjo or Coahuayana rivers, and includes multiple mountain ranges (Piscila, Volcancillos, La Palmera, El Camichín and Copales) (http://www.inafed.gob.mx/work/enciclopedia/EMM06colima/mediofisico.html – accessed October 10, 2019).

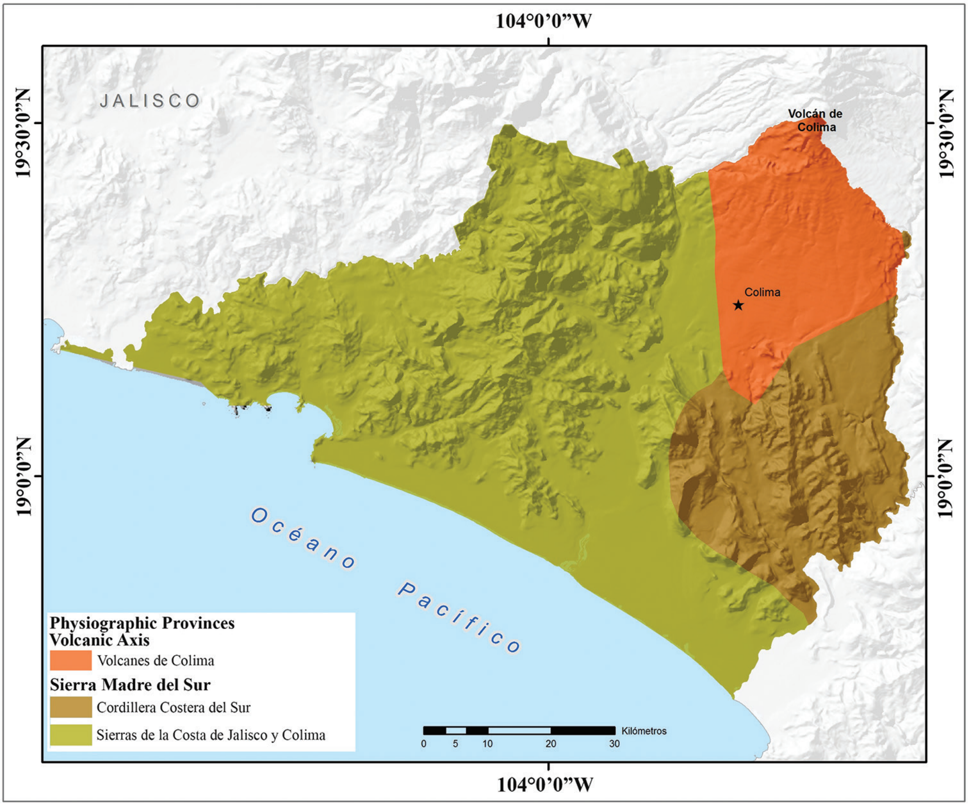

Figure 3. Physiographic provinces of the state of Colima, Mexico (modified from Cervantes-Zamora et al. 1990).

The climate of Colima is very diverse (Fig. 4), although relatively high humidity predominates throughout the state. In northern Colima the climate is warm sub-humid, whereas in the mountains there is a sub-humid semi-warm climate and the plains of Tecomán have a semi-dry warm climate. In the coastal area and in the Armería river basin the climate is warm and humid. The average annual temperature ranges around 25 °C, with the maximum of 38 °C and the minimum of 7 °C. Average annual rainfall is 983 mm. Colima's climate is greatly influenced by the presence of mountains to the west, north and east. The mountain range of Picila creates the southern border of the Valley of Colima, and to the south, the plains of Tecomán end in a low and sandy coast. These mountains, due to their latitude and exposure, allow rainfall to be greater and the climate to differ from the lower elevation parts of the state. In the coastal zone and in the Armería river basin the climate is warm and humid, whereas in the higher elevations in the southern zone it is warm and temperate.

The occurrence of various tropical and temperate floristic elements coupled with variations in the physical environment has resulted in an intricate and complex mosaic of plant associations in Colima (Fig. 5; Schaldach 1963; INEGI 2017). The types of vegetation present in the state are several types of tropical forest, palm

Figure 4. Climate map of the state of Colima, Mexico (modified from García – Comisión Nacional para el Conocimiento y Uso de la Biodiversidad 1998).

groves, savanna, mangrove, coniferous forest, as well as areas of irrigated agriculture. The different types of tropical forest occupy most Colima's area (74%). Medium subdeciduous forest covers 57% of the state and is present in all municipalities, with the dominant species being highly branched canopy trees, 15 to 25 m high and 50 to 75% of species losing their leaves in the dry season. Medium subperenifolious forest covers 15.4% of Colima. The vegetation is characterized by a height of 20 to 25 m, with 50 to 75% of the species being evergreens. Low deciduous forest covers 1.3% of the area of Colima. The dominant vegetation is low trees from 8 to 12 m high, with abundant leaves that fall in the dry season. Prickly low deciduous forest covers only 1.0% of Colima. It is characterized by the presence of deciduous trees 4 to 8 m tall with thorns. Palmar is only found in the municipalities of Manza-

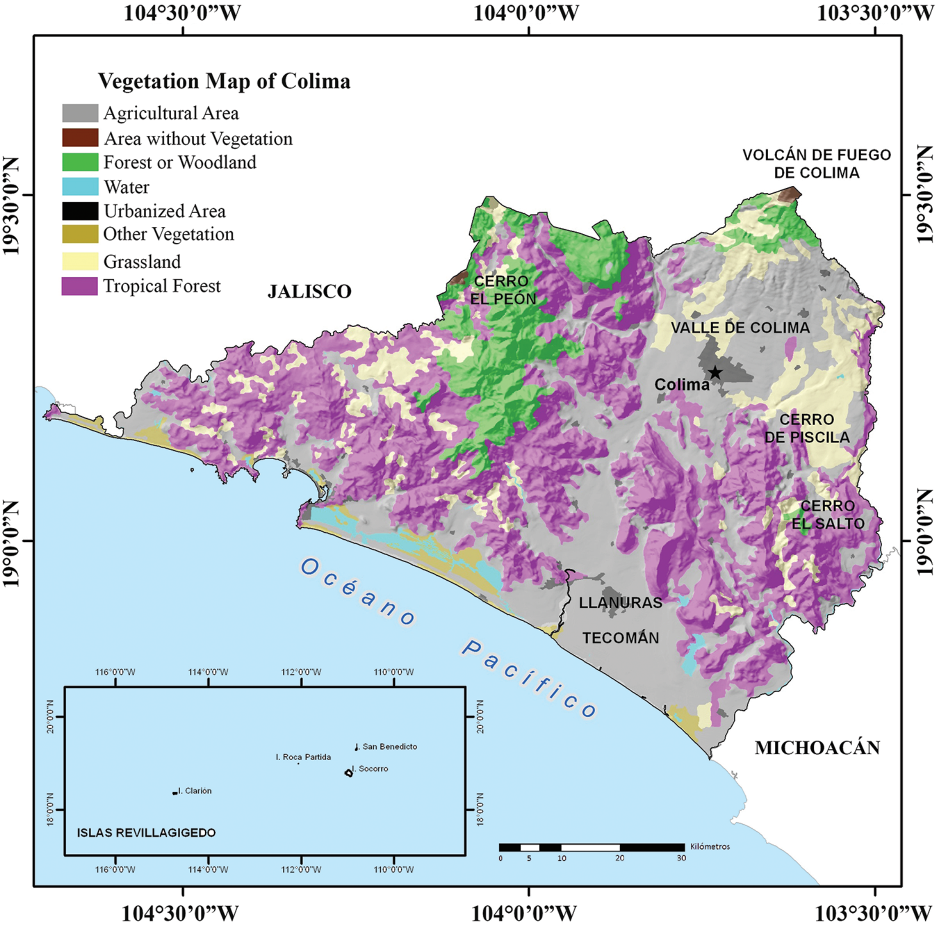

Figure 5. Vegetation map of the state of Colima, Mexico (modified from Dirección General de Geografía – INEGI 2005).

nillo and Armería. Mangrove is distributed in the coastal area with coastal lagoons. Savanna is characterized by widely dispersed trees and grasses and is a product of logging or burning of primary communities. Different types of coniferous forests occupy 10.6% of the state's area.

At the highest elevations in northern Colima, > 50% of the area is forested, so that the agricultural area is limited to 42,700 ha (12,000 ha of irrigation and 30,700 ha of temporary), and due to the topography < 30% can be subjected to mechanized agriculture. The coastal region is characterized as being more favorable for agriculture, including the Tecomán region where the largest area with irrigation infrastructure and plantations with perennial crops is concentrated and mechanized agriculture is used over an area of 92,700 ha (58,400 ha of irrigation and 34,300 ha of temporary).

Recent taxonomic changes

Acevedo et al. (2016) demonstrated that there were two evolutionary lineages within *Rhinella marina*, one eastern and one western Andean. The eastern populations retained the name *R. marina*, and the western populations were given the revalidated name *R. horribilis*. Duellman et al. (2016) treated two major Hylid clades as genera: *Hyla* which is restricted to the Old World, and *Dryophytes* which is distributed primarily in the New World, including species in Mexico. Reyes-Velasco et al. (2015) described a new species of *Eleutherodactylus* from the Sierra de Manantlán in northern Colima and southwestern Jalisco (*Eleutherodactylus grunwaldi*). In addition, Grünwald et al. (2018) described two new species of *Eleutherodactylus* from Colima, one endemic to the state (*Eleutherodactylus manantlanensis*) and one limited to Colima and western Michoacán (*E. colimotl*). Grünwald et al. (2018) also regarded *E. nivicolimae* as a synonym of *E. rufescens*. Frost et al. (2006) recommended the use of the name *Lithobates* for most New World species of *Rana*, including those in Mexico. However, Yuan et al. (2016) retained all the species of the genera suggested by Frost et al. (2006), including *Lithobates*, in the traditional genus *Rana*, based on clear monophyly of a larger group that includes all of these genera. We therefore follow Yuan et al. (2016) and AmphibiaWeb (2019) in using *Rana* instead of *Lithobates*.

Plestiodon indubitus was originally described by Taylor (1933), however Dixon (1969) regarded it as a subspecies of *P. brevirostris*. Feria-Ortiz et al. (2011) subsequently elevated it to full species status, and suggested that the western populations of *P. b. indubitus* from Colima and Jalisco likely represent an undescribed species. We tentatively assign the name *indubitus* to the Colima population until a new name is available. Originally *Holcosus sinister* was described as a subspecies of *H. undulatus* by Smith and Laufe (1946), but it has recently been elevated to full species status by Meza-Lázaro and Nieto-Montes de Oca (2015). Card et al. (2016) resurrected the name *sigma* for the population from María Madre Island, Tres Marías Islands, Nayarit, Mexico described by Smith (1943) as *Constrictor* (= *Boa*) *constrictor sigma*, which was regarded as a junior synonym of *B. c. imperator* by Zweifel (1960). Card et al. (2016) recognized the *Boa* populations from the slopes of the Mexican Pacific as *Boa sigma*, and this is followed here. *Epictia bakewelli* was described as a species by Oliver (1937), and was regarded as a subspecies of *E. goudotti* by Peters et al. (1970). However, Wallach et al. (2014) considered it a full species, and McCranie and Hedges (2016) confirmed its status as a full species. Originally *Rena dugesii* was described as a species by Bocourt (1881), but for a long time it was regarded as a subspecies of *Rena humilis*, however it has recently been regarded as a full species (Wallach et al. 2014). Bryson et al. (2014) described a new species of *Crotalus* from western Jalisco and the Sierra de Manantlán of southwestern Jalisco and northern Colima (*Crotalus campbelli*). They also recognized *C. armstrongi* as a species, which was originally described as a subspecies of *C. triseriatus*.

Methods

We generated our list of the amphibians and reptiles of Colima using our own field work, a thorough examination of the available literature, checking the amphibian and reptile records for Colima in VertNet.org, and consulting databases from the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (National Commission for the Understanding and Use of Biodiversity; CONABIO), including records from museum collections listed in Appendix 1.

The amphibian names we use follow Frost (2019) and AmphibiaWeb (2019) (http://amphibiaweb.org) and the reptile names we use follow Uetz and Hošek (2019). We include species in the list only if we could confirm records, either by direct observation or through documented museum records or vouchers. We created species accumulation curves the total herpetofauna, amphibians, and reptiles using the year of the first recorded observation for each species. Species accumulation curves may provide reasonable estimates of the potential species richness of amphibians and reptiles (see Raxworthy et al. 2012). We recorded the conservation status of each species based on the IUCN Red List 2019-2 (IUCN 2019), listing in SEMARNAT (2010), and Environmental Vulnerability Scores (Wilson et al. 2013a,b; Johnson et al. 2015). We determined the number of species shared between Colima and its neighboring states using recent lists of amphibians and reptiles for Jalisco (Cruz-Sáenz et al. 2017) and Michoacán (Alvarado-Díaz et al. 2013).

Results and discussion

A total of 153 species of amphibians and reptiles (three introduced) is found in Colima. Thirty-nine of these species are amphibians (36 anurans [one introduced], two salamanders, and one caecilian), and 114 are reptiles (one crocodilian, 41 lizards [two introduced], 64 snakes, and eight turtles) (Tables 1, 2). These represent 37 families: 12 amphibians (nine anurans, two salamanders, one caecilians), and 25 reptiles (one crocodilian, 12 lizards [one of them introduced], eight snakes, and four turtles); 92 genera: 20 amphibians (17 anurans, two salamanders, one caecilian), and 72 reptiles (one crocodile, 22 lacertilia [two of the introduced], 42 snakes, and six turtles. The introduced amphibian is the American Bullfrog (*Rana catesbeiana*), and the two introduced lizards are species of the family Gekkonidae: the Stump-toed Gecko (*Gehyra mutilata*) and the Common House Gecko (*Hemidactylus frenatus*). There are also 20 species (eight amphibians and 12 reptiles) that potentially occur within the state of Colima (Table 3). Most of these are species from the northern slope of Nevado de Colima in Jalisco, and it is highly likely that they also occur on the southern slope of this volcano in Colima. Some other species have been recorded in extreme southwestern Jalisco, near the border with Colima and it is likely that they occur in extreme western Colima. There are a few other species that might occur in eastern or southern

Table 1. Amphibians and reptiles of Colima with distributional information and conservation status. Physiographic region: (1 = Volcanes de Colima; 2 = Sierras de la Costa de Jalisco y Colima; 3 = Cordillera Costera del Sur; 4 = Marine; 5 = Islands); IUCN Status: (DD = Data Deficient; LC = Least Concern, V = Vulnerable, NT = Near Threatened; E = Endangered; CE = Critically Endangered; NE = not Evaluated) according to the IUCN Red List (The IUCN Red List of Threatened Species, Version 2019-2 (www.iucnredlist.org; accessed 2 October 2019); conservation status in Mexico according to SEMARNAT (2010): (P = in danger of extinction, A = threatened, Pr = subject to special protection, NL – not listed); Environmental Vulnerability Score: (EVS: low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20) from Wilson et al. (2013a,b) and Johnson et al. (2015). Global Distribution: 0 = Endemic to Colima; 1 = Endemic to Mexico; 2 = Shared between the US and Mexico; 3 = widely distributed from Mexico to Central or South America; 4 = widely distributed from the US to Central or South America; 5 = circumglobal distribution; 6 = Pacific and Indian Oceans; IN = Introduced to Colima. Date in which the first record appeared; and Source of the record.

