

# *Epitomapta simentalae* sp. n., a new species of apodous sea cucumber from the Central Eastern Pacific coast of Mexico (Echinodermata, Holothuroidea, Apodida)

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## Abstract

*Epitomapta simentalae* sp. n. occurs in depths of 4–10 m off the Mexican Central Pacific coast. It is distinctive in having twelve tentacles, each tentacle with two or three pairs of digits and four to six sensory cups, lacking papillae or oval bumps and in reaching a maximum length of 50 mm in life.

## Keywords

Leptosynaptinae; Synaptidae; Taxonomy

## Resumen

*Epitomapta simentalae* sp. n. fue recolectada a profundidades de entre 4–10 m en el Pacífico Central Tropical. Se distingue de sus congéneres por poseer doce tentáculos, cada tentáculo tiene dos o tres pares de dígitos, los cuales a su vez cuentan con cuatro a seis copas sensoriales, no posee papilas o protuberancias ovaladas sobre la superficie del cuerpo y por tener tallas desde los 4 hasta los 50 mm de largo total en ejemplares vivos.

## Palabras clave

Leptosynaptinae; Synaptidae; Taxonomía

## Introduction

Sea cucumbers of the family Synaptidae (order Apodida), while resolved as a non-monophyletic group in recent molecular analyses (Miller et al. 2017), includes the genus *Epitomapta*, a shallow water transisthmian taxonomic group of burrowing apodous sea cucumbers. The genus was created by Heding (1928) to include the previously described *Epitomapta roseola* (Verrill, 1873) and his new species *E. tabogae* Heding, 1928. Heding based the new genus on the presence of notched rather than perforated radial pieces of the calcareous ring.

The genus is represented by three nominal species, including the new one described here. In 1952 Cherbonnier described *Epitomapta knysnaensis* from the South African coasts but in 1989 Thandar and Rowe transferred the species into the genus *Leptosynapta* on the basis of new collections from the type locality and on the reexamination of the type material.

## Materials and methods

Specimens were collected by SCUBA diving (4–10 m depth). They were relaxed in a solution of 4% magnesium chloride and seawater. Fixation was made using 70% ethanol. Ossicles were extracted from the body wall (anterior, medium and posterior region) and tentacles. The tissue was dissolved in fresh household bleach [5–6.5%] in centrifuge tubes. After centrifugation at 1000 rpm for 10 minutes, bleach was pipetted off and the ossicles were rinsed and centrifuged with distilled water that was pipetted off afterwards. The same process was done with 70, 80, and 95% ethanol. Absolute ethanol was added to the ossicles, and finally a small aliquot was taken and placed to dry on a cylindrical double-coated conductive carbon tape stub. Then it was sputter coated with gold 2.5 kV in the ionizer Polaron E3000 for 3 minutes and photographed using a Hitachi S-2460N scanning electron microscope (SEM). Ciliated funnels were detached from the internal body wall using tweezers and dehydrated by critical point drying and placed on a carbon tape stub. Specimens were deposited at the following scientific collections: Colección Nacional de Equinodermos “Dra. Ma. Elena Caso Munoz”, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Ciudad de México and Smithsonian Institution, Natural History Museum, Washington, D.C., United States.

Abbreviations used in the text:

**ICML-UNAM** Colección Nacional de Equinodermos, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México;  
**USNM** Smithsonian Institution, Washington, D.C., United States Natural History Museum.

## Taxonomy

**Order Apodida Brandt, 1835**

**Family Synaptidae Burmeister, 1837**

**Subfamily Leptosynaptinae Smirnov, 1989**

**Diagnosis.** Synaptidae with 10, 11 or 12 pinnate tentacles, with one to nine digits on each side. Digits increase in size from base to tip of tentacle. Anchor plate develops from a rod which lies at a right angle to stock of developing anchor. Anchor plates with small number of holes, usually seven (6+1) in main part of the plate: six holes form a circle around a central hole. Articular end of plate usually has a “ledge” for contact with anchor keel. Anchor arms serrated, rarely naked, and without minute knobs on vertex (Smirnov 1989).

### **Genus *Epitomapta* Heding, 1928**

**Emended diagnosis.** Tentacles pinnate, usually 12. Digits from two to five pairs on each side (rarely two or none). Sense organs never in the form of pigment-eyes, but occur as minute cups on inner face of stalk of tentacles. Calcareous ring well developed. The radial pieces are not perforated for the passage of nerves, but with a notch in the anterior margin. Cartilaginous ring absent. Polian vesicle usually single. Stone canal single, unbranched. Ciliated funnels are of different shapes and are attached to the body wall, not to mesenteries. The calcareous deposits in the body wall are anchors, anchor plates and miliary granules; in the tentacles large rods. Stock of anchors finely toothed, but not branched; arms usually with teeth on the outer edge; vertex smooth. Anchor plates oval or somewhat elongated, with large central hole, surrounded by six large holes, usually more or less dentate, and two large and several small smooth holes at the narrow posterior end, but without an arched bow crossing the outer surface; at the broad end there are often additional dentate holes (modified from Heding 1928).