	Physiographic region	IUCN status	SEMARNAT	EVS	Global distribution	Year of first record	Source
Class Amphibia (39)							
Order Anura (36)							
Family Bufonidae (5)							
Anaxyrus compactilis (Wiegmann, 1833)	1, 2, 3	LC	NL	H (14)	1	1935	FMNH 103444
Incilius marmoreus (Wiegmann, 1833)	1, 2, 3	LC	NL	M (11)	1	1905	UAZ 11613
Incilius mazatlanensis (Taylor, 1940)	1, 2	LC	NL	M (12)	1	1965	UAZ 33286
Incilius occidentalis (Camerano, 1879)	1	LC	NL	M (11)	1	1961	UCM 61121
Rhinella horribilis (Wiegmann, 1833)	1, 2, 3,	NE	NL	NE	4	1901	MNHN RA 1901.341
Family Craugastoridae (2)							
Craugastor occidentalis (Taylor, 1941)	1, 2	DD	NL	M (13)	1	1958	UTEP H-14317
Craugastor vocalis (Taylor, 1940)	1	LC	NL	M (13)	1	1970	FSM-UF 66361
Family Eleutherodactylidae (7)							
Eleutherodactylus angustidigitorum (Taylor, 1940)	1	VU	Pr	H (17)	1	1964	LACM 25450
Eleutherodactylus colimotl Grünwald, Reyes- Velasco, Franz-Chávez, Morales-Flores, Ahumada-Carrillo, Jones & Boissinot, 2018	2, 3	NE	NL	NE	1	2015	Grünwald et al. (2018)
Eleutherodactylus grunwaldi Reyes-Velasco, Ahumada-Carrillo, Burkhardt, & Devitt, 2015	2	NE	NL	NE	1	2011	Reyes-Velasco et al. (2015)
Eleutherodactylus manantlanensis Grünwald, Reyes-Velasco, Franz-Chávez, Morales-Flores, Ahumada-Carrillo, Jones & Boissinot, 2018	2	NE	NL	NE	0	2014	Grünwald et al. (2018)
Eleutherodactylus modestus (Taylor, 1942)	1, 2	VU	Pr	H (16)	1	1935	USNM 139729
Eleutherodactylus nitidus (Peters, 1870)	2, 3	LC	NL	M (12)	1	1956	UMMZ 114311
Eleutherodactylus rufescens (Duellman &Dixon, 1959)	1, 2	CR	Pr	H (17)	1	1963	USNM 161162
Family Hylidae (10)							
Dendropsophus sartori (Smith, 1951)	2, 3	LC	A	H (14)	1	1960	MVZ 71221
Dryophytes arenicolor Cope, 1866	2, 3	LC	NL	L (7)	2	1973	UTEP H-10258

	Physiographic region	IUCN status	SEMARNAT	EVS	Global distribution	Year of first record	Source
Dryophytes eximius (Baird, 1854)	1, 2	LC	NL	M (10)	1	1975	UTEP H-10387
Exerodonta smaragdina (Taylor, 1940)	2	LC	Pr	M (12)	1	1953	UMMZ 110873
Exerodonta sumichrasti Brocchi, 1879	1	LC	NL	L (9)	1	NA	USNM 57518
Smilisca baudini (Duméril & Bibron, 1841)	1, 2, 3	LC	NL	L (3)	4	1902	USNM 57555
Smilisca fodiens (Boulenger, 1882)	1	LC	NL	L (8)	2	1919	MCZ A-6683
Tlalocohyla smithii (Boulenger, 1902)	1, 2, 3	LC	NL	M (11)	1	1960	MVZ 71216
Trachycephalus vermiculatus (Cope, 1877)	1, 2	NE	NL	L (4)	3	1935	UMMZ 80018
Triprion spatulatus Günther, 1882	1, 2, 3	LC	NL	M (13)	1	1958	UAZ 12869
Family Leptodactylidae (2)							
Leptodactylus fragilis (Brocchi, 1877)	1, 2	LC	NL	L (5)	4	1958	UTEP H-14352
Leptodactylus melanonotus (Hallowell, 1861)	1, 2, 3	LC	NL	L (6)	3	1902	USNM 57765
Family Microhylidae (2)							
Hypopachus ustus (Cope, 1866)	1, 2	LC	Pr	L (7)	3	1935	UMMZ 79998
Hypopachus variolosus (Cope, 1866)	1, 2	LC	NL	L (4)	3	1935	USNM 118658
Family Phyllomedusidae (1)							
Agalychnis dacnicolor (Cope, 1864)	1, 2	LC	NL	M (13)	1	1963	FSM-UF 109279
Family Ranidae (6)							
Rana berlandieri Baird, 1859	2	LC	Pr	L(7)	2	1919	FMNH 1628
Rana catesbeiana Shaw, 1802	N/A	N/A	N/A	N/A	IN	2018	https://www.youtube
Rana forreri Boulenger, 1883	1, 2	LC	Pr	L (3)	4	1964	CAS 97107
Rana neovolcanica Hillis & Frost, 1985	1	NT	A	M (13)	1	2016	Cruz-Saenz et al. (2018)
Rana pustulosa Boulenger, 1883	1, 2	LC	Pr	L (3)	1	1700	MCZ A-20941
Rana zweifeli Hillis, Frost & Webb, 1984	1	LC	NL	M (11)	1	1982	MNHUK 194432
Family Scaphiopodidae (1)							
Spea multiplicata (Cope, 1863)	1	LC	NL	L (6)	2	1960	TNHC 19343
Order Caudata (2)							
Family Ambystomatidae (1)							
Ambystoma velasci Dugès, 1888	1, 2	LC	Pr	M (10)	1	1868	MNHN RA 1868.175
Family Plethodontidae (1)							
Isthmura belli (Gray, 1850)	2	VU	A	M (12)	1	1970	MCZ A-85395
Order Gymnophiona (1)							
Family Caecilidae (1)							
Dermophis oaxacae (Mertens, 1930)	1, 2	DD	Pr	M (12)	1	1970	FSM-UF 61604
Class Reptilia (112)							
Order Crocodylia (1)							
Family Crocodylidae (1)							
Crocodylus acutus (Cuvier, 1807)	1, 2	VU	Pr	H (14)	4	1892	USNM 52336
Order Squamata (107)							
Suborder Lacertilia (41)							
Family Anguidae (3)							
Barisia imbricata (Wiegmann, 1828)	2	LC	Pr	H (14)	1	1965	UAZ 32849
Elgaria kingii Gray, 1838	2	LC	Pr	M (10)	2	1993	MZFC 6811
Gerrhonotus liocephalus Wiegmann, 1828	1, 2	LC	Pr	L (6)	1	1868	MNHN RA 1868.153

	Physiographic region	IUCN status	SEMARNAT	EVS	Global distribution	Year of first record	Source
Family Corytophanidae (2)							
Basiliscus vittatus Wiegmann, 1828	2	LC	NL	L (7)	3	1935	UMMZ 80147
Laemanctus longipes Wiegmann, 1834	2	LC	Pr	L (9)	3	1904	FMNH 1353
Family Dactyloidae (1)							
Anolis nebulosus (Wiegmann, 1834)	1, 2, 3	LC	NL	M (13)	1	1863	USNM 63700
Family Eublepharidae (1)							
Coleonyx elegans Gray, 1845	2, 3	LC	A	L (9)	3	1960	MNHUK 62400
Family Gekkonidae (2)							
Gehyra mutilata (Wiegmann, 1834)	1, 2, 3	N/A	N/A	N/A	IN	1976	AMNH R-163687
Hemidactylus frenatus Duméril & Bribon, 1836	2	N/A	N/A	N/A	IN	1960	MVZ 71229
Family Helodermatidae (1)							
Heloderma horridum (Wiegmann, 1829)	1, 2, 3	LC	A	M (11)	3	1818	MVZ 79417
Family Iguanidae (2)							
Ctenosaura pectinata (Wiegmann, 1834)	1, 2, 3	NE	NL	H (15)	1	1863	MCZ R-24902
Iguana iguana (Linnaeus, 1758)	1, 2, 3	LC	Pr	M (12)	3	1863	USNM 63699
Family Phrynosomatidae (14)							
Phrynosoma asio Cope, 1864	1, 2	LC	Pr	M (11)	1	1935	UMMZ 80067
Phrynosoma orbiculare (Linnaeus, 1758)	1	LC	A	M (12)	1	1870	Duméril and Bocourt (1870)
Sceloporus bulleri Boulenger, 1895	2	LC	NL	H (15)	1	2004	Reyes-Velasco et al. (2009)
Sceloporus dugesii Bocourt, 1874	1, 2	LC	NL	M (13)	1	1969	ASNHC 13801
Sceloporus horridus Wiegmann, 1834	1, 2, 3	LC	NL	M (12)	1	1863	USNM 31389
Sceloporus melanorhinus Bocourt, 1876	1, 2	LC	NL	L (9)	3	1863	USNM 31496
Sceloporus nelsoni Cochran, 1923	2	LC	NL	M (13)	1	1964	LACM 74288
Sceloporus pyrocephalus Cope, 1864	1, 2, 3	LC	NL	M (12)	1	1863	USNM 31449
Sceloporus torquatus Wiegmann, 1828	2	LC	NL	M (11)	1	1863	MNHN RA 0.2922
Sceloporus utiformis Cope, 1864	1, 2, 3	LC	NL	H (15)	1	1902	USNM 58811
Sceloporus unicanthalis Smith, 1937	2	NE	NL	H (16)	1	2005	Reyes-Velasco et al. (2009)
Urosaurus auriculatus (Cope, 1871)	5	EN	NL	H (16)	0	1871	Cope (1871)
Urosaurus bicarinatus (Duméril, 1856)	1, 2, 3	LC	NL	M (12)	1	1919	CAS 54904
Urosaurus clarionensis (Townsend, 1890)	5	VU	NL	H (17)	0	1890	Townsend (1890)
Family Phyllodactylidae (3)							
Phyllodactylus davisi Dixon, 1964	1, 2	LC	A	H (16)	1	1930	MVZ 12186
Phyllodactylus lanei Smith, 1935	1, 2	LC	NL	H (15)	1	1937	Oliver (1937)
Phyllodactylus tuberculosus Wiegmann, 1834	2	LC	NL	L (8)	3	NA	MNHN RA 0.1657
Family Scincidae (5)							
Marisora brachypoda (Taylor, 1956)	1, 2, 3	LC	NL	L (6)	3	1962	LACM 5987
Plestiodon indubitus (Taylor, 1933)	2	NE	NL	H (15)	1	1923	MCZ R-135422
Plestiodon colimensis (Taylor, 1935)	1	DD	Pr	H (14)	1	1935	Taylor (1936)
Plestiodon parvulus (Taylor, 1933)	2	DD	NL	H (15)	1	1935	UMMZ 80108
Scincella assata (Cope, 1864)	1, 2	LC	NL	L (7)	3	1935	UMMZ 80106
Family Teiidae (6)							
Aspidoscelis communis (Cope, 1878)	1, 2, 3	LC	Pr	H (14)	1	1920	LACM 7956

	Physiographic region	IUCN status	SEMARNAT	EVS	Global distribution	Year of first record	Source
Aspidoscelis costatus (Cope, 1878)	1, 2	LC	Pr	M (11)	1	1863	USNM 31610
Aspidoscelis deppii (Wiegmann, 1834)	1, 2	LC	NL	L (8)	3	1959	UAZ 06297
Aspidoscelis guttatus (Wiegmann, 1834)	2	LC	NL	M (12)	1	1969	ASNHC 13965
Aspidoscelis lineattissimus (Cope, 1878)	1, 2, 3	LC	Pr	H (14)	1	1957	UCM 14659
Holcosus sinister (Wiegmann, 1834)	1, 2, 3	NE	NL	M (13)	1	1920	LACM 7956
Family Xantusidae (1)							
Lepidophyma tarascae Bezy, Webb & Álvarez, 1982	3	DD	A	H (14)	1	2005	Reyes-Velasco et al. (2009)
Suborder Serpentes (66)							
Family Boidae (1)							
Boa sigma Smith, 1943	1, 2	NE	NL	H (15)	1	1863	USNM 62024
Family Colubridae (23)							
Conopsis biserialis (Taylor & Smith, 1942)	2	LC	A	M (13)	1	2004	Reyes-Velasco et al. (2009)
Drymarchon melanurus (Duméril, Bibron & Duméril, 1854)	1, 2	LC	NL	L (6)	3	1902	CM S7254
Drymobius margaritiferus (Schlegel, 1837)	1, 2	LC	NL	L (6)	3	1902	CM S7252
Geagras redimitus Cope, 1875	2	DD	Pr	H (14)	1	1962	MVZ 75805
Lampropeltis polyzona Cope, 1860	1	LC	NL	L(7)	1	1863	MCZ R-27105
Leptophis diplotropis (Günther, 1872)	2	LC	A	H (14)	1	1962	MVZ 75804
Masticophis anthonyi (Stejneger, 1901)	5	CR	A	H (17)	0	1901	Stejneger (1901)
Masticophis bilineatus (Jan, 1863)	1, 2	LC	NL	M (11)	2	1914	MCZ R-11409
Masticophis mentovarius (Duméril, Bibron & Duméril, 1854)	1, 2	LC	A	L (6)	3	1863	USNM 32234
Mastigodryas melanolomus (Cope, 1868)	1, 2	LC	NL	L (6)	3	1902	USNM 56283
Oxybelis aeneus (Wagler, 1824)	2	LC	NL	L (5)	4	1892	USNM 46606
Pituophis deppei (Dumeril, 1853)	1	LC	A	H (14)	1	1868	MNHN RA 1868.157
Pseudoficimia frontalis (Cope, 1864)	1, 2, 3	LC	NL	M (13)	1	1956	UMMZ 114482
Salvadora lemniscata (Cope, 1895)	2	LC	Pr	H (15)	1	1971	CAS 132121
Salvadora mexicana (Duméril, Bibron & Duméril, 1854)	1, 2, 3	LC	Pr	H (15)	1	1863	USNM 61969
Senticolis triaspis (Cope, 1866)	1, 2	LC	NL	L (6)	4	1935	UMMZ 80210
Sonora michoacanensi (Dugès, 1884)	2	LC	NL	H (14)	1	1966	Harris and Simmons (1970)
Symphimus leucostomus Cope, 1869	3	LC	Pr	H (14)	1	2004	Reyes-Velasco et al. (2009)
Tantilla bocourti (Günther, 1895)	3	LC	NL	L (9)	1	1960	MVZ 72202
Tantilla calamarina Cope, 1866	1, 2	LC	Pr	M (12)	1	1935	UMMZ 80224
Tantilla ceboruca Canseco-Marquéz, Smith, Ponce-Campos, Flores-Villela & Campbell, 2007	1	NE	NL	H (16)	1	2004	Reyes-Velasco et al. (2012)
Trimorphodon biscutatus (Duméril, Bibron & Duméril, 1854)	1, 2, 3	NE	NL	L (7)	3	1818	MVZ 72194
Trimorphodon tau Cope, 870	1, 2	LC	NL	M (13)	1	1956	UMMZ 114479
Family Dipsadidae (21)							
Clelia scytalina (Cope, 1867)	1, 2	LC	NL	M (13)	3	1963	MVZ 76355
Coniophanes lateritius Cope, 1862	2	DD	NL	M (13)	1	2005	Reyes-Velasco et al. (2009)
Conophis vittatus Peters, 1860	1, 2	LC	NL	M (11)	1	1961	FSM-UF 42088

	Physiographic region	IUCN status	SEMARNAT	EVS	Global distribution	Year of first record	Source
Dipsas gaigeae (Oliver, 1937)	2	LC	Pr	H (17)	1	1935	UMMZ 80221
Enulius flavitorques (Cope, 1868)	2	LC	NL	L (5)	3	1959	UAZ 20369
Geophis dugesii Boucourt, 1883	1	LC	NL	M (13)	1	1914	MCZ R-11422
Geophis sieboldi (Jan, 1862)	1	DD	Pr	M (13)	1	2012	Ahumada-Carrillo et al. (2014)
Hypsiglena torquata (Günther, 1860)	1, 2	LC	Pr	L (8)	1	1968	MNHN RA 1868.162
Hypsiglena unaocularus Tanner, 1946	5	NE	NL	NE	0	1946	Tanner (1946)
Imantodes gemmistratus (Cope, 1861)	1, 2	LC	Pr	L (6)	3	1935	UMMZ 80215
Leptodeira maculata (Hallowell, 1861)	1, 2	LC	Pr	L (7)	1	1863	USNM 31486
Leptodeira septentrionalis (Kennicott, 1859)	2	LC	NL	L (8)	4	1935	UMMZ Herps 80220
Leptodeira splendida Günther, 1895	1, 2	LC	NL	H (14)	1	1914	MCZ R-11411
Leptodeira uribei (Ramírez-Bautista & Smith, 1992)	2	LC	NL	H (17)	1	2004	Reyes-Velasco et al. (2009)
Manolepis putnami (Jan, 1863)	2	LC	NL	M (13)	1	1863	USNM 31478
Pseudoleptodeira latifasciata (Günther, 1894)	3	LC	Pr	H (14)	1	1961	MNHUK 63423
Rhadinaea hesperia Bailey, 1940	2	LC	Pr	M (10)	1	1935	UMMZ 80226
Rhadinaea taeniata (Peters, 1863)	1, 2	LC	NL	M (13)	1	1969	CAS 121078
Sibon nebulatus (Linnaeus, 1758)	1, 3	NE	NL	L (5)	3	1960	USNM 196500
Tropidodipsas annulifera Boulenger, 1894	1, 2, 3	LC	Pr	M (13)	1	2004	Reyes-Velasco et al. (2009)
Tropidodipsas philippii (Jan, 1863)	1, 2	LC	Pr	H (14)	1	1914	MCZ R-11410
Family Elapidae (6)							
Hydrophis platurus (Linnaeus, 1766)	4	LC	NL	NE	6	1956	UMMZ 114561
Micrurus browni Schmidt & Smith, 1943	2	LC	Pr	L (8)	3	1976	NLU 40764
Micrurus distans Kennicott, 1860	1, 2	LC	Pr	H (14)	1	1914	MCZ R-11416
Micrurus laticollaris Peters, 1870	1, 2, 3	LC	Pr	H (14)	1	1951	MNHUK 32546
Micrurus proximans Smith & Chrapliwy, 1958	2	LC	Pr	H (18)	1	2008	Reyes-Velasco et al. (2012)
Micrurus tener Baird & Girard, 1953	1	LC	NL	M (11)	2	2004	Reyes-Velasco et al. (2009)
Family Leptotyphlopidae (2)							
Epictia bakewelli (Oliver, 1937)	1, 2	NE	NL	NE	1	1935	UMMZ 80228
Rena dugesii (Bocourt, 1881)	2	NE	NL	NE	2	1868	MNHN RA 1868.154
Family Loxocemidae (1)							
Loxocemus bicolor Cope, 1861	1, 2	LC	Pr	M (10)	3	1863	USNM 61924
Family Natricidae (4)							
Storeria storerioides (Cope, 1866)	2	LC	NL	M (11)	1	2004	Reyes-Velasco et al. (2009)
Thamnophis cyrtopsis (Kennicott, 1860)	1	LC	A	L (7)	4	1964	LSUMZ 7846
Thamnophis melanogaster (Wiegmann, 1830)	1	EN	A	H (15)	1	1868	MNHN RA 1868.161
Thamnophis validus (Kennicott, 1860)	2	NE	NL	M (12)	1	1961	MNHUK 63428
Family Viperidae (6)							
Agkistrodon bilineatus Günther, 1863	1, 2	NT	Pr	M (11)	3	1928	UMMZ 68433
Crotalus basiliscus (Cope, 1864)	1, 2	LC	Pr	H (16)	1	1864	Cope (1864)
Crotalus campbelli Bryson, Linkem, Dorcas, Lathrop, Jones, Alvarado-Díaz, Grünwald & Murphy, 2014	2	NE	NL	H (17)	1	2004	Reyes-Velasco et al. (2009)

	Physiographic region	IUCN status	SEMARNAT	EVS	Global distribution	Year of first record	Source
Crotalus lannomi Tanner, 1966	2	DD	A	H (19)	1	2008	Reyes-Velasco et al. (2010)
Crotalus pusillus Klauber, 1952	1	EN	A	H (18)	1	2008	Reyes-Velasco et al. (2012)
Porthidium hespere (Campbell, 1976)	2, 3	DD	Pr	H (18)	1	1973	Campbell (1976)
Order Testudines (8)							
Family Cheloniidae (3)							
Caretta caretta (Linnaeus, 1758)	4	VU	P	NE	5	NA	UMMZ40350
Chelonia mydas (Linnaeus, 1758)	4	EN	P	NE	5	1905	CAS 8532
Lepidochelys olivacea (Eschscholtz, 1829)	4	VU	P	NE	5	1964	LACM 8111
Family Dermochelyidae (1)							
Dermochelys coriacea (Vandelli, 1761)	4	VU	P	NE	5	1971	AMNH R-172553
Family Geoemydidae (2)							
Rhinoclemmys pulcherrima (Gray, 1855)	1, 2	NE	NL	L (8)	3	1935	UMMZ 80348
Rhinoclemmys rubida (Cope, 1870)	1, 2	NT	Pr	H (14)	1	1902	CAS 14085
Family Kinosternidae (2)							
Kinosternon chimalhuaca Berry, Seidel &Iverson, 1997	2	LC	NL	H (16)	1	1997	Berry et al. (1997)
Kinosternon integrum LeConte, 1854	1, 2	LC	Pr	M (11)	1	1892	USNM 50990

Colima, near the border with the states of Jalisco and Michoacán. We are confident that with more samples in these areas with low accessibility they will be recorded in Colima, resulting in a much richer herpetological species list. This conclusion of a richer herpetofauna in Colima than currently documented is supported by the species accumulation curves we generated (Fig. 6). The species accumulation curves show a general and continuous increase in the number of species known in Colima during the first half of the 20th century followed by a plateau in the second half of the 20th century; however, there has been a rapid upturn in new species being documented in the 21st century, suggesting the total number of amphibians and reptiles in Colima is likely to be higher, perhaps substantially, than the 153 species we document here. These results make it clear that continued exploration and surveying of the amphibians and reptiles of Colima are needed to establish a firm understanding of their richness in the state.

General distribution

Twenty-six of the 39 species of amphibians that inhabit Colima are endemic to Mexico (Table 1). One to northern Colima near the state line with Jalisco at the Sierra de Manantlán (*Eleutherodactylus manatlanensis*). Seven are restricted to small areas in northern, eastern, or southern Colima and adjacent Jalisco or Michoacán, or both. Twelve are species typical of the Mexican Pacific, extending from northwestern Mexico (Chihuahua, Sonora or Sinaloa) to the Balsas Depression or even Oaxaca or Chiapas. Four have a wide distribution in northern and central Mexico, and two occur along

Table 2. Summary of native species present in Colima by Family, Order or Suborder, and Class. Status summary indicates the number of species found in each IUCN conservation status in the Order DD, LC, VU, NT, EN, CE (see Table 1 for abbreviations; in some cases species have not been assigned a status by the IUCN and therefore these may not add up to the total number of species in a taxon). Mean EVS is the mean Environmental Vulnerability Score, scores ≥ 14 are considered high vulnerability (Wilson et al. 2013a, b) and conservation status in Mexico according to SEMARNAT (2010) in the Order NL, Pr, A, P (see Table 1 for abbreviations).

Scientific name	Genera	Species	IUCN	mean EVS	SEMARNAT
Class Amphibia					
Order Anura	**17**	**35**	**1, 25, 2, 1, 0, 1**	**9.8**	**25, 8, 2, 0**
Bufonidae	3	5	0, 4, 0, 0, 0, 0	12	5, 0, 0, 0
Craugastoridae	1	2	1, 1, 0, 0, 0, 0	13	2, 0, 0, 0
Eleutherodactylidae	1	7	0, 1, 2, 0, 0, 1	15.5	4, 3, 0, 0
Hylidae	7	10	0, 9, 0, 0, 0, 0	9.1	8, 1, 1, 0
Leptodactylidae	1	2	0, 2, 0, 0, 0, 0	5.5	2, 0, 0, 0
Microhylidae	1	2	0, 2, 0, 0, 0, 0	5.5	1, 1, 0, 0
Phyllomedusidae	1	1	0, 1, 0, 0, 0, 0	13	1, 0, 0, 0
Ranidae	1	5	0, 4, 0, 1, 0, 0	7.4	1, 3, 1, 0
Scaphiopodidae	1	1	0, 1, 0, 0, 0, 0	6	1, 0, 0, 0
Order Caudata	**2**	**2**	**0, 1, 1, 0, 0, 0**	**11**	**0, 1, 1, 0**
Ambystomatidae	1	1	0, 1, 0, 0, 0, 0	10	0, 1, 0, 0
Plethodontidae	1	1	0, 0, 1, 0, 0, 0	12	0, 0, 1, 0
Order Gymnophiona	**1**	**1**	**1, 0, 0, 0, 0, 0**	**12**	**0, 1, 0, 0**
Caecilidae	1	1	1, 0, 0, 0, 0, 0	12	0, 1, 0, 0
Subtotal	**20**	**38**	**2, 26, 3, 1, 0, 1**	**10.0**	**25, 10, 3, 0**
Class Reptilia					
Order Crocodylia	**1**	**1**	**0, 0, 1, 0, 0, 0**	**14**	**0, 1, 0, 0**
Crocodylidae	1	1	0, 0, 1, 0, 0, 0	14	0, 1, 0, 0
Order Squamata	**62**	**103**	**8, 77, 1, 1, 3, 1**	**12**	**57, 32, 14, 0**
Suborder Lacertilia	**20**	**39**	**3, 30, 1, 0, 1, 0**	**12.1**	**24, 10, 5, 0**
Anguidae	3	3	0, 3, 0, 0, 0, 0	10	0, 3, 0, 0
Corytophanidae	2	2	0, 2, 0, 0, 0, 0	8	1, 1, 0, 0
Dactyloidae	1	1	0, 1, 0, 0, 0, 0	13	1, 0, 0, 0
Eublepharidae	1	1	0, 1, 0, 0, 0, 0	9	0, 0, 1, 0
Helodermatidae	1	1	0, 1, 0, 0, 0, 0	11	0, 0, 1, 0
Iguanidae	2	2	0, 1, 0, 0, 0, 0	13.5	1, 1, 0, 0
Phrynosomatidae	3	14	0, 11, 1, 0, 1, 0	13.1	12, 1, 1, 0
Phyllodactylidae	1	3	0, 3, 0, 0, 0, 0	13	2, 0, 1, 0
Scincidae	3	5	2, 2, 0, 0, 0, 0	11.4	4, 1, 0, 0
Teiidae	2	6	0, 5, 0, 0, 0, 0	12	3, 3, 0, 0
Xantusidae	1	1	1, 0, 0, 0, 0, 0	14	0, 0, 1, 0
Suborder Serpentes	**42**	**64**	**5, 47, 0, 1, 2, 1**	**11.9**	**33, 22, 9, 0**
Boidae	1	1	0, 0, 0, 0, 0, 0	15	1, 0, 0, 0
Colubridae	17	23	1, 19, 0, 0, 0, 1	11	13, 5, 5, 0
Dipsadidae	14	21	2, 18, 0, 0, 0, 0	11.4	12, 9, 0, 0
Elapidae	2	6	0, 6, 0, 0, 0, 0	13	2, 4, 0, 0
Leptotyphlopidae	2	2	0, 0, 0, 0, 0, 0	NE	2, 0, 0, 0
Loxocemidae	1	1	0, 1, 0, 0, 0, 0	10	0, 1, 0, 0
Natricidae	2	4	0, 2, 0, 0, 1, 0	11.3	2, 0, 2, 0
Viperidae	3	6	2, 1, 0, 1, 1, 0	16.5	1, 3, 2, 0
Order Testudines	**6**	**8**	**0, 2, 3, 1, 1, 0**	**12.3**	**2, 2, 0, 4**
Cheloniidae	3	3	0, 0, 2, 0, 1, 0	NE	0, 0, 0, 3
Dermochelyidae	1	1	0, 0, 1, 0, 0, 0	NE	0, 0, 0, 1
Geoemydidae	1	2	0, 0, 0, 1, 0, 0	11	1, 1, 0, 0
Kinosternidae	1	2	0, 2, 0, 0, 0, 0	13.5	1, 1, 0, 0
Subtotal	**69**	**112**	**8, 79, 5, 2, 4, 1**	**12**	**59, 35, 14, 4**
Total	**89**	**150**	**10, 105, 8, 3, 4, 2**	**11.5**	**84, 45, 17, 4**

Table 3. List of amphibians and reptiles that potentially occur in Colima.

Taxon	Explanation
Class Amphibia	
Order Anura	
Bufonidae	
Incilius perplexus (Taylor, 1943)	Likely to occur in eastern Colima
Craugastoridae	
Craugastor augusti (Dugès, 1879)	Likely to occur in extreme western Colima
Craugastor hobartsmithi (Taylor, 1937)	Likely to occur in extreme western Colima
Craugastor pygmaeus (Taylor, 1937)	Likely to occur in northwestern and southern Colima
Hylidae	
Sarcohyla bistincta (Cope, 1877)	Likely to occur through the state but the coastal area
Ranidae	
Rana megapoda Taylor, 1942	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Rana psilonota Webb, 2001	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Order Caudata	
Plethodontidae	
Pseudoeurycea leprosa (Cope, 1869)	Likely to occur in southern Colima
Class Reptilia	
Order Squamata	
Suborder Lacertilia	
Phrynosomatidae	
Sceloporus grammicus Wiegmann, 1828	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Sceloporus heterolepis Boulenger, 1895	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Order Squamata	
Suborder Serpentes	
Colubridae	
Salvadora bairdi Jan, 1860	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Sonora mutabilis Stickel, 1943	Likely to occur in northeastern-eastern Colima
Dipsadidae	
Geophis bicolor Günther, 1868	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Geophis nigrocinctus Duellman, 1959	Likely to occur in northern Colima
Geophis petersi Boulenger, 1894	Likely to occur in northern Colima
Geophis tarascae Hartweg, 1959	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Viperidae	
Crotalus armstrongi (Campbell, 1979)	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Crotalus culminatus Klauber, 1952	Likely to occur in southern Colima, near the border with Coahuayana, Michoacán
Crotalus polystictus (Cope, 1865)	Likely to occur in northeastern Colima, in the Volcanes de Colima physiographic region
Order Testudines	
Cheloniidae	
Eretmochelys imbricata (Linnaeus, 1766)	Likely to occur in the coastline of the state

both coasts of Mexico. Of the 14 species not endemic to Mexico, four are distributed from the US to central or southern Mexico, four are distributed from the US to Central or South America, four are found in Mexico and Central or South America, and one is an introduced species, the American Bullfrog (*Rana catesbeiana*).

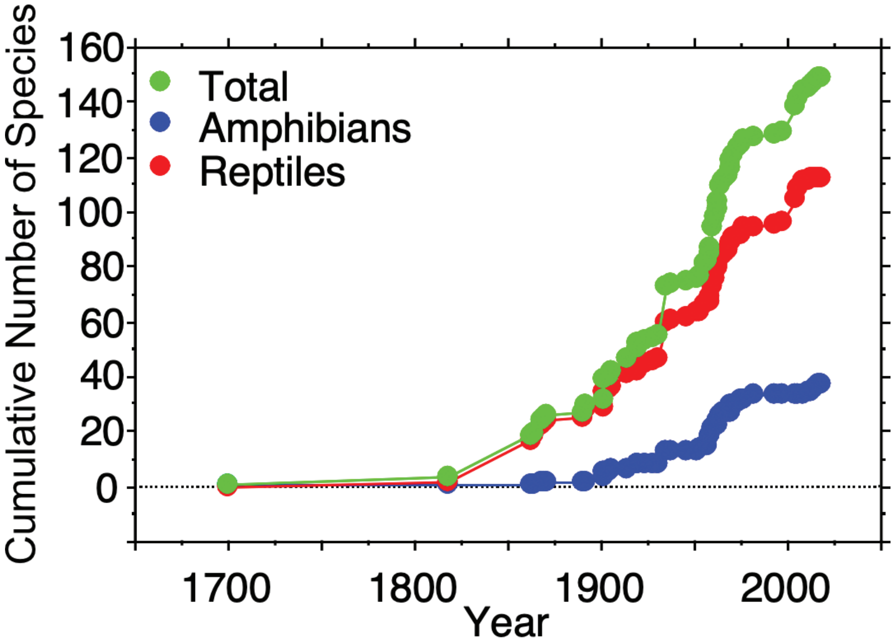

Figure 6. Species accumulation curves for total herpetofauna, amphibians, and reptiles of Colima, Mexico.

The American Crocodile (*Crocodylus acutus*) is widely distributed from the eastern US to South America, including the Caribbean. Two of the 41 species of lizards that occur in Colima are endemic to islands of the Revillagigedo Archipielago (*Urosaurus auriculatus* on Socorro Island and *U. clarionensis* on Clarion Island), and 24 are endemic to Mexico (Table 1). Of the 15 lizard species not endemic to Mexico that inhabit Colima, two are introduced, one is found in the US and Mexico, one is found from the US to Central America, and 11 have a wide distribution that includes Mexico and Central or South America (Table 1). Two of the 64 species of snakes found in Colima are endemic to Isla Clarion in the Revillagigedo Archipielago (*Masticophis anthonyi* and *Hypsiglena unaocularis*) (Table 1). Forty-two of the snake species found in Colima are endemic to Mexico. Of the 22 snake species not endemic to Mexico that occur in Colima, three are distributed from the US to Mexico, four from the US to Central or South America, 12 from Mexico to Central or South America, and one marine species is distributed in the Pacific and Indian Oceans (Table 1). Three of the eight species of turtles found in Colima are endemic to Mexico (Table 1). One is distributed from Mexico to Central America, and four are sea turtles that have a circumglobal distribution (Table 1).