**Type species.** *Epitomapta tabogae* Heding, 1928 (original designation).

### ***Epitomapta simentalae* sp. n.**

<http://zoobank.org/7C6055AD-EC7E-4C8E-B702-7F31E264729D>

Figs 1–4

**Type material.** Holotype ICML-UNAM 5.169.0, 19 mm total length (TL), Caleta, Acapulco Bay, Guerrero, Mexico, Pacific Ocean 16°49.812'N, 99°59.062'W, 10 m depth, 8 May 2008, coll. F. A. Solís-Marín.

Paratypes: USNM 1114315, 10 specimens, same data as the holotype; ICML-UNAM 5.169.1, 11 specimens, same data as the holotype; ICML-UNAM 5.169.2, 63 specimens, Caleta, Acapulco Bay, Guerrero, Mexico, Pacific Ocean 16°49.812'N,



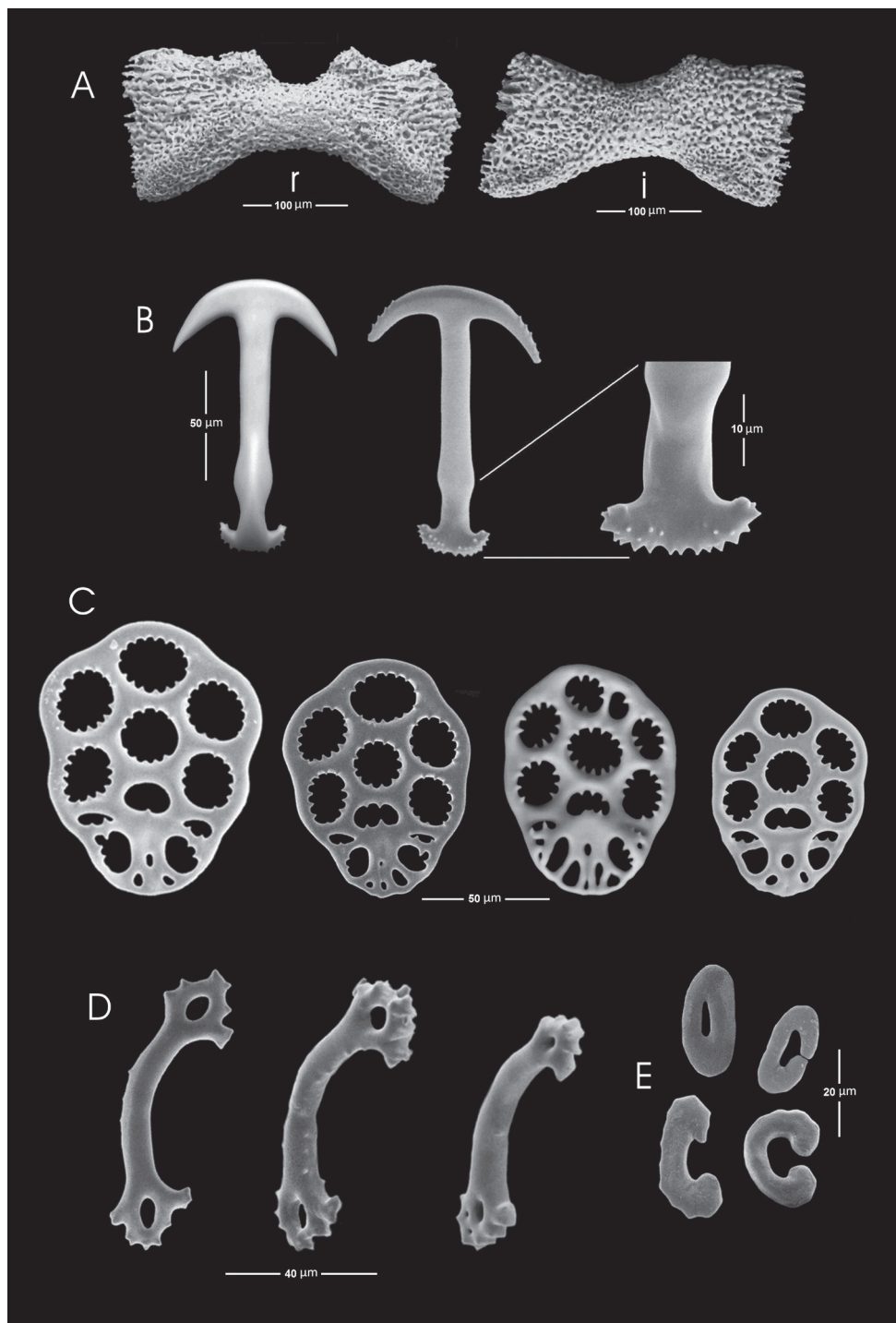
**Figure 1.** *Epitomapta simentalae* sp. n. Holotype ICML-UNAM 5.169.0. Lateral view from preserved specimen.