Conservation status

Of the amphibians and reptiles found in Colima, 12.9% are IUCN listed (i.e., Vulnerable, Near Threatened, Endangered, or Critically Endangered), and 14.0% are placed

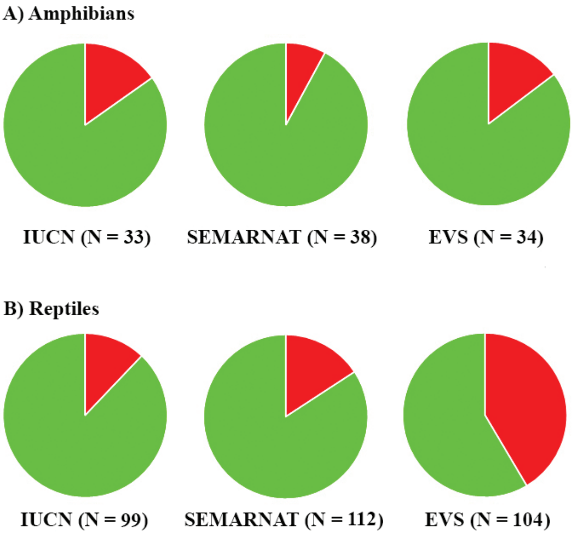

Figure 7. Proportion of **A** amphibians and **B** reptiles listed in protected categories on the IUCN Red List, SEMARNAT, and high EVS. Green is proportion in Data Deficient and Least Concern (IUCN); Not Listed and Subject to Special Protection (we regarded the category of Subject to Special Protection in SEMARNAT equivalent to Least Concern in IUCN) (SEMARNAT); or low or medium EVS. Red is percentage in protected categories or high EVS. N is the number of species assessed.

in a protected category by SEMARNAT (excluding NL and Pr, this last category is equivalent to the LC category of IUCN), and 34.1% are categorized as high risk by the EVS (Tables 1, 2). For amphibians, 15.2% are IUCN listed, 7.9% are protected by SEMARNAT, and 14.7% are at high risk according to the EVS (Fig. 7; Tables 1, 2). For reptiles, 12.1% are listed by the IUCN, 16.1% are protected by SEMARNAT, and 40.4% are at high risk according to the EVS (Fig. 7; Tables 1, 2). These results suggest that the herpetofauna as a whole of Colima is considered to be a relatively low conservation concern at a global scale (i.e., IUCN listing) and national level (i.e., SEMARNAT listing, EVS). However, the EVS categories suggest that, at a national level, the reptiles of Colima are at higher risk than the other assessments suggest and are at higher risk than the amphibians of Colima. In addition, there are several specific taxa that, based on their IUCN listing, SEMARNAT category, or their EVS, are of conservation concern. These include species in the families Eleutherodactylidae, Ranidae, Plethodontidae, Crocodylidae, Eublepharidae, Helodermatidae, Iguanidae, Phrynosomatidae, Phyllodactylidae, Xantusidae, Colubridae, Natricidae, Viperidae, Cheloniidae, and Dermochelyidae (Tables 1, 2). In particular, the family Eleutherodactylidae in Colima is of great conservation concern; this family has seven species, three of them are IUCN listed and are at great risk according to their EVS (*Eleutherodactylus angustidigitorum*, *E. modestus*, and *E. rufescens*). Another three have not been evaluated by the IUCN or EVS due to their recent description (*E. colimotl*, *E. grunwaldi*, and *E. manantlanensis*); however, due to their limited distribution it is almost certain that once they are evaluated, they will be considered in some category of the IUCN and with a high risk

EVS, therefore the family Eleutherodactylidae in Colima would be represented by six species (86% = 6/7) at high conservation risk. Because the summarized conservation statuses are global or national-level assessments, the conservation status of at least some species of amphibians and reptiles in Colima are probably not accurately assessed by these measures. Additional assessments at the state level will be required to establish conservation or management needs for the herpetofauna of Colima.

Using the data in Table 1, we summarized the conservation status of amphibian and reptile taxa in each physiographic region found in Colima. For IUCN listing, 13.8% of the amphibians in the Volcanes de Colima physiographic region are listed; 10.0% in the Sierras de la Costa de Jalisco y Colima; and none in the Cordillera Costera del Sur. For SEMARNAT categories, 3.4% of amphibian species in the Volcanes de Colima are listed; 6.7% in the Sierras de la Costa de Jalisco y Colima; and 9.1% in the Cordillera Costera del Sur. For EVS, 13.8% of the amphibians in the Volcanes de Colima in the high-risk category; 13.3% in the Sierras de la Costa de Jalisco y Colima, and 18.2% in the Cordillera Costera del Sur. For IUCN listings, relatively few species of reptiles are placed in the protected categories for most of the physiographic regions (Volcanes de Colima, 7.8%; Sierras de la Costa de Jalisco y Colima, 3.4%; Cordillera Costera del Sur, 0%). Reptiles in the Marine (80%) and Revillagigedo Archipelago (75%) regions show relatively high percentages of species in protected categories. Similar patterns hold for SEMARNAT listings with 12.5% of reptiles in the Volcanes de Colima, 8.0% from Sierras de la Costa de Jalisco y Colima, and 12.5% from the Cordillera Costera del Sur, 80% in the Marine region, and 25% in the Revillagigedo Archipelago region in the protected SEMARNAT categories. For the EVS assesments of reptile species, 31.3% were in the high category in Volcanes de Colima, 35.6% in the Sierras de la Costa de Jalisco y Colima, 41.7% in the Cordillera Costera del Sur. None of the five species in the marine region were evaluated for EVS, and 75% of the species in the Revillagigedo Archipelago were in the high EVS category. Based on our summary of conservation status, the reptiles in the Marine and Revillagigedo Archipelago regions are the most threatened taxa of the Colima herpetofauna.

Comparison with neighboring states

For amphibians, Colima shares 92.1% of its species with Jalisco, and it shares 86.8% of its species with Michoacán (Table 4). Species in eight of the 12 families of amphibians present in Colima are fully shared with Jalisco and Michoacán. The percentage of shared reptile species is slightly smaller; however, overlap in species lists is still very high. Colima shares 84.8% of its reptile species with Jalisco, and 82.1% with Michoacán. Species in 13 of the 25 families of reptiles present in Colima are fully shared with Jalisco and Michoacán. Only ten of the species found in Colima (two amphibians and eight reptiles) do not occur in either Jalisco or Michoacán, four of which are species endemic to the Revillagigedo Archipelago (*Urosaurus auriculatus*, *U. clarionensis*, *Masticophis anthonyi*, and *Hypsiglena unaocularus*), one is endemic to northern Colima (*Eleutherodactylus manantlanensis*), three

Table 4. Summary of the numbers of species shared between Colima and neighboring Mexican states (not including introduced species). The percent of Colima species shared by a neighboring state are given in parentheses. Total refers to the total number of species found in Colima and two neighboring states (i.e., regional species pool) and the number in parentheses in this column is the percent of the regional species pool found in Colima. – indicates either Colima or the neighboring state has no species in the taxonomic group, thus no value for shared species is provided.

Taxon	Colima	Jalisco	Michoacán	Total
Class Amphibia	38	35 (92.1)	33 (86.8)	73 (52.1)
Order Anura	35	32 (91.4)	30 (85.7)	61 (57.4)
Bufonidae	5	5 (100)	4 (80)	10 (50)
Craugastoridae	2	2 (100)	2 (100)	5 (40)
Eleutherodactylidae	7	6 (85.7)	5 (71.4)	15 (46.6)
Hylidae	10	9 (90.0)	8 (80.0)	13 (76.9)
Leptodactylidae	2	2 (100)	2 (100)	2 (100)
Microhylidae	2	2 (100)	2 (100)	2 (100)
Phyllomedusidae	1	1 (100)	1 (100)	1 (100)
Ranidae	5	4 (80)	5 (100)	11 (45.5)
Rhinophrynidae	0	–	0 (0)	1 (0)
Scaphiopodidae	1	1 (100)	1 (100)	1 (100)
Order Caudata	2	2 (100)	2 (100)	11 (18.2)
Ambystomatidae	1	1 (100)	1 (100)	8 (12.5)
Plethodontidae	1	1 (100)	1 (100)	3 (33.3)
Order Gymnophiona	1	1 (100)	1 (100)	1 (100)
Caecilidae	1	1 (100)	1 (100)	1 (100)
Class Reptilia	112	95 (84.8)	92 (82.1)	211 (53.1)
Order Crocodylia	1	1 (100)	1 (100)	1 (100)
Crocodylidae	1	1 (100)	1 (100)	1 (100)
Order Squamata	103	86 (83.5)	85 (82.5)	198 (52)
Suborder Lacertilia	39	30 (76.9)	30 (76.9)	77 (50.6)
Anguidae	3	3 (100)	3 (100)	7 (42.9)
Corytophanidae	2	1 (50)	1 (50)	2 (100)
Dactyloidae	1	1 (100)	1 (100)	2 (50)
Eublepharidae	1	1 (100)	1 (100)	1 (100)
Helodermatidae	1	1 (100)	1 (100)	1 (100)
Iguanidae	2	2 (100)	2 (100)	3 (66.7)
Phrynosomatidae	14	11 (78.6)	9 (64.3)	34 (41.2)
Phyllodactylidae	3	1 (33.3)	2 (66.7)	6 (50)
Scincidae	5	4 (80)	4 (80)	10 (50)
Teiidae	6	5 (83.6)	5 (83.6)	9 (66.7)
Xantusidae	1	0 (0)	1 (100)	2 (50)
Suborder Serpentes	64	56 (87.5)	55 (85.9)	121 (52.9)
Boidae	1	1 (100)	1 (100)	1 (100)
Colubridae	23	19 (82.6)	19 (82.6)	39 (59)
Dipsadidae	21	20 (95.2)	19 (90.5)	41 (51.2)
Elapidae	6	5 (83.6)	4 (66.7)	7 (85.7)
Leptotyphlopidae	2	1 (50)	2 (100)	4 (50)
Loxocemidae	1	1 (100)	1 (100)	1 (100)
Natricidae	4	4 (100)	4 (100)	12 (33.3)
Viperidae	6	5 (83.3)	5 (83.3)	16 (37.5)
Order Testudines	8	8 (100)	6 (75)	12 (66.7)
Cheloniidae	3	3 (100)	2 (66.7)	4 (75)
Dermochelyidae	1	1 (100)	1 (100)	1 (100)
Emydidae	0	0 (0)	–	2 (0)
Geoemydidae	2	2 (100)	2 (100)	2 (100)
Kinosternidae	2	2 (100)	1 (50)	3 (66.7)
Total	**150**	**130 (86.7)**	**125 (83.3)**	**284 (52.8)**

have spotty distributions along the Pacific Coast of Mexico (*Phyllodactylus tuberculosus*) or in south-southeastern Mexico (*Laemanctus longipes* and *Aspidoscelis guttatus*), and two have isolated records in Colima, with the bulk of their distribution in southeastern Mexico (*Exerodonta sumichrasti* and *Salvadora lemniscata*). The high level of similarity in the herpetofauna between Colima and its two neighbors is due in part to the small size of Colima compared with each one of these two state (7.2% of Jalisco, 9.6% of the Michoacán). In addition, and perhaps more importantly, Colima is completely surrounded by Jalisco and Michoacán, and shares the same physiographic regions and habitat types with them.

Conclusions

Colima is home to a rich herpetofauna, especially relative to its small size, and is likely richer than currently known. Its herpetofauna contains a relatively high number of species that are endemic to Mexico, and thus is an important state for the Mexican herpetofauna. Based on IUCN and SEMARNAT listings, the conservation status of the amphibians and reptiles would appear to be relatively low, but the EVS assessments suggest this may not be a completely accurate impression. Of particular concern are the marine species and those species found in the Revillagigedo Archipelago. Colima shares the vast majority of its species with the neighboring states of Jalisco and Michoacán, suggesting that these three states may make a useful unit for understanding and creating conservation and management plans and strategies for their amphibians and reptiles.

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Appendix I

Museum collections included in the CONABIO database examined for records of Colima amphibians and reptiles or that house specimens of the first record of a species in Colima.

AMNH	Collection of Herpetology, Herpetology Department, American Museum of Natural History
ASNHC	Amphibians and Reptiles Collection, Angelo State Natural History Collection

CAS	Collection of Herpetology, Herpetology Department, California Academy of Sciences
CMNH	Collection of Herpetology, Amphibian and Reptile Section, Carnegie Museum of Natural History, Pittsburgh
FMNH	Division of Amphibians and Reptiles, Field Museum of Natural History
FSM-UF	Collection of Herpetology, Florida State Museum, University of Florida
LACM	Collection of Herpetology, Herpetology Section, Natural History Museum of Los Angeles County
LSUMZ	Collection of Herpetology, Museum of Zoology, Biological Science Division, Louisiana State University
MCZ	Collection of Herpetology, Museum of Comparative Zoology, Harvard University Cambridge
MNHN	Collection of Reptiles and Amphibians, Muséum National D'Histoire Naturelle
MNHUK	Museum of Natural History, Division of Herpetology, University of Kansas
MVZ	Collection of Herpetology, Museum of Vertebrate Zoology, Division of Biological Sciences, University of California Berkeley
MZFC-UNAM	Colección Herpetológica, Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias UNAM
NLU	Northeastern Louisiana University
TNHC	Collection of Herpetology, Texas Natural History Collection, University of Texas Austin
UAZ	Amphibians and Reptiles Collections, University of Arizona
UCM	Collection of Herpetology, University of Colorado Museum
UMMZ	Collection of Herpetology, Museum of Zoology, University of Michigan Ann Arbor
USNM	Collection of Herpetology, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution
UTEP	Collection of Herpetology, Laboratory of Environmental Biology, Biological Science Department, University of Texas – El Paso

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A new species of cryptic Bush frog (Anura, Rhacophoridae, *Raorchestes*) from northeastern Bangladesh

Hassan Al-Razi[1], Marjan Maria[1], Sabir Bin Muzaffar[2]

1 *Faculty of Life and Earth Science, Department of Zoology, Jagannath University, Dhaka, Bangladesh*
2 *Department of Biology, United Arab Emirates University, Al Ain, United Arab Emirates*

Corresponding author: Hassan Al-Razi (chayan1999@yahoo.com)

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Abstract

Raorchestes is a speciose genus of bush frogs with high diversity occurring in the Western Ghats of India. Relatively fewer species have been recorded across India, through Bangladesh, southern China, into Vietnam and Peninsular Malaysia. Many bush frogs are morphologically cryptic and therefore remain undescribed. Here, a new species, *Raorchestes rezakhani* **sp. nov.**, is described from northeastern Bangladesh based on morphological characters, genetics, and bioacoustics. The 16S rRNA gene distinguished this species from 48 known species of this genus. Bayesian Inference and Maximum Likelihood analyses indicated that the new species was most similar to *R. tuberohumerus*, a species found in the Western Ghats, and to *R. gryllus*, a species found in Vietnam. Bioacoustics indicated that their calls were similar in pattern to most *Raorchestes* species, although number of pulses, duration of pulses, pulse intervals and amplitude differentiated it from a few other species. It is suggested that northeastern India, Bangladesh, northern Myanmar, and southern China represent important, relatively unexplored areas that could yield additional species of *Raorchestes*. Since many remaining habitat patches in Bangladesh are under severe threat from deforestation, efforts should be made to protect these last patches from further degradation.

Keywords

Amphibian, bush frog, DNA, herpetofauna, *Raorchestes rezakhani* sp. nov.

Introduction

Raorchestes Biju et al., 2010, is a genus of bush frogs belonging to the family Rhacophoridae, that extends in distribution from southwestern India through northeastern India, Bangladesh, Myanmar, southern China, and into Laos, Vietnam, and Peninsular Malaysia (Biju and Bossuyt 2009; IUCN 2016; Vijayakumar et al. 2016; Frost 2019). The genus is particularly speciose in the Western Ghats of India, where more than 50 of the 63 recorded species occur (Biju et al. 2010; Vijayakumar et al. 2014; Priti et al. 2016; Vijayakumar et al. 2016; Boruah et al. 2018). In addition, a few species have been recorded from the Eastern Ghats, Eastern Himalayas, and northeastern India, southern China and adjoining regions (Vijayakumar et al. 2016; Boruah et al. 2018; Wu et al. 2019, Frost 2020). Many species in the genus are cryptic (morphologically difficult to distinguish from congenerics) and, as a result, remain undescribed (Priti et al. 2016; Vijayakumar et al. 2016; Boruah et al. 2018; Wu et al. 2019). The genus is characterized within the Rhacophoridae by small size (15–45 mm snout-vent length), absence of vomerine teeth, transparent gular pouch, and direct development (Biju et al. 2010). Their advertisement calls consists of repetitive 'treenk.. treenk.. treenk' with variation in number of pulses, duration of calls, interval between calls and amplitude, which may be used to distinguish between species (Priti et al. 2016). Thus, an integrative approach using morphological traits, bioacoustics and molecular variation has been used to distinguish cryptic species (Priti et al. 2016; Boruah et al. 2018).