99°59.062'W, 10 m depth, 28 October 2006, coll. F. A. Solís-Marín, Y. Yerye, Honey-Escandón, M., A. Martínez Melo; ICML-UNAM 5.169.3, 20 specimens, Caleta, Acapulco Bay, Guerrero, Mexico, Pacific Ocean 16°49'N, 99°59'W, 4 m depth, 2 March 2006, coll. F. A. Solís-Marín, C. S. Frontana Uribe; ICML-UNAM 5.169.4, 27 specimens, Caleta, Acapulco Bay, Guerrero, Mexico, Pacific Ocean 16°49'N, 99°59'W, 9 m depth, 27 September 2006, coll. F. A. Solís-Marín, B. Urbano, M. A. Torres; ICML-UNAM 5.169.5, 5 specimens, Caleta, Acapulco Bay, Guerrero, Mexico, Pacific Ocean 16°49'N, 99°59'W, 8 m depth, 21 March 2009, coll. F. A. Solís-Marín and J.A. Díaz-Jáuregui.

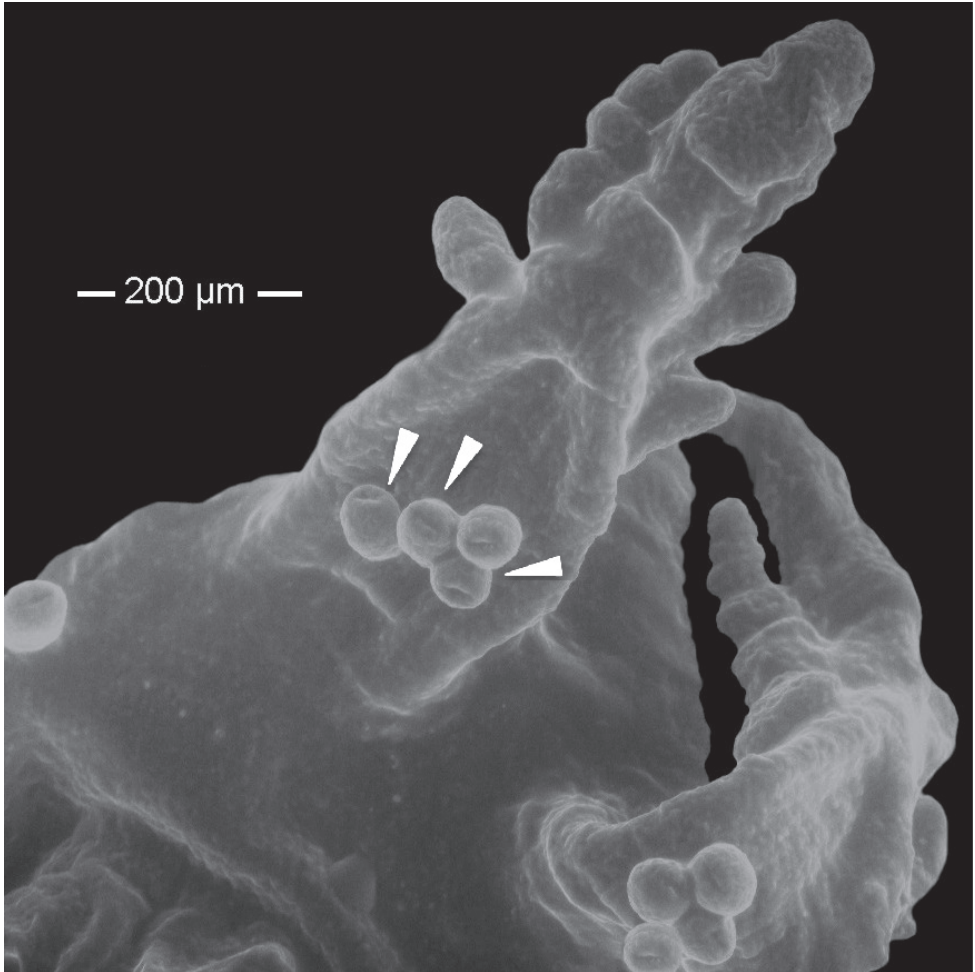
**Type locality.** Caleta, Acapulco Bay, Guerrero, Mexico, Pacific Ocean 16°49.812'N, 99°59.062'W.

**Diagnosis.** Body wall smooth, lacking papillae or oval bumps. Tentacles 12, each with two or three pairs of digits and a terminal digit; up to six sensory cups on each tentacle. One Polian vesicle. Stone canal single, unbranched. Anchor and anchor plates of one kind, large, anchors usually exceeding 120  $\mu\text{m}$  in length, plates exceeding 100  $\mu\text{m}$  in length. Miliary granules numerous, in form of C-shaped rods with enlarged ends and O-shaped ossicles present only in the longitudinal muscles. Tentacle ossicles curved spiny rods with perforated ends.

**Holotype description.** 19 mm total length (TL). When preserved is uniformly whitish, body wall translucent when expanded (Fig. 1); color in life pink to light purple. Gonads well developed, yellowish in preserved specimens (Fig. 1). Anchors (Fig. 2B) project through body wall. Tentacles 12, each with two to three pairs of digits and a terminal digit; digits increase in length distally, and terminal digit is longest. Inner (oral) surfaces of tentacles with double row of well-developed sensory cups; up to six sensory cups on each tentacle (Fig. 3). Ciliated funnels of various shapes (Fig. 4) occur on the body wall, not on the mesenteries. There are two longitudinal rows of ciliated funnels, each row attached to



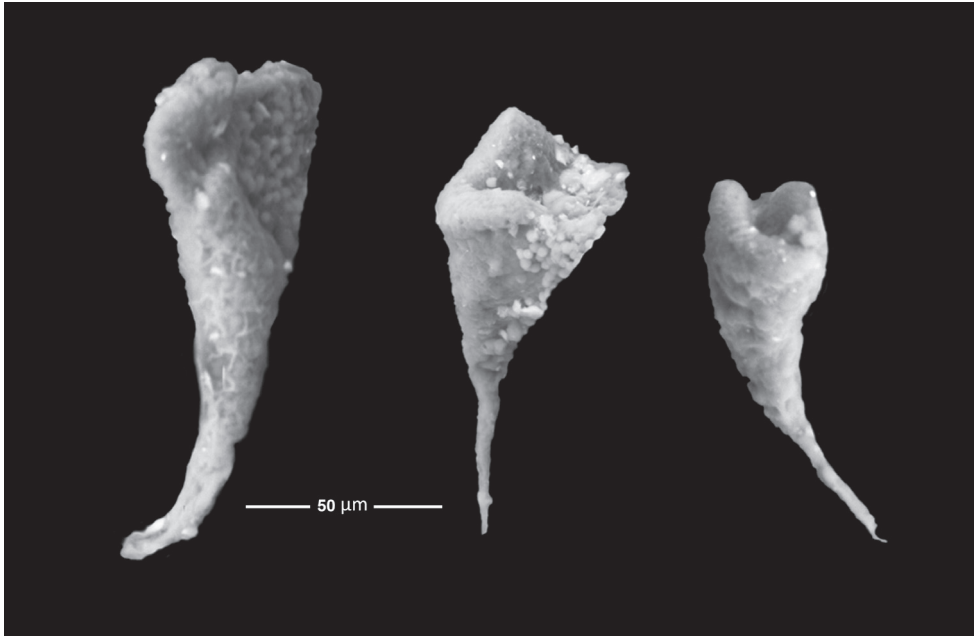
**Figure 2.** *Epitomapta simentalae* sp. n. Holotype ICML-UNAM 5.169.0, **A** Calcareous ring, r= radial piece, i= inter-radial piece **B** Anchors from mid-body, showing the detail of the posterior part **C** Anchor plates from mid-body **D** Rods from tentacles **E** Military granules from the body wall.



**Figure 3.** *Epitomapta simentalae* sp. n. Holotype ICML-UNAM 5.169.0. Detail of the tentacular crown showing the sensory cups.

one side of one longitudinal muscle. Polian vesicle single. Stone canal single, unbranched. Calcareous ring simple, well developed (Fig. 2A); the radial pieces (Fig. 2Ar) have a cavity in the central region more conspicuous than that in inter-radial pieces (Fig. 2Ai).

*Ossicles.* Body wall deposits, anchors, and anchor plates of one kind (Fig. 2B–C). Anchors and plates at anterior, middle and posterior body wall essentially similar, although developmental stages of these ossicles more numerous posteriorly; anchors of this region (in a ventral view) have the right arm slightly more elongated than the left. Anchors average 120  $\mu\text{m}$  in length. Arms carry up to six conspicuous teeth. Stock unbranched, but equipped with numerous small sharp projections (Fig. 2B). Anchor plates elongated, approximately oval, with numerous toothed perforations. Anchor plates average 100  $\mu\text{m}$  in length and 90  $\mu\text{m}$  in greatest width (Fig. 2C). Miliary granules numerous, present only in the epithelium covering the longitudinal muscles, highly variable in shape, but generally the miliary granules tending to be enlarged; C and O-shaped bodies are distinguishable



**Figure 4.** *Epitomapta simentalae* sp. n. Paratype ICML-UNAM 5.169.3. Ciliated funnels showing their different sizes and shapes.