Bangladesh falls within the Indo-Malayan realm, with forests classified as tropical moist, tropical evergreen and several other less-extensive forest types (Champion and Seth 1968; Slik et al. 2018). Broad similarities exist between forest patches in northeastern and southeastern Bangladesh and the surrounding Indian States of Meghalaya, Tripura, Mizoram and Nagaland, and adjoining northern Myanmar and southern China (Slik et al. 2018). Three species of *Raorchestes*, namely the Darjeeling bush frog *Raorchestes annandalii* (Boulenger, 1906), the Karin bubble-nest frog *R. parvulus* (Boulenger, 1893) and, most recently, the Longchuan bush frog *R. longchuanensis* (Yan and Li 1978) have been recorded from Bangladesh (Ghose and Bhuiyan 2012; IUCN 2015; Khan 2015; Al Razi et al. 2020). *Raorchestes parvulus* has a distribution from northeastern India, Bangladesh through Southeast Asia extending up to Vietnam and Peninsular Malaysia (Khan 2015; IUCN 2015; IUCN-SSC 2016). In fact, *R. parvulus* has been confused with *R. longchuanensis* reported originally from southern China (Frost 2020) and recently from northeastern Bangladesh (Al-Razi et al. 2020). Furthermore, Thai populations of *R. parvulus* are possibly separate species (Frost 2020). Thus, *R. parvulus* has been regarded as members of a species complex (Khan 2015; IUCN 2015; IUCN-SSC 2016). It has been speculated that *R. longchuanensis* has a wider distribution in northeastern India and northern Myanmar (Al-Razi et al. 2020). *Raorchestes annandalii*, on the other hand, has a more restricted distribution in southeastern Bangladesh, northeastern India, and Nepal (Bardoloi et al. 2004; IUCN 2015). The region of northeastern India that surrounds Bangladesh hosts at least four species of *Raorchestes* and other related genera. This suggests that the region represent a zone of diversification of bush frogs. It is possible that *R. annandalii* represents a northern complex of related species and *R. parvulus* are part of a more southern species complex (Frost 2020). Here we describe a new species of bush frog from northeastern Bangladesh based on bioacoustics, morphology, and molecular characterization.

Materials and methods

Study area

We conducted this study in Adampur Reserve Forest (24°13.410'N, 91°54.836'E) and Lawachara National Park (24.330755N, 91.789396E), two small forest patches of northeastern Bangladesh (Fig. 1). Both forests are semi-evergreen, and local climate and hydrologic patterns are similar, but their sizes and disturbance patterns differ (Quazi and Ticktin 2016). The topography of the study area is hilly, with elevations ranging from 50–100 m a.s.l. (Islam et al. 2007). Annual temperature ranges from 9 °C (January) to 32 °C (August-October), and nearly 80% of the annual average rainfall (3,334 mm) occurs between the months of May and October (Quazi and Ticktin 2016). Numerous streams and swampy areas crisscross the region. The landscape is categorized into hill forests, scrublands, and mixed bamboo forests (IUCN 2015). Northeast Bangladesh shares an international border with India, and two of the Indian states, Tripura, and Assam, are adjacent to northeast Bangladesh (Fig. 1).

Specimen collection

We collected four adult calling males from April to October 2019. We euthanized and fixed the specimens in 95% ethanol for 5 hrs and stored them in 70% ethanol. We tentatively designated the specimens to the genus *Raorchestes* based on small size (18.85–20.90 mm snout-vent length), absence of vomerine teeth, transparent gular pouch, and advertisement calls consisting of repetitive 'treenk.. treenk.. treenk' which is characteristic of *Raorchestes* (following Biju et al. 2010). Thigh-muscle samples for genetic analysis were collected before fixing the specimen. We recorded the color of living specimens and recorded natural history observations at the type locality during specimen collections. As the frog was very small, cryptic, and very difficult to find, we were able to collect only four specimens. We deposited the specimens in the Shahid Rafique Special Specimen Collection (**SRSSC**), Department of Zoology, Jagannath University, Dhaka. Since the SRSSC is a newly established part of the Zoological Museum, the catalogue numbers for specimens retain the original codes adopted by the Zoological Museum, namely JnUZool.

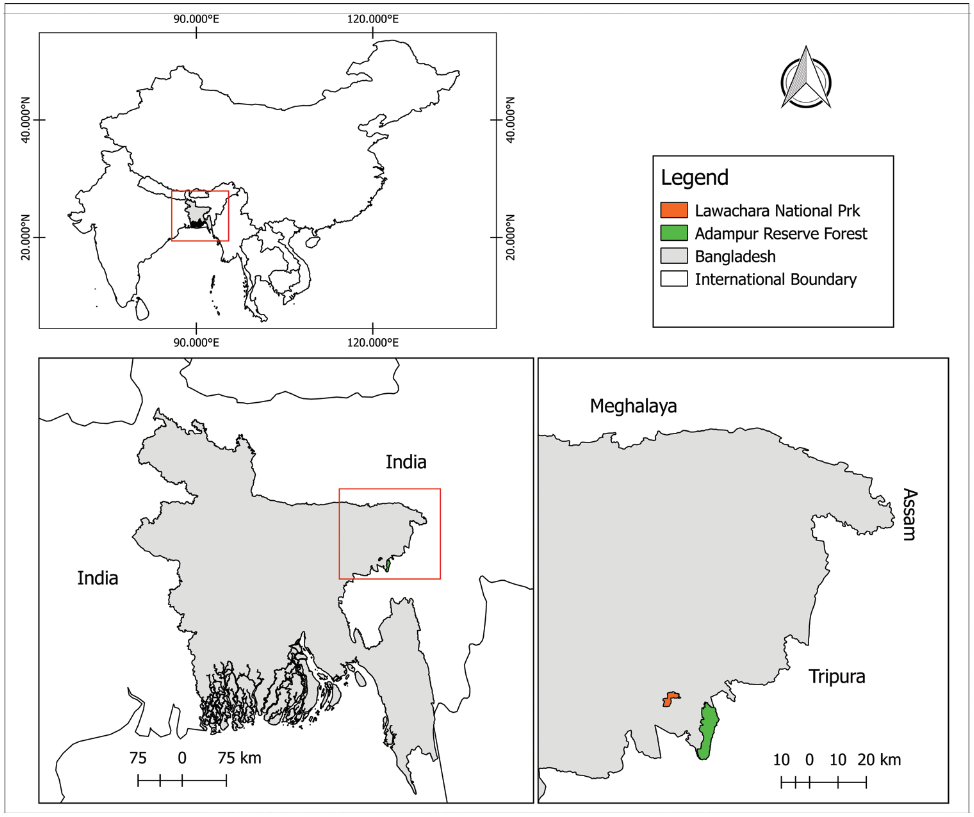

Figure 1. Map showing the type location of *Raorchestes rezakhani* sp. nov. in northeastern Bangladesh as well as adjoining areas.

Morphometrics

We measured the following from the left side of the specimens with digital calipers (to the nearest 0.10 mm):

ED	eye diameter (horizontal diameter of the eye);
EN	eye-nostril distance (distance between anterior canthus of eye and the posterior edge of nostril);
FD I to IV	width of 1st to 4th finger disks (measured at the widest point on the finger disk);
FL I to FL IV	lengths of 1st to 4th fingers (from the tip of the respective finger to where it; connects with the palm);
FOL	foot length (from the distal end of tarsus tip of Toe IV);
HAL	hand length (from distal end of radioulna to tip of distal finger III);
HL	head length (distance between tip of the snout to the rear of the mandible);
HW	head width (at angle of jaw);
IND	internarial distance (least distance between inner edge of the nostrils);

IOD	interorbital distance (least distance between proximal edges of upper eyelids);
NS	nostril-Snout distance (distance from the anterior edge of nostril to the tip of the snout);
ShL	shank length (distance between knee and heel);
SL	snout length (from anterior canthus of eye to tip of snout);
SVL	snout-vent length (from tip of snout to vent);
TD	tympanum diameter (maximum diameter of the tympanum);
TD I to V	width of 1st to 5th toe disks (the greatest horizontal distance between the edges of toe disks);
TL	thigh length (distance from the middle of vent to knee);
TL I to V	lengths of 1st to 5th toes (from base of proximal subarticular tubercle to tip of the respective toe);
UEW	upper eyelid width (maximum transverse distance of the upper eyelid.

We compared morphological characters based on morphometric measurements provided in the following published papers (Al-Razi et al. 2020; Kuramoto and Joshy 2003; Padhye et al. 2015; Orlov et al. 2012):

In addition, we compared eleven linear, morphometric variables of four species using Principle Components Analysis (PCA) (McGarigal et al. 2000; Sokal and Rohlf 2012), using PAST (version 3.8). All linear morphometric variables were transformed by subtracting each variable from the mean of that variable (McGarigal et al. 2000; Sokal and Rohlf 2012). We derived eleven principle components, since there were eleven variables, each representing a linear combination of all eleven variables. We generated Eigenvalues and their relative weightings to determine the relative contribution of the variables towards each principle component (McGarigal et al. 2000). Loadings of each of the eleven variables in relation to each of the eleven principle components were used to determine relative effect of individual morphological characters on each principle component. We visualized the differences in the species compared using a scatter plot of principle components that explained the greatest variance in the data (McGarigal et al. 2000).

DNA Extraction and amplification

We extracted DNA from the muscle samples using a standard protocol described in Vences et al. (2012) for DNA extraction. We amplified mitochondrial 16S ribosomal RNA gene. The PCR amplification and sequencing of the 16S rRNA gene were done following Palumbi et al. (1991) and Bossuyt et al. (2004) respectively. We used primers 5’ -GCCTGTTTATCAAAAACAT-3’ (16Sar-L) and 5’ -CCGGTCTGAACTCAGATCACGT-3’ (16Sbr-H) as forward and reverse primers for 16S (Palumbi et al. 1991) for this study. We performed PCR amplifications in a 20 µl reaction volume; Master Mix 10 µl, T DNA (Concentration 25–65 ng/ µl) 1 µl, Primer F (Concentration 10–20 pMol) 1 µl, Primer R (Concentration 10–20 pMol) 1 µl and nuclease-free water 7 µl with the following cycling conditions: an initial denaturing step at 95 °C for 3 min; 40 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s and extending at 72 °C for 45 s, and a final extension step of 72 °C for 5 min. We sent the amplified product to First Base Laboratories, Malaysia for sequencing. The sequences were checked manually using the program Chromas lite 2.01 (http://www.technelysium.com.au/chromas_lite.html). The sequences were submitted to GenBank (Accession no: MN072374, MN072375, MN615901, MN615902).

Phylogenetic analyses

We compared the new sequences to the GenBank sequences using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to confirm their genetic identity and determine similar species that allow the evaluation of the phylogenetic position of the new taxon. Homologous sequences of other *Raorchestes* species were obtained from GenBank (Table 1). *Kurixalus eiffingeri* Boettger, 1895 was selected as outgroup based on Yu et al. (2013). Sequences were aligned using the MUSCLE tool in MEGA 7 (Kumar et al. 2016), alignments were checked visually, and both ends of the sequence were trimmed to avoid low quality base pairs. Alignment gaps were treated as missing data. The best substitution model (GTR+I+G) was selected using the Akaike Information Criterion (AIC) and Bayesian information criteria (BIC) in jModelTest v2.1.2. Maximum likelihood phylogenetic analyses were performed using the RAxML v4.0 Geneious plugin (Stamatakis 2006) with 1,000 bootstrap replicates. Bayesian phylogenetic inference analysis were performed in MrBayes 3.2.4 (Ronquist et al. 2012). We performed an MCMC Bayesian analysis that consisted of two simultaneous runs of 1 million generations and sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs). The trees were visualized and edited in FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree). Additionally, pairwise genetic distances (uncorrected *p*) of 21 species under the genus *Raorchestes* including the new species were calculated for 16S using MEGA 7.0 (Kumar et al. 2016).

Call recording and analysis

The call of a single male individual (JnUZool- A0519) was recorded with a Sony ICD-PX240 digital sound recorder with sampling rate of 48 kHz and 32-bit resolution on 10 May 2019. The device was approximately 1–1.5 m away from the calling male. Air temperature and humidity were taken by a digital hygrometer. For the call analysis we used Raven Pro Ver. 1.5 (Charif et al. 2010; Bioacoustics Research Program 2011). We measured call-group duration, inter-call group interval, duration of intervals between pulses, call duration, pulse rate and dominant frequency comprising of 25 call groups.

Table 1. Species of *Raorchestes* and the outgroup and their associated GenBank accession numbers that were used in the phylogenetic analysis.

	Species	Location	Voucher	GenBank16S rRNA accession numbers	Source
1	*R. rezakhani* sp. nov.	Maulovibazar, Bangladesh	JnUZool-A0319	MN072374	This study
2	*R. rezakhani* sp. nov.	Maulovibazar, Bangladesh	JnUZool-A0419	MN072375	This study
3	*R. rezakhani* sp. nov.	Maulovibazar, Bangladesh	JnUZool-A0619	MN615901	This study
4	*R. rezakhani* sp. nov.	Maulovibazar, Bangladesh	JnUZool-A0519	MN615902	This study
5	*R. longchuanensis*	Habigonj, Bangladesh	JnUZool-A0317	MN193414	Al-Razi et al. 2020
6	*R. longchuanensis*	Habigonj, Bangladesh	JnUZool-A0117	MN193412	Al-Razi et al. 2020
7	*R. ghatei*	Satara, Maharashtra, India	WILD-AMP-13-100	KF366385	Padhye et al. 2013
8	*R. ghatei*	Satara, Maharashtra, India	ZSI-WRC A/1484	KF366384	Padhye et al. 2013
9	*R. ghatei*	Satara, Maharashtra, India	WILD-AMP-13-104	KF366387	Padhye et al. 2013
10	*Raorchestes* sp. R3	Riwai, Meghalaya,India	–	MG980284	Boruah et al. 2018
11	*Raorchestes* sp. R4	Mawlynong, Meghalaya,India	–	MG980285	Boruah et al. 2018
12	*R. shillongensis*	Malki forest, Meghalaya,India	–	MG980282	Boruah et al. 2018
13	*R. shillongensis*	Malki forest, Meghalaya,India	–	MG980283	Boruah et al. 2018
14	*R. gryllus*	Pac Ban, Vietnam	ROM30288	GQ285674	Li et al. 2009
15	*R. menglaensis*	Yunnan, China	KIZ060821286	EU924621	Yu et al. 2009
16	*R. bombayensis*	Uttara Kannada, Karnataka, India	1362PhiBom	EU450019	Biju and Bossuyt 2009
17	*R. bombayensis*	Uttara Kannada, Karnataka, India	WILD-13-AMP-230	KF767502	Padhye et al. 2013
18	*R. tuberohumerus*	Western Ghats, India	CESF424 16S	KM596574	Vijayakumar et al. 2009
19	*R. tuberohumerus*	Western Ghats, India	0073PhiTub	EU450004	Biju and Bossuyt 2009
20	*R. sanctisilvaticus*	Eastern Ghats, India	SKD244	MH915511	Mirza et al. 2019
21	*R. sanctisilvaticus*	Eastern Ghats, India	SKD240	MH915509	Mirza et al. 2019
22	*R. ponmudi*	Western Ghats, India	1451PhiPonb	EU450026	Biju and Bossuyt 2009
23	*R. ponmudi*	Western Ghats, India	1121PhiPon	EU450011	Biju and Bossuyt 2009
24	*R. ponmudi*	Western Ghats, India	0030PhiBed	EU449998	Biju and Bossuyt 2009
25	*R. indigo*	Western Ghats, India	CESF138	KM596557	Vijayakumar et al. 2009
26	*R. parvulus*	southern Yunnan, China	KIZ 20160374	MK564634	Yu et al. 2019
27	*R. parvulus*	southern Yunnan, China	KIZ 20160366	MK564630	Yu et al. 2019
28	*R. theuerkaufi*	Western Ghats, India	CESF1342	JX092693	Vijayakumar et al. 2009
29	*R. signatus*	Western Ghats, India	CESF1666	KM596562	Vijayakumar et al. 2009
30	*R. signatus*	Western Ghats, India	CESF1662	KM596561	Vijayakumar et al. 2009
31	*R. tinniens*	Munnar, Kerala, India	SDBDU2010.274	KU169991	Biju et al. 2016
32	*R. tinniens*	Western Ghats, India	0058PhiTin	EU450001	Biju and Bossuyt 2009
33	*R. marki*	Western Ghats, India	CESF467	JX092719	Vijayakumar et al. 2009
34	*R. chromasynchysi*	Western Ghats, India	CESF1127	JX092667	Vijayakumar et al. 2009
35	*R. chromasynchysi*	Western Ghats, India	CESF1203	KM596543	Vijayakumar et al. 2009
36	*R. charius*	Karnataka, India	SDBDU2011.814	KU169985	Biju et al. 2016
37	*R. charius*	Sri Lanka	–	AY141840	Meegaskumbura et al. 2002
38	*R. primarrumfi*	Western Ghats, India	CESF442	KM596575	Vijayakumar et al. 2009
39	*R. chalazodes*	Western Ghats, India	BRA-2014	KJ619643	Unpublished
40	*R.* sp.	Western Ghats, India	CESF403	JX092710	Vijayakumar et al. 2009
41	*R.* sp.	Western Ghats, India	CESF427	JX092714	Vijayakumar et al. 2009
42	*R.* sp.	Western Ghats, India	SPV-2014b	KM596563	Vijayakumar et al. 2009
43	*R. lechiya*	Western Ghats, India	CB-2015a	KT359622	Zachariah et al. 2016
44	*R. lechiya*	Western Ghats, India	CB-2015a	KT359623	Zachariah et al. 2016
45	*R.* sp.	Western Ghats, India	SPV-2014b	KM596563	Vijayakumar et al. 2009
46	*Kurixalus eiffingeri*	Okinawa Islands, Japan	A120	DQ468673	Wu et al. 2016