Granules up to approximately 30  $\mu\text{m}$  in length (Fig. 2E). Stems of tentacles with ossicles similar to miliary granules of longitudinal muscle epithelium but tending to be slightly smaller. In tentacle digits spiny rods of up to 90  $\mu\text{m}$  length, with perforated ends (Fig. 2D).

*Paratype variations.* Specimens ranges from 4–43 mm TL. Sensory cups vary in number, fewer (2–3) in smaller specimens (4–15 mm TL).

**Ethymology.** *Epitomapta simentalae* sp. n. is named in honor of Dr Delia Rosalba Simental Crespo, a scientist, entrepreneur and echinoderm enthusiast, who supports research programs and marine expeditions providing passion, funding, equipment, and travel support to scientists who are involved in research and conservation efforts related to the echinoderms in the Mexican marine waters.

**Ecology.** *Epitomapta simentalae* sp. n. occurs at 4–10 m depth, burrowed approximately 2 cm deep in in well-aerated quartz sand.

**Reproduction.** *Epitomapta simentalae* sp. n. is a gonochoric species; females have lecithotrophic eggs between 140 and 150  $\mu\text{m}$  in diameter; ripe gonads occupy about 80% of the celomic cavity. Neither brooding nor external sexual dimorphism was observed.

**Geographical distribution.** Known only from Caleta, Acapulco Bay, Guerrero.

## Discussion

*Epitomapta simentalae* sp. n. is very similar to its Caribbean congener *E. roseola*, differing in the number of pairs of digits present on the tentacles (2–4 in *E. roseola* and 2–3 in *E. simentalae* sp. n.), and in the number of sensory cups per tentacle (2–5 in *E. roseola* and 4–6

in *E. simentalae* sp. n.). In addition to the geographical distribution, *E. simentalae* sp. n. is smaller (<50 mm) than *E. roseola* (30–120 mm) (Heding 1928, Miranda et al. 2015).

*Epitomapta simentalae* sp. n. clearly differs from *E. tabogae* and *E. roseola* in lacking papillae or oval bumps all over its body wall; the number of sensory cups per tentacle (8–14 in *E. tabogae* and 4–6 in *E. simentalae* sp. nov), and in the number of pairs of digits present on the tentacles (5–6 in *E. tabogae* and 2–3 in *E. simentalae* sp. n.). *Epitomapta tabogae* is distributed throughout the Gulf of California (Solís-Marín et al. 2009) whereas *E. simentalae* sp. n. is currently known only from the Central Eastern Pacific coast of Mexico. *Epitomapta roseola* was previously described for the Caribbean (Bermuda) (Heding 1928), and later recorded in Connecticut, Massachusetts to Florida (USA) (Hendler et al. 1995) and recently reported for the South American coast (Brazil) (Miranda et al. 2015).

The anchors of the body wall in *E. simentalae* sp. n. are similar in shape to those of *E. roseola*, but differ in size, being approximately 90–150 µm length and 70–90 µm width in *E. simentalae* sp. n. (Fig. 2B); the anchors of the posterior region of the body wall in both species are similar and can reach up to 150 µm in length and 70 µm width; anchors from the anterior end of the body wall in *E. roseola* measure almost 120 µm in length and 70 µm in width (Heding 1928), while in *E. simentalae* sp. n. they measure from 90–150 µm length and 70 µm width. On the other hand, the anchors of the Pacific *E. tabogae* are 200 µm in length and 100 µm width in the posterior region of the body, and 170 µm length and 100 µm width in the anterior region of the body (Heding 1928); *E. tabogae* has the largest anchors in this genus (Heding 1928).

In *Epitomapta simentalae* sp. n. the anchor plates are 100 µm in length and 90 µm in width.

*Epitomapta simentalae* sp. n. is clearly distinguished from other species of the genus in lacking papillae or oval bumps in the body wall, a character that had been used to differentiate species of the genus by various authors (see Heding 1928 and Hendler et al. 1995).

**Key to the genus *Epitomapta***

- 1 Papillae or oval bumps present all over the body wall ..... **2**
- Papillae or oval bumps absent. With 2–3 pairs of tentacle digits, each tentacle with 4–6 sensory cups. Miliary granules “C” and “O”-shaped bodies; no papillae or oval bumps present in the body wall ..... ***E. simentalae* sp. n.**
- 2 Atlantic Ocean. With 7 pairs of tentacle digits, each tentacle with 2–5 sensory cups. Anchors of body wall exceed 120 µm in length (up to 150 µm). Miliary granules in the shape of small oval rings and very few C-shaped bodies ..... ***E. roseola***
- Pacific Ocean. With 5–6 pairs of tentacle digits, each tentacle with 8–14 sensory cups. Anchors of body wall exceed 120 µm in length (up to 200 µm). Miliary granules in the shape of oval rings and very few C-shaped bodies.... ***E. tabogae***



## Acknowledgements

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# *Neocaridina fonticulata*, a new land-locked freshwater shrimp from Hengchun Peninsula, Taiwan (Decapoda, Caridea, Atyidae)

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## Abstract

A new species of land-locked freshwater shrimp, *Neocaridina fonticulata* **sp. n.** (Atyidae), is described from Kenting, Hengchun Peninsula, Pingtung County, southern Taiwan. This new species can be distinguished from its congeners by rostrum structure, pereopods, and male first and second pleopods. The molecular evidence of mitochondrial cytochrome oxidase subunit I (COI) also supports the establishment of a new species. This is the third endemic species of *Neocaridina* known from Taiwan.

## Keywords

*Neocaridina fonticulata*, mitochondrial cytochrome oxidase subunit I, new species, morphology

## Introduction

The genus *Neocaridina* Kubo, 1938 is a group of small-sized shrimps with a land-locked habit, inhabiting in the middle and upper reaches of rivers in East Asia, with more than 30 species recorded (Liang 2004, De Grave and Fransen 2011, Shih et al. 2017). In the

East Asian arc, three species have been reported from Taiwan, viz. *N. davidi* (Bouvier, 1904), *N. ketagalan* Shih & Cai, 2007 and *N. saccam* Shih & Cai, 2007; two species from the Ryukyus, viz. *N. ishigakiensis* (Fujino & Shokita, 1975) and *N. iriomotensis* Naruse, Shokita & Cai, 2006; and two species from the main islands of Japan, viz. *N. denticulata* (De Haan, 1844) and *N. ikiensis* Shih, Cai, Niwa & Nakahara, 2017, with several introduced species reported (Naruse et al. 2006, Shih and Cai 2007, Shih et al. 2017).

A recent survey of the species diversity of freshwater shrimps of Taiwan showed an undescribed species from southern Taiwan with different morphological characters compared to other known species of *Neocaridina*, which was supported by molecular evidence. This species is herein described as a new species, endemic to Taiwan Island, which brings the total number of Taiwanese species of *Neocaridina* to four.

## Materials and methods

Specimens of the genus *Neocaridina* examined in this study were collected from a spring in Sheding, Kenting, Hengchun Peninsula, Pingtung County, Taiwan and preserved in 70%–95% ethanol after collection. Some specimens were selected and illustrated with the help of a drawing tube attached to a Nikon stereo microscope (model SMZ 1000), and deposited in the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (**NCHUZOOL**) and the Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore, Singapore (formerly the Raffles Museum of Biodiversity Research) (**ZRC**). Carapace length is abbreviated cl, and the mode refers to the most frequently number occurring. The rostral formula was counted based on all specimens available. The egg measurements were based on five eggs each from four ovigerous females (see material examined).

Sequences of mitochondrial cytochrome oxidase subunit I (COI) were obtained following the method described by Shih et al. (2017), with the primers LCO1490 and HCO2198 (Folmer et al. 1994). Sequences were obtained by automated sequencing (Applied Biosystems 3730xl DNA Analyzer), after verification with the complementary strand. Sequences obtained have been deposited in the DNA Data Bank of Japan (DDBJ) and were analyzed with other sequences published in Shih and Cai (2007) and Shih et al. (2017).

The best-fitting model for sequence evolution was determined by MrModeltest (version 2.2, Nylander 2005), selected by the Akaike information criterion (AIC). The obtained best model was HKY + G, and was subsequently used for the Bayesian inference (BI) analysis. The BI analysis was performed with MrBayes (version 3.2.3, Ronquist et al. 2012). The search was run with four chains for 10 million generations and four independent runs, with trees sampled every 1000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended 0.01 (Ronquist et al. 2005) and the first 1150 trees were discarded as the burnin accordingly. The maximum likelihood (ML) analysis was conducted in RAxML (vers. 7.2.6, Stamatakis 2006). The model GTR + G (i.e.,

GTRGAMMA) was used with 100 runs, and found the best ML tree by comparing the likelihood scores. The robustness of the ML tree was evaluated by 1000 bootstrap pseudoreplicates under the model GTRGAMMA.