Results

Molecular data

The ML and BI analyses resulted in essentially identical topologies and were integrated in the consensus tree (Fig. 2), in which the maximum nodes were sufficiently supported with the Bayesian posterior probabilities (BPP) > 0.90 and the bootstrap supports (BS) for maximum likelihood analysis > 70 and a few poorly supported basal nodes. Both Bayesian and Maximum Likelihood analyses strongly supported that the new species is in the genus *Raorchestes*. The uncorrected p-distances for the 16S rRNA gene

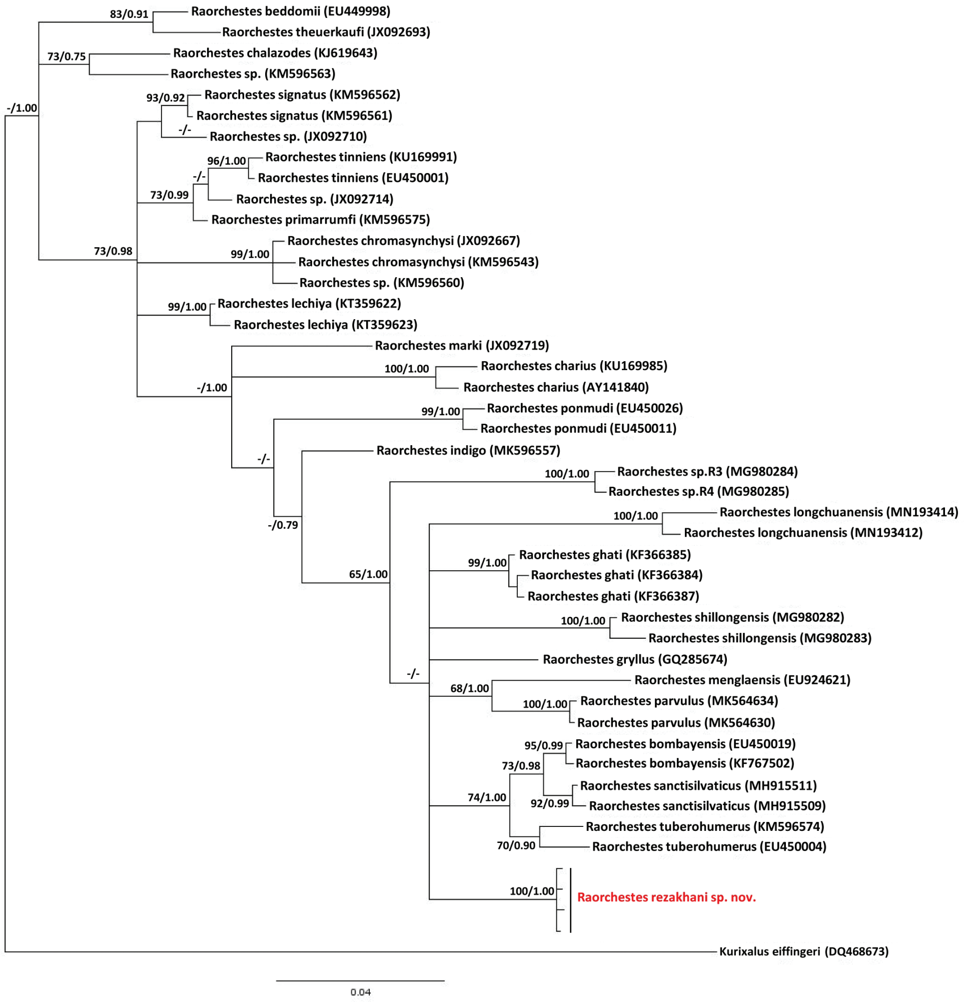

Figure 2. Bayesian Inference and Maximum Likelihood phylogenies, showing the placement of *Raorchestes rezakhani* sp. nov. in relation to other congeneric species. The Bayesian Posterior Probabilities (BPP) > 0.75 and the bootstrap supports for Maximum Likelihood analysis (ML) > 60 were retained.

that are interpreted as interspecific distances were lowest between *R. bombayensis* Annandale 1919 and *R. sanctisilvaticus* Das and Chanda 1997 (*p* = 1.4%, Table 2). The highest interspecific distances were between *R. tinniens* (Jerdon 1854) and *R. ghatei* Padhye et al. 2013 (*p* = 10.6%, Table 2). The newly discovered species was most similar to *R. tuberohumerus* (Kuramoto and Joshy 2003) (*p* = 4.6%) followed by *R. gryllus* (Smith 1924) (*p* = 4.7%) at the gene fragment examined. In addition, *R. rezakhani* sp. nov. differed considerably from *R. longchuanensis* (*p* = 5.5%), *R. shillongensis* (*p* = 5.5%) and R. parvulus (*p* = 6.6%, Table 2). The average divergence (p-distance) between the new species and other congeneric species ranged from 4.6% to 9.6% (Table 2). This level of divergence in the 16S rRNA gene is typically seen in many other frog species pairs, thereby justifying the status of *R. rezakhani* sp. nov. as a new species (Fouquet et al. 2007).

Raorchestes rezakhani **sp. nov.**

http://zoobank.org/CDDA555B-9B29-4D94-B5E6-BA560ECD8DB3
Figures 4, 5
Suggested English name: Reza Khan's bush frog

Type. ***Holotype*** (Figs 4A, B, 5). JnUZool-A0419, an adult male from Lawachara National Park, Kamalgonj, Moulavibazar, Bangladesh (24°20.746'N, 91°47.945'E, ca. 59 m a.s.l., Fig. 1), collected on 26 April 2019 by Hassan Al-Razi and Marjan Maria.

Paratypes (Fig. 4C, D). Three specimens: adult male (JnUZool-A0319) same locality as the holotype; two adult males (JnUZool-A0519, JnUZool-A0619) from the Adampur, Rajkandhi Reserved Forest, Kamalgonj, Moulavibazar (24°14.878'N, 91°54.002'E, ca. 64 m a.s.l., Fig. 1), on 10 May 2019 by Hassan Al-Razi.

Generic placement. We assign this species to *Raorchestes* based on molecular characterization of the 16S rRNA gene.

Etymology. We take great pleasure in naming the new species as a patronym for one of the pioneers in the field of wildlife research in Bangladesh, Dr. Mohammad Ali Reza Khan.

Diagnosis. A species of *Raorchestes* having the following unique combination of characters: (1) relatively small size (adult males = 18.85–20.90 mm SVL); (2) head wider than long (HW/HL 1.55; range 1.53–1.56, *N* = 4); (3) dark brown, granular dorsum bearing small, horny spicules; (4) vomerine teeth absent; (5) single transparent vocal sac while calling; (6) snout projecting, sub-elliptical in ventral aspect, and subequal to or smaller than horizontal diameter of eye; (7) tympanum indistinct; (8) supratympanic fold weakly distinct; (9) finger and toe discs well developed and rounded; (FD IV 0.50–0.60, TD IV 0.56–0.65 mm); (10) both inner and outer metacarpal and metatarsal tubercles absent; (11) nostril is closer to tip of snout than to eye (NS 0.63–0.90, EN 1.10–1.25 mm); (12) Tongue without papilla (13) venter pale white, with minute dark gray flecks present in the vocal sac region. Details of these measurements are provided in Table 3.

Table 2. Intraspecific and interspecific genetic divergence. Uncorrected p-distances between 16S rRNA sequences of closely related 20 species of *Raorchestes*.

	Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*R. rezakhani* sp. nov.	–																				
2	*R. longchuanensis*	0.055	–																			
3	*R. shillongensis*	0.055	0.072	–																		
4	*R. ghatei*	0.065	0.085	0.061	–																	
5	*R. gryllus*	0.047	0.067	0.054	0.058	–																
6	*R. menglaensis*	0.059	0.066	0.054	0.065	0.065	–															
7	*R. bombayensis*	0.061	0.074	0.044	0.071	0.056	0.063	–														
8	*R. tuberohumerus*	0.046	0.058	0.037	0.063	0.053	0.051	0.019	–													
9	*R. sanctisilvaticus*	0.061	0.067	0.047	0.070	0.058	0.062	0.014	0.014	–												
10	*R. ponmudi*	0.074	0.077	0.067	0.096	0.087	0.086	0.084	0.076	0.084												
11	*R. beddomii*	0.072	0.082	0.07	0.095	0.084	0.087	0.081	0.07	0.082	0.061	–										
12	*R. indigo*	0.054	0.063	0.039	0.069	0.059	0.058	0.055	0.053	0.054	0.054	0.064	–									
13	*R. parvulus*	0.066	0.065	0.06	0.072	0.058	0.047	0.044	0.045	0.042	0.083	0.088	0.061	–								
14	*R. theuerkaufi*	0.078	0.088	0.078	0.093	0.088	0.088	0.077	0.064	0.075	0.067	0.021	0.062	0.082	–							
15	*R. signatus*	0.082	0.097	0.073	0.088	0.087	0.076	0.078	0.074	0.078	0.072	0.060	0.043	0.081	0.063	–						
16	*R. tinniens*	0.084	0.105	0.075	0.106	0.099	0.088	0.078	0.072	0.08	0.082	0.071	0.048	0.097	0.077	0.028	–					
17	*R. marki*	0.069	0.069	0.067	0.077	0.071	0.066	0.067	0.061	0.062	0.064	0.066	0.047	0.059	0.064	0.051	0.056	–				
18	*R. chromasynchysi*	0.082	0.084	0.064	0.078	0.085	0.068	0.08	0.074	0.083	0.073	0.060	0.055	0.085	0.064	0.044	0.050	0.051	–			
19	*R. charius*	0.096	0.105	0.071	0.096	0.091	0.08	0.084	0.070	0.087	0.085	0.063	0.064	0.091	0.073	0.078	0.080	0.058	0.075	–		
20	*R. primarrumfi*	0.073	0.085	0.059	0.084	0.073	0.076	0.063	0.070	0.066	0.058	0.049	0.046	0.077	0.054	0.021	0.012	0.054	0.038	0.058	–	
21	*R. chalazodes*	0.081	0.091	0.085	0.085	0.085	0.079	0.079	0.076	0.079	0.087	0.058	0.065	0.085	0.058	0.044	0.057	0.057	0.052	0.077	0.049	–

Table 3. Morphological measurements (in mm) of the four specimens of *Raorchestes rezakhani* sp. nov.

	Characters	Abbreviation	Holotype	Paratype			Mean ± SD
			JnUZool-A0419	JnUZool-A0319	JnUZool- A0519	JnUZool-A0619	
1	Snout–vent length	SVL	20.30	20.90	20.20	18.85	20.06 ± 0.87
2	Head length	HL	4.50	4.60	4.55	4.50	4.54 ± 0.05
3	Head width	HW	7.00	7.05	7.10	6.95	7.03 ± 0.06
4	Eye diameter	ED	2.45	2.70	2.50	2.65	2.58 ± 0.12
5	Tympanum diameter	TD	1.10	1.22	1.21	1.16	1.17 ± 0.05
6	Eye–nostril distance	EN	1.25	1.20	1.10	1.20	1.19 ± 0.06
7	Snout length	SL	2.24	2.25	2.24	2.22	2.24 ± 0.01
8	Nostril-Snout distance	NS	0.80	0.90	0.85	0.63	0.80 ± 0.12
9	Interorbital distance	IOD	2.20	2.40	2.25	2.20	2.26 ± 0.09
10	Internarial distance	IND	1.70	1.65	1.60	1.70	1.66 ± 0.05
11	Upper eyelid width	UEW	1.45	1.50	1.40	1.55	1.48 ± 0.06
12	Thigh length	TL	10.03	9.20	10.10	10.00	9.83 ± 0.42
13	Shank length	ShL	10.10	10.10	11.90	10.20	10.58 ± 0.88
14	Foot length	FOL	7.95	6.60	7.85	7.95	7.59 ± 0.66
15	Hand length	HAL	4.90	4.35	4.95	4.90	4.78 ± 0.28
16	Fore limb length	FLL	4.70	4.70	5.0	5.0	4.85 ± 0.17
17	Finger I disk width	FD I	0.25	0.20	0.20	0.20	0.21 ± 0.02
18	Finger II disk width	FD II	0.45	0.40	0.40	0.40	0.41 ± 0.03
19	Finger III disk width	FD III	0.75	0.70	0.75	0.70	0.73 ± 0.03
20	Finger IV disk width	FD IV	0.50	0.50	0.60	0.50	0.53 ± 0.05
21	Finger I length	FL I	1.20	1.05	1.10	1.20	1.14 ± 0.07
22	Finger II length	FL II	1.75	1.80	1.70	1.80	1.76 ± 0.05
23	Finger III length	FL III	3.40	3.05	3.55	3.55	3.39 ± 0.24
24	Finger IV length	FL IV	2.15	1.95	2.20	2.25	2.14 ± 0.13
25	Toe I length	TL I	1.15	1.00	1.00	1.15	1.08 ± 0.09
26	Toe II length	TL II	2.10	1.90	2.05	1.90	1.99 ± 0.10
27	Toe III length	TL III	3.20	2.90	3.10	3.00	3.05 ± 0.13
28	Toe IV length	TL IV	4.25	4.00	4.10	4.30	4.16 ± 0.14
29	Toe V length	TL V	3.05	2.95	3.15	3.05	3.05 ± 0.08
30	Toe I disk width	TD I	0.30	0.20	0.30	0.25	0.26 ± 0.05
31	Toe II disk width	TD II	0.35	0.25	0.35	0.30	0.31 ± 0.05
32	Toe III disk width	TD III	0.50	0.40	0.45	0.50	0.46 ± 0.05
33	Toe IV disk width	TD IV	0.65	0.56	0.60	0.60	0.60 ± 0.04
34	Toe V disk width	TD V	0.60	0.45	0.50	0.50	0.51 ± 0.06

Description of holotype. A small frog (SVL = 20.30, Fig. 5, Table 3, all measurements in mm); head wider than long (HW = 7.0; HL = 4.5); snout sub-elliptical in ventral aspect, shorter than eye diameter (ED = 2.45; SL = 2.24). Canthus rostralis sharply rounded; loreal region slightly concave. Interorbital region flat and larger (IOD = 2.20) than the upper eyelid (UEW = 1.45 mm) or internarial distance (IND = 1.70). Nostrils oval (dorsally compressed), without flap, directed laterally, closer to tip of snout than to eye (NS = 0.80; EN = 1.20). Tympanum indistinct, oval (TD = 1.10), close to eye, supratympanic fold weakly distinct, extends from eye to the end of the tympanum. Vocal sac single, sub-gular, translucent. Tongue bifid, lingual papilla absent. Eyes relatively large (ED = 2.45), protruding; pupil horizontal.

Forelimb length shorter than hand length (FLL = 4.70; HAL = 4.90). Relative lengths of fingers I < II < IV < III (FL I = 1.20; FL II = 1.75; FL III = 3.40; FL IV = 2.15). Fingertips with well-developed discs (FD I = 0.25, FD II = 0.45, FD III = 1.1, FD IV = 1.2) bearing circum-marginal grooves. Dermal fringe absent on fingers. Webbing between fingers absent. Subarticular tubercles weak, number of subarticular tubercles in fingers: I = 1, II = 1, III = 1, IV = 1, rounded. Supernumerary tubercles indistinct. Nuptial pad absent.

Hind limbs long, shank shorter than thigh (ShL = 10.03; TL = 10.10), longer than foot (FOL = 7.95). Relative toe length I < II < V < III < IV (ToL I = 1.15; ToL II = 2.10, ToL III = 3.20; ToL IV = 4.25; ToL V = 3.05). Toes with well-developed discs (TD I = 0.30, TD II = 0.35, TD III = 0.50, TD IV = 0.65, TD V = 0.60). Webbing moderate, webbing formula (fingers: I2-2^{+}II1¾-2^{+}III1½-3IV2¾-2^{-}V) (Fig. 5D, E). Inner and outer metatarsal tubercles absent, subarticular tubercle present (toe: I = 1, II = 1, III = 2, IV = 3, V = 2). Supernumerary tubercles absent.

In preservative, dorsum dark gray; loreal and tympanic regions lighter; forelimbs and hind limbs with black bands. Venter uniform cream white, vocal sac with dark gray flecks. Webbing cream; ventral side of feet and hands light gray with small black spots.

In life, dorsum grayish brown with dark brown specks; “)-(“ or “)(“ shaped blackish mark present on the mid dorsum; blackish line between upper eyelids; snout much darker, loreal and tympanic region blackish; iris dark golden brown. Dorsal side of hind limbs with several black bands; forelimbs with single band these bands are also present in the other members of this genus. Fingers and toes discs reddish or whitish. Abdomen brownish, with few black spots. Vocal sac translucent whitish, with a few black flecks. A few dark spots present near fore limbs. Foot webbing grayish.