Other analyses, including the nucleotide composition, variable and parsimony informative positions, Kimura 2-parameter (K2P) distance (Kimura 1980) and p-distance between haplotypes were calculated using MEGA (version 5.2.2, Tamura et al. 2011).

## Systematic account

### Family Atyidae De Haan, 1849

### *Neocaridina* Kubo, 1938

### *Neocaridina fonticulata* sp. n.

<http://zoobank.org/5F96C596-4AF1-43E1-971B-BA49C15D5E1F>

Figures 1–4

**Material examined.** Holotype: male, cl 3.4 mm, NCHUZOOOL 15004, a spring at Sheding, Kenting, Pingtung County, Taiwan, 21°57'26.7"N, 120°48'35.5"E, elevation of 150 m, coll. H.-T. Shih and Y. C. Gan, 1 July 2015. Paratypes: 13 males, cl 2.5–3.3 mm, NCHUZOOOL 15005, 5 females, cl 2.6–3.8 mm, 2 ovigerous females, cl 3.6–3.7 mm, NCHUZOOOL 15006; 1 male, cl 4.2 mm, NCHUZOOOL 15007; 1 male, cl 3.9 mm, NCHUZOOOL 15008; 7 males, cl 2.7–3.3 mm, 2 females, cl 3.6–3.8 mm, 2 ovigerous females, cl 3.5–3.6 mm, ZRC 2018.1013, same collection data as for holotype. 1 male, cl 3.5 mm, 1 damaged specimen, cl 4.4 mm, NCHUZOOOL 15009, Sheding, Kenting, Pingtung County, Taiwan, 5 May 2015, coll. Y. C. Gan.

**Other material.** 3 males, 9 females, NCHUZOOOL 15010, Sheding, Kenting, Pingtung County, Taiwan, coll. Y. C. Gan, 5 May 2015. 3 males, 11 females, 2 ovigerous females, NCHUZOOOL 15011, two damaged males, ZRC 2018.1014, same collection data as for holotype.

**Comparative material.** *Neocaridina ikiensis*: 1 male, cl 4.6 mm, ZRC 2017.0960, 1 female, cl 5.1 mm, ZRC 2017.0961, 8 males, cl 3.0–5.4 mm, 8 females, cl 2.9–5.1 mm, ZRC 2017.0962, small stream at Kugiyama-hure, Gonoura Town, Iki City, Nagasaki Prefecture, Japan, coll. Yasuhiko Nakahara, 23 November 2015.

**Diagnosis.** Rostrum short, straight, slightly sloping downwards, reaching mostly to end of 1<sup>st</sup> segment of antennular peduncle, rostral formula 1–3+8–15/1–4. Pterygostomian margin armed with an indistinct spine. 1<sup>st</sup> pereopod carpus 1.2–1.5 × as long as high; chela 2.0–2.1 × as long as broad; fingers slightly longer than palm. 2<sup>nd</sup> pereopod carpus 1.1–1.2 × as long as chela, 3.9–4.3 × as long as high; chela 2.1–2.3 × as long as broad; fingers 1.3–1.4 × as long as palm. 3<sup>rd</sup> pereopod with propodus straight in females, slightly incurved in males, 2.7–3.0 × as long as dactylus; dactylus terminating in two claws, 4–6 accessory spines on flexor margin, strongly incurved in males. 5<sup>th</sup> pereopod propodus 2.7–2.8 × as long as dactylus, dactylus terminating in

one claw, with 46–54 spinules on flexor margin. Endopod of male 1<sup>st</sup> pleopod extending to  $0.8 \times$  exopod length, inflated at distal  $\frac{3}{4}$ , pyriform,  $1.7 \times$  as long as wide, appendix interna at base of inflated part, short. Appendix masculina of male 2<sup>nd</sup> pleopod cylindrical, reaching to 0.7 length of endopod, appendix interna reaching to 0.6 length of appendix masculina. Uropodal diaeresis with 13–14 movable spinules. Eggs  $1.10 \times 0.68$  to  $1.20 \times 0.75$  mm in diameter.

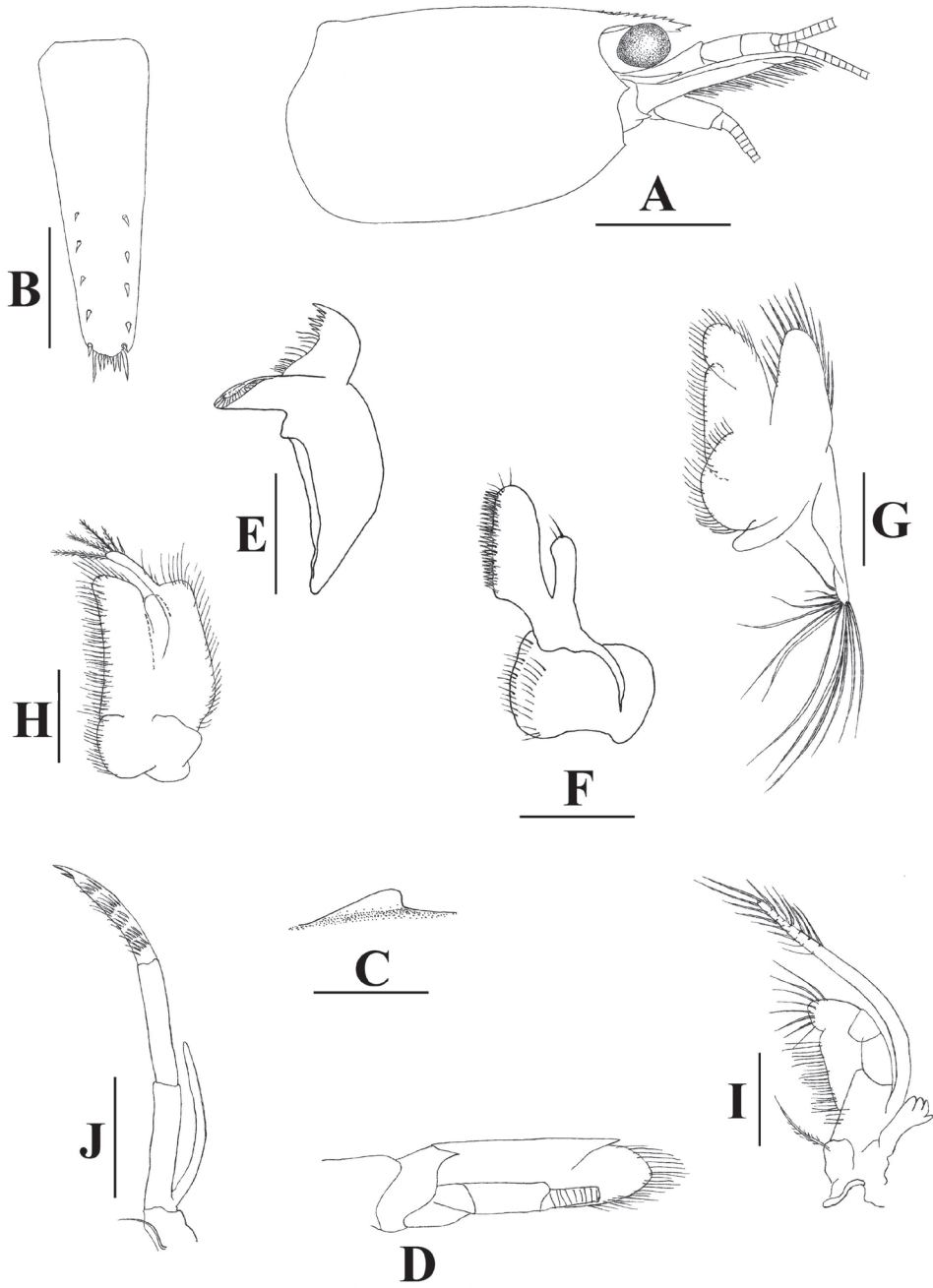
**Description.** Rostrum short, straight, slightly sloping downwards, without distinct postrostral ridge, reaching slightly short of or slightly beyond end of 1<sup>st</sup> segment of antennular peduncle, occasionally reaching to, rarely beyond end of 2<sup>nd</sup> segment of antennular peduncle; armed dorsally with 9–18 (mode 13–15) very small teeth, including 1–3 (mode 2) on carapace, ventrally with 1–4 small teeth (mode 2–3). Antennal spine fused with inferior orbital angle. Pterygostomian margin sub-rectangular, armed with an indistinct spine.

Sixth pleomere in male 0.43cl,  $1.40 \times$  as long as 5<sup>th</sup> pleomere, slightly shorter than telson; 6<sup>th</sup> pleomere in female 0.48cl,  $1.38 \times$  as long as 5<sup>th</sup> pleomere, slightly shorter than telson. Telson  $3.0 \times$  as long as wide, with four or five pairs of dorsal spinules and one pair of dorsolateral spinules; posterior margin rounded, lined with four or five pairs of simple setae, lateral pair distinctly longer than intermediate pairs. Pre-anal carina moderately high, lacking spine.

Eyes well developed, anterior corneal margin reaching to  $0.6 \times$  length of basal segment of antennular peduncle. Antennular peduncle  $0.6 \times$  as long as carapace; basal segment of antennular peduncle longer than combined length of 2<sup>nd</sup> and 3<sup>rd