Variation. Because all specimens were males, sexual dimorphism could not be determined. Details of morphometric variation observed in four individuals are provided in Table 3. All of the specimens are almost similar except the size and the coloration. One of the four specimens (JnUZool-A0619) is smaller than others. For two specimens (JnUZool-A0619, JnUZool- A0519) the ventral dark gray flecks are more than others. The)(shape is present on the dorsum of three specimens where for one specimen (JnUZool- A0519) it is shaped “)-(“. Some individuals have a greater proportion of dark gray spots on the ventral surface. Detailed comparisons between *R. rezakhani* sp. nov. and other species of *Raorchestes* are provided below.

Bioacoustics analyses. An advertisement call of the paratype (JnUZool-A0519) from the Lawachara National Park were recorded at an ambient air temperature of 27.8 °C, 97% relative humidity. Advertisement calls occurred without call groups (Fig. 3). The duration of the analyzed call was 16 s. The number of notes within this call was 25, and number of pulses within a note varied from 5–11 (8.84 ± 1.70 *SD*). Note duration was 0.183 – 0.379 s. The interval between notes was 0.222 – 0.592 s (0.323 ± 0.098 *SD*, *N* = 24). These intervals increase gradually within a call (mean interval for first five notes = 0.2422, mid five notes = 0.2784, last five notes = 0.4754). Pulse duration was 0.003–0.029 s (0.013 ± 0.007 *SD*, *N* = 205 pulses), duration of intervals between pulses was 0.005–0.127 s (0.027 ± 0.017 *SD*, *N* = 179 intervals).

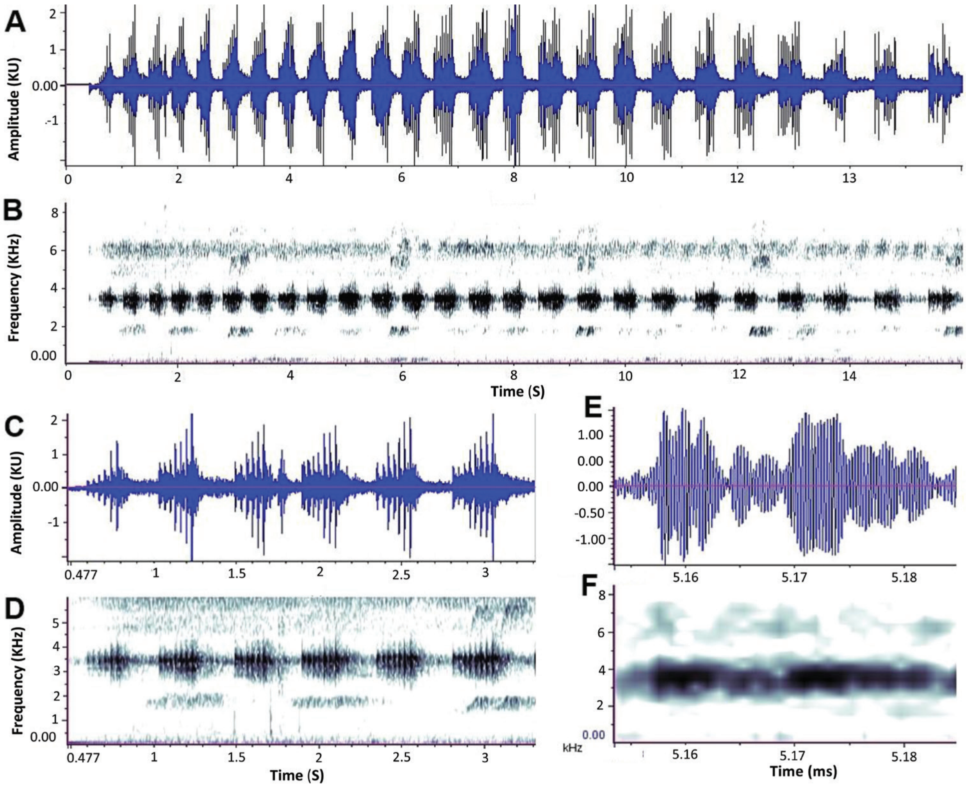

Figure 3. Advertisement call of *Raorchestes rezakhani* sp. nov. showing 25 notes that vary in amplitude **A** waveform of 25 notes **B** shows variation in frequency **C** shows waveform of first six notes of the call; and **D** shows a spectrogram of the six notes **E** shows a pulse of fourth note and **F** shows the spectrogram of pulse of fourth note.

Pulse rate was 10–19/s (14.27 ± 2.49 *SD*, *N* = 15 seconds interval). The advertisement call had a dominant frequency at 4.32–4.77 kHz (4.55 ± 0.12 *SD*, *N* = 25). To the human ear, the calls sounded similar to cricket calls.

Distribution and natural history. *Raorchestes rezakhani* sp. nov. was recorded from the semi-evergreen forests of northeastern Bangladesh. They were active with the onset of the rainy season in the month of April. We did not hear calls of this species after August. Frogs were found inside the primary and secondary forest mainly on the edge of streams and near man-made trails. They often use the hilly slopes during calling. Individuals perch on leaves and branches of small trees and on bamboo trunks (with diameters of 1.5–4 cm). Vocalizing individuals were perched 1–1.5 m above the forest floor. We usually heard the calls immediately after the sunset (ca. 1815 h in April) although calling activity started a little earlier when it was raining.

Comparisons. Based on morphology, we compared *Raorchestes rezakhani* sp. nov. with some other member of this genus. This new species is differs from *R. amboli* (Biju & Bossuyt, 2009), *R. anili* (Biju & Bossuyt, 2006), *R. charius* (Rao, 1937),

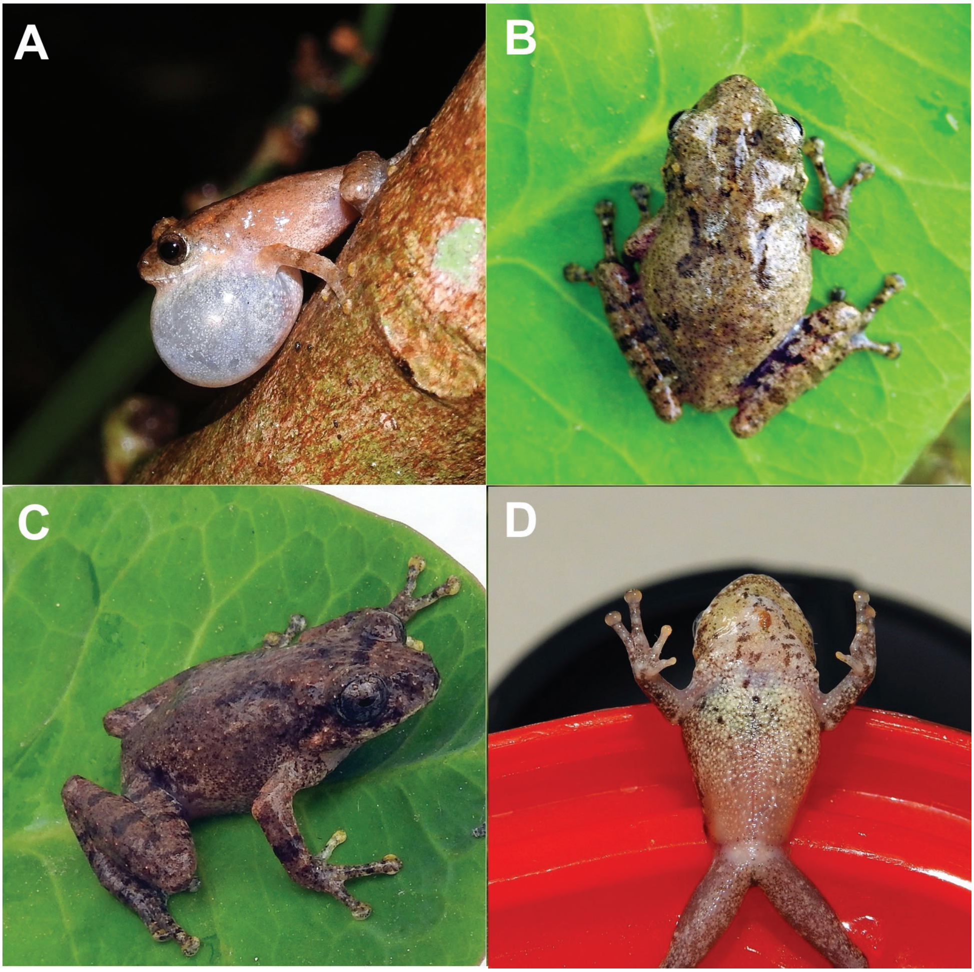

Figure 4. Color variation in *R. rezakhani* sp. nov. **A** holotype, showing single transparent vocal sac during advertisement call (**B** holotype with brown dorsum and ")-(" mark; **C** dorsolateral view of paratype (JnUZool- A0519) **D** ventral view of paratype (JnUZool- A0519), showing small dark brown spots.

R. chlorosomma (Biju & Bossuyt, 2009), *R. flaviventris* (Boulenger, 1882), *R. glandulosus* (Jerdon, 1853), *R. jayarami* (Biju & Bossuyt, 2009), *R. kaikatti* (Biju & Bossuyt, 2009), *R. luteolus* (Kuramoto & Joshy, 2003), *R. munnarensis* (Biju & Bossuyt, 2009), *R. nerostagona* (Biju & Bossuyt, 2005), *R. ochlandrae* (Gururaj et al., 2007), *R. ponmudi* (Biju & Bossuyt, 2005), *R. signatus* (Boulenger, 1882), *R. sushili* (Biju & Bossuyt, 2009), *R. wynaadensis* (Jerdon, 1853), *R. kakachi* Seshadri et al., 2012, *R. crustai* Zachariah et al., 2011, *R. johnceei* Zachariah et al., 2011, *R. theuerkaufi* Zachariah et al., 2011, *R. thodai* Zachariah et al., 2011, *R. gryllus* (Smith 1924) by its smaller size. SVL of male individuals of these species ranged from 24.9–36.8 mm whereas *Raorchestes rezakhani* sp. nov. is 20.06 mm. *Raorchestes rezakhani* sp. nov. is quite similar to *R. longchuanensis* Yang et al. 1979 but differs for the following characters:

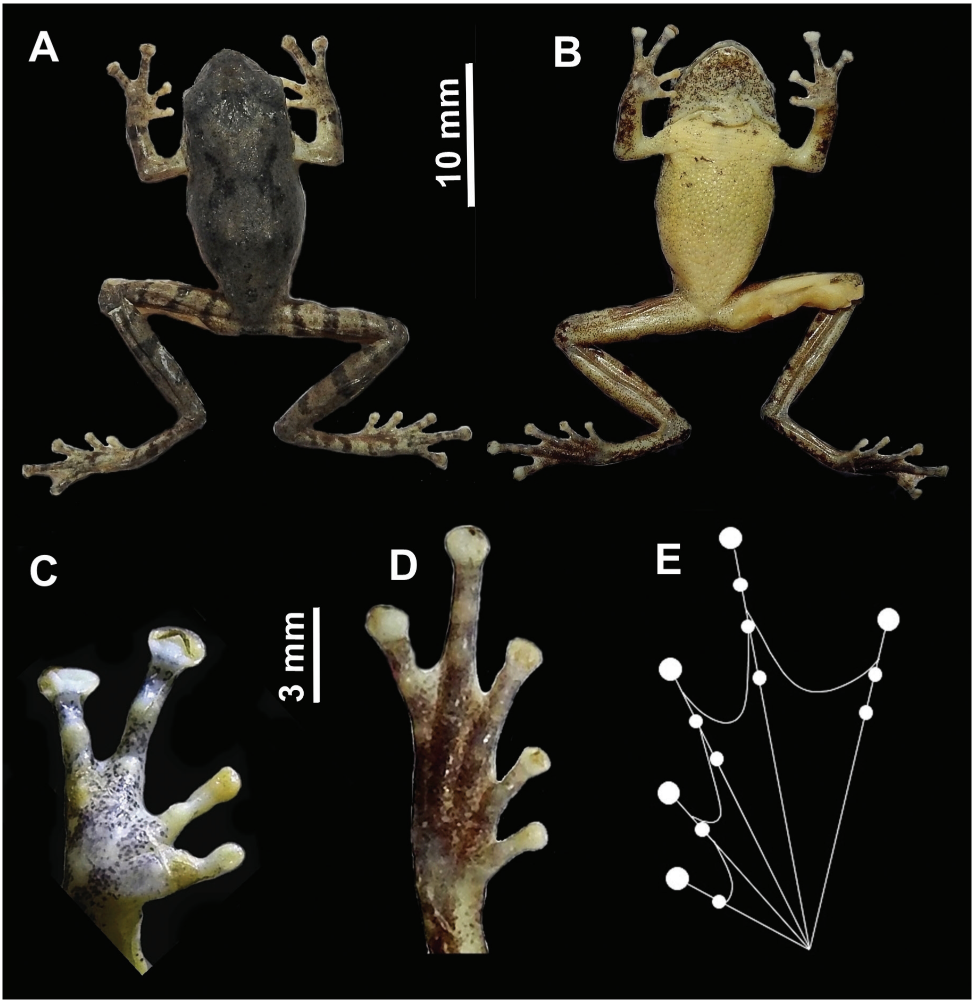

Figure 5. Holotype of *R. rezakhani* sp. nov. **A** dorsal view **B** ventral view **C** ventral view of right hand **D** ventral view of right foot **E** web pattern in foot.

tympanum indistinct in males (vs. distinct); snout sub-elliptical (vs. pointed); thigh shorter than the tibia/shank, TL/ShL = 93% (vs. Thigh slightly longer than the tibia/shank, TL/ShL = 97%); inner metatarsal tubercles absent (vs. present) [Yang et al. 1979; Al-Razi et al. 2020]. *Raorchestes rezakhani* sp. nov. differs from *R. tuberohumerus* in: snout sub-elliptical (vs. slightly pointed); relative lengths of fingers I < II < IV < III (vs. I < IV < II < III); thigh shorter than the tibia/shank, TL/ShL = 93% (vs. thigh longer than the tibia/shank, ShL/TL = 96%); inner metatarsal tubercles absent (vs. present); supernumerary tubercles feebly distinct (vs. distinct) [Kuramoto and Joshy 2003; Padhye et al. 2015]. *Raorchestes rezakhani* sp. nov**.** differs from *R. gryllus* in: snout sub-elliptical (vs. pointed); tympanum indistinct in males (vs. large and rounded); relative toe lengths I < II < V < III < IV (vs. I < II < III < V < IV); subarticular tubercles in finger weakly distinct I = 1, II = 1, III = 1, IV = 1 (vs. distinct I = 1, II = 1, III = 2, IV = 1) [Smith 1924; Orlov et al. 2012]. *Raorchestes rezakhani* sp. nov. is also similar to *R. shillongensis* (Pillai & Chanda, 1973) but differs in: SVL of male 20.06 ± 0.87 (vs. 16.51 ± 1.29); head wider than long, HL/HW = 61% (vs. length slightly greater than the width, HW/HL = 98%); snout length shorter than the eye diameter (vs. slightly longer than eye diameter); subarticular tubercles in finger weakly distinct, I = 1, II = 1, III = 1, IV = 1 (vs. distinct, I = 1, II = 1, III = 2, IV = 1) [Pillai and Chanda 1973; Boruah et al. 2018]. *Raorchestes rezakhani* sp. nov. is very similar to *R. parvulus* but differs in: forearm and hand length (9.05–9.95 mm) generally shorter than half body size (vs. longer than the half body size); relative toe length I < II < V < III < IV (vs. I < II < III < V < IV); toe subarticular tubercle: I = 1, II = 1, III = 2, IV = 3, V = 2 (vs. I = 1, II = 1, III = 2, IV = 2, V = 1); inner metatarsal tubercles absent (vs. present) [Boulenger 1893; Yu et al. 2019]. *Raorchestes rezakhani* sp. nov. differs from *R. sahai* (Sarkar & Ray, 2006) in: smaller SVL (18.85–20.90 vs. 25–26 mm); nostril closer to tip of snout than to eye, NS/EN = 67% (vs. equidistance from the tip of the snout and the eye NS/EN = 100%); snout length shorter than the eye diameter, SL/ED = 87% (vs. slightly longer than eye diameter, ED/SL = 81%); interorbital distance larger than the upper eyelid UEW/IOD= 65% (vs. equal to the upper eyelid, UEW/IOD = 100%) [Sarkar and Ray 2006]. *Raorchestes rezakhani* sp. nov. differs from *R. annandalii* in: snout sub-elliptical (vs. pointed); nostril closer to tip of snout than to eye, NS/EN = 67% (vs. equidistant from the tip of the snout and the eye, NS/EN = 100%); inner metatarsal tubercles absent (vs. feebly distinct); ShL longer than TL, TL/ShL = 93% (vs. ShL shorter than TL) [Boulenger 1906; Chanda 1994]. *Raorchestes rezakhani* sp. nov**.** differs from *R. menglaensis* (Kou 1990) in: male with external single subgular vocal sac (vs. internal single subgular vocal sac); outer metatarsal tubercle absent (vs. present); [Padhye et al. 2013; Kou 1990]. This new species differs from *R. garo* (Boulenger 1919) in: SVL 18.85–20.90 (vs. 13–16 mm); eye diameter larger than the interorbital distance, IOD/ED = 88% (vs. less than interorbital distance, ED/IOD = 92%); dark line present between eyelids (vs. absent); nostril closer to tip of snout than to eye, NS/EN = 67% (vs. equidistance from the tip of the snout and the eye or slightly closer to the tip of snout); tympanum indistinct (vs. distinct); inner metatarsal tubercles absent (vs. present) [Boulenger 1919; Chanda 2002]. *Raorchestes rezakhani* sp. nov. differs from *R. kempiae* (Boulenger 1919) in: SVL 18.85–20.90 (vs. 13–17.5 mm); nostril closer to tip of snout than to eye (vs. equidistant from the tip of the snout and the eye); tympanic fold indistinct (vs. distinct) [Boulenger 1919, Chanda 1994, 2002].

Principle Components Analysis showed that the specimens of *R. rezakhani* sp. nov. did not overlap with *R. longchuanensis*, *R. tuberohumerus*, or *R. gryllus* (Fig. 6). Eigenvalues indicated that PC1 accounted for more than 91% of the variation in the data while PC2 contributed another 5% (Table 4). Thus, the inclusion of further principle components would not add substantially to the characterization of these species based on these variables. Loading of individual morphological variables indicated that SVL, HL, HW, THL and TL strongly influenced PC1, ED, SL, UEW and THL strongly influenced PC2, while HL and TL strongly influenced PC3, that helped to segregate the *R. rezakhani* sp. nov. from the remaining three species (Table 5).

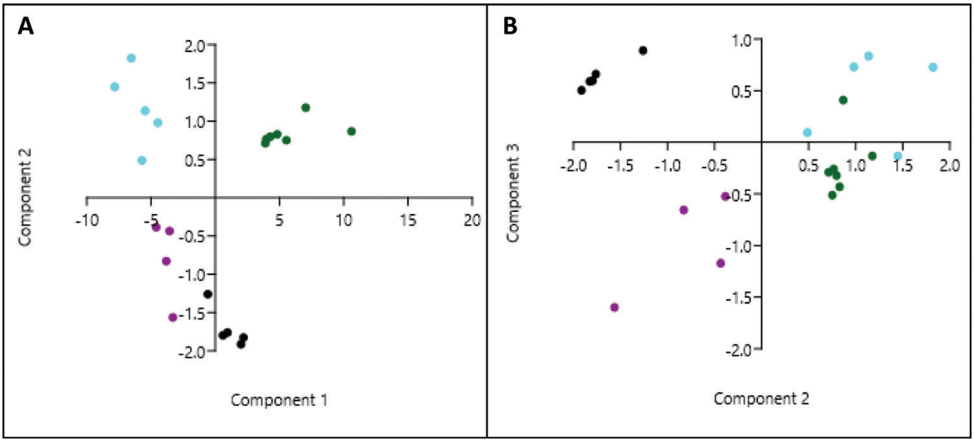

Figure 6. A Scatterplot of principle component axes 1 and 2 and **B** principle component axes 2 and 3. *R. tuberohumerus* (light blue), *R. gryllus* (green), *R. longchuanensis* (black), and *R. rezakhani* sp. nov. (purple).

Table 4. Eigen analysis showing relative contributions of each Principle Component towards the characterization of each species.

Principle component	Eigen value	% variance
1	26.07	91.15
2	1.57	5.50
3	0.47	1.66
4	0.28	0.98
5	0.07	0.25
6	0.06	0.21
7	0.04	0.15
8	0.01	0.04
9	0.0064	0.02
10	0.0043	0.01
11	0.0019	0.006

Table 5. Loading plot showing individual loadings of each measured variable in *Raorchestes tuberohumerus*, *R. gryllus*, *R. longchuanensis*, and *R. rezakhani* sp. nov. against four principle components.

Variable	PC 1	PC 2	PC 3	PC 4
SVL	0.60	-0.24	-0.25	-0.69
HL	0.40	0.40	0.47	-0.07
HW	0.31	0.04	0.12	0.10
ED	0.23	0.39	-0.36	0.21
EN	0.09	-0.03	0.23	0.03
SL	0.18	0.27	0.11	0.19
IOD	0.15	0.06	0.24	0.01
IND	0.04	-0.05	0.32	0.00
UEW	0.18	0.50	-0.26	0.14
THL	0.32	-0.43	0.33	0.41
TL	0.35	-0.34	-0.41	0.49

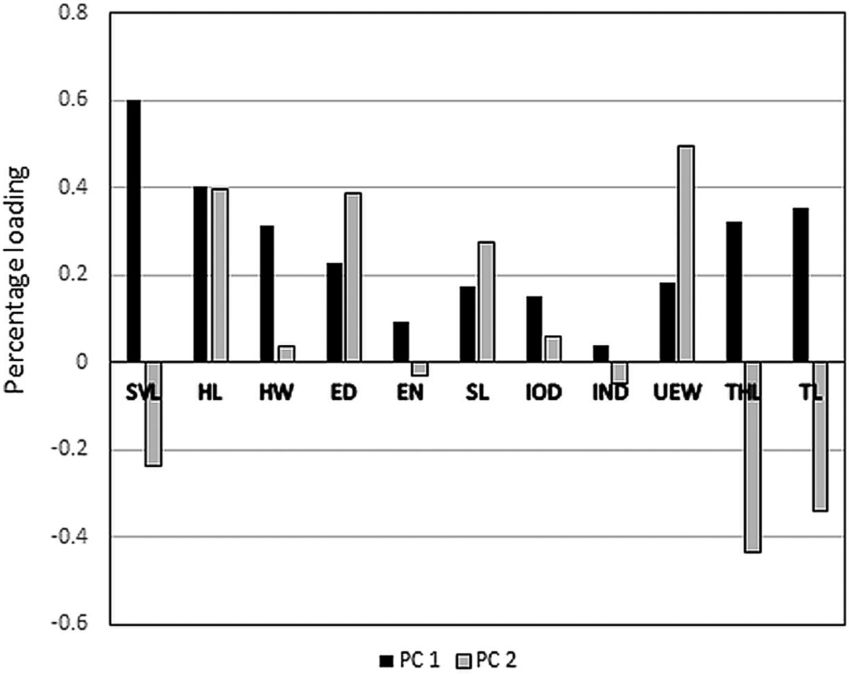

Figure 7. Plot showing individual loadings of each morphometric variable in relation to PC1, which accounted for over 91% of the variation in the data.

Discussion

Our discovery of a new species of *Raorchestes* is not unexpected (Reza 2014; Khan 2015; IUCN 2015). Our recent report of *R. longchuanensis* from northeastern Bangladesh (Al-Razi et al. 2020) supports the suggestion of the authors of the species, who stated that it was very likely to occur outside of Longchuan (the type locality) as well as nearby provinces in southern China (Yang et al. 2004). We suggested that the broad similarities between southern China, northern Myanmar, several northeastern states of India, and northeastern Bangladesh with their relative proximity to each other would suggest that many species may occur across this region (Al-Razi et al. 2020). Slik et al. (2018) recently classified the world's forest types using phylogenetic similarities into five floristic regions. Two of these five regions, namely the Indo-Pacific and the Subtropical floristic regions, are of interest. The Indo-Pacific region spans across the Indian subcontinent and through Myanmar into the rest of Southeast Asia. In addition, the Subtropical floristic region spans from northeastern India, northern Myanmar, through southern China (where it has significant overlaps with the Indo-Pacific floristic region) further into eastern China (Slik et al. 2018). The Indian subcontinental fauna differs considerably compared to the Southeast Asian fauna, making the entire region of great interest to diversification of biota.

The Western Ghats region of India is a global biodiversity hotspot (Gadgil 1996). The region has undergone biodiversity loss along with changes in land use that has contributed towards the creation of geographic barriers within the last few decades (Gadgil 1996). The diversification of frogs in the Western Ghats has generally been attributed to long-term ecological change over extended geological time scales (Priti et al. 2016). Due to its status as a biodiversity hotspot, considerable research attention has been placed on this region, resulting in more discoveries in the anuran fauna. On the other hand, the taxonomic challenges as well as the lack of funding for dedicated studies examining species diversity in Bangladesh could have precluded the detection of cryptic species until recently (Reza 2014; Khan 2015; IUCN 2015; Frost 2020). This is also true for the northeastern regions of India, where relatively few studies have been done on cryptic anurans (Ao et al. 2003; Vijayakumar et al. 2016; Boruah et al. 2018). Myanmar has only recently been opened up to biological exploration and we anticipate that more species will be found from this region. Renewed interest, especially with respect to anuran biodiversity and the relative availability and cost-effectiveness of molecular tools, have made it easier to target cryptic species for identification. We anticipate that further extensive surveys followed by molecular characterization, and bioacoustics data could aid in discovering additional species and delineating their occurrence in the region (Vijaykumar et al. 2014; Priti et al. 2016).

Northeastern India, particularly Meghalaya and parts of Assam, are separated by the river Brahmaputra, that effectively creates differences in forest type (Champion and Seth 1968). Areas south of the river have more subtropical influence, compared to areas north of the river, which are climatically affected by the Himalayas and its foothills, due to variation in local climatic patterns (Champion and Seth 1968). Thus, there are forested areas in Meghalaya, Assam, Tripura and Mizoram states of India and Bangladesh with variation in niche types affected by local climates that could have encouraged diversification of *Raorchestes* or other forest-dwelling genera (Ahmed et al. 2009; Vijayakumar et al. 2016).

The similarities between *R. rezakhani* sp. nov., *R. tuberohumerus*, and *R. gryllus* could offer some insight into the diversification of the *Raorchestes* in the region. *Raorchestes tuberohumerus* is distributed in the Western Ghats while *R. gryllus* is limited in distribution to central Vietnam and Laos (Frost 2020). *Raorchestes shillongensis*, restricted to a small part of Megalaya state of India, is mostly closely related to either *R. tuberohumerus* (p = 3.7%) and *R. indigo* (p = 3.9%), a species also found in the Western Ghats (Frost 2020). Thus, we suggest that the *Raorchestes* species in northeastern India and surrounding regions may have separated from Western Ghats species giving rise to *R. shillongensis* and *R. rezakhani* sp. nov. relatively recently (Vijayakumar et al. 2016). Ancestors of *Raorchestes parvulus* may have diverged from Western Ghats stock even earlier (difference compared to *R. bombayensis* was 4.4%) and from Eastern Ghats and Deccan plateau species (difference compared to *R. sanctisilvaticus* was 4.2%). Our analysis indicates that *R. longchuanensis* is quite distinct from *R. parvulus* (p = 6.5%). *Raorchestes rezakhani* sp. nov. is also significantly different both morphologically and genetically from *R. parvulus*, providing support of the idea that *R. parvulus* is part of a Southeast Asian species complex. The status of *Raorchestes annandalii* is not clear since there are no sequences of 16S rRNA genes for this species in GenBank. They are morphologically distinct from *R. rezakhani* sp. nov. and we speculate that they could be part of a species complex associated with northeast India and northern Myanmar, and may include species such as *R. shillongensis*, *R. longchuanensis*, and *R. rezakhani* sp. nov. as closely related congenerics. Further genetic analyses could clarify their status in relation to the evolution and biogeography of *Raorchestes* in the region. We speculate that *R. rezakhani* sp. nov. may be found in other adjoining areas including the northeastern states of India and northern Myanmar due to close affiliations of the habitat types in this floristic region.

It is also important to note that Bangladesh retains some forest patches that are of high value to biodiversity. The two areas in the northeast, Lawachara National Park and Adampur reserve forest contain high bird and mammal diversity. Six of the ten species of primates in Bangladesh occur there in numbers higher than elsewhere in the country (Al-Razi et al. 2019; Al-Razi and Maria 2019). Lawachara is legally protected whereas Adampur is under the management of the Forest Department of the Ministry of Environment and Forests of Bangladesh, but not under formal protected areas status. Illegal logging, fuel wood collection, and hunting occurs in these areas (Muzaffar et al. 2011; Islam et al. 2013). Although a signatory to the Convention on Biological Diversity, all forested and other wilderness areas suffer from poor implementation of the principles of ecosystem management (Muzaffar et al. 2011). Lawachara has a total area of about 12 km^2 and Adampur has an area of about 71.9 km^2, making both of them relatively small patches. Despite all odds, our finding of new species and previous studies on primates suggest that viable populations of varied species persist in these areas (Muzaffar et al. 2007, 2011; Al-Razi et al. 2020). Thus, efforts must be made to protect these remaining forest patches, which may still retain undiscovered new species, as documented in this study.

Ethics statement

Fieldwork and sampling were carried out in Adampur Reserve Forest and Lawachara National Park, with permission from Forest Department Bangladesh (Permit no. 22.01.0000.101.23.2019.2940). Individuals were euthanized and muscle tissue was collected in strict accordance with protocols approved by the Forest Department solely for scientific research. The sampling is unlikely to affect population size of the species since the bare minimum of specimens were collected.

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CORRIGENDA

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Corrigenda: A biodiversity hotspot for Microgastrinae (Hymenoptera, Braconidae) in North America: annotated species checklist for Ottawa, Canada

Samuel Lauzon[1], Jose Fernandez-Triana[2]

1 *Direction de la recherche forestière, Ministère des Forêts, de la Faune et des Parcs, Québec, Canada* **2** *Canadian National Collection of Insects, Ottawa, Canada*

Corresponding author: Samuel Lauzon (samuel.lauzon@mffp.gouv.qc.ca)

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In a paper about the biodiversity of Microgastrinae (Hymenoptera: Braconidae) in Ottawa Canada (Fernandez-Triana et al. 2016) some figure captions are incorrect. That includes three cases where the species name shown does not correspond with the actual species being depicted in those figures. To correct those mistakes, we detail below the correct captions for the corresponding figures.

The caption of Figure 8 (page 17):

Figure 8. *Apanteles laricellae*. **A** Habitus, lateral **B** head, frontal **C** wings **D** head and mesosoma (partially), dorsal **E** metasoma, lateral **F** metasoma, dorsal **F** mesosoma, dorsal.

Should read:

Figure 8. *Apanteles petrovae*. **A** Habitus, lateral **B** head, frontal **C** wings **D** cocoon **E** head and mesosoma (partially), dorsal **F** head and antenna, ventral **G** metasoma, dorsal.

The caption of Figure 9 (page 18):

Figure 9. *Apanteles morrisi.* **A** Habitus, lateral **B** head, frontal **C** wings **D** head and mesosoma, dorsal **E** metasoma dorsal **F** head and antenna, dorsal.

Should read:

Figure 9. *Apanteles laricellae*. **A** Habitus, lateral **B** head, frontal **C** wings **D** head and mesosoma (partially), dorsal **E** metasoma, lateral **F** metasoma, dorsal **G** mesosoma, dorsal.

The caption of Figure 10 (page 19):

Figure 10. *Apanteles petrovae.* **A** Habitus, lateral **B** head, frontal **C** wings **D** cocoon **E** head and mesosoma (partially), dorsal **F** head and antenna, ventrally **G** metasoma, dorsal.

Should read:

Figure 10. *Apanteles morrisi*. **A** Habitus, lateral **B** head, frontal **C** wings **D** head and mesosoma, dorsal **E** metasoma, dorsal **F** head and antenna, dorsal.

The caption of Figure 12 (page 28):

Figure 12. *Choeras consimilis*. **A** Habitus, lateral **B** wings **C** head, frontal **D** metasoma dorsal **F** head and mesosoma, dorsal.

Should read:

Figure 12. *Choeras consimilis*. **A** Habitus, lateral **B** wings **C** head, frontal **D** metasoma dorsal **E** head and mesosoma, dorsal.

The caption of Figure 21 (page 48):

Figure 21. *Dolichogenidea paralechiae*. **A** Habitus, lateral **B** head, frontal **C** wings **D** mesosoma and metasoma (partially), dorsal **E** metasoma, lateral **F** metasoma, dorsal **E** head and mesosoma, dorsal.

Should read:

Figure 21. *Dolichogenidea paralechiae*. **A** Habitus, lateral **B** head, frontal **C** wings **D** mesosoma and metasoma (partially), dorsal **E** metasoma, lateral **F** metasoma, dorsal **G** head and mesosoma, dorsal.

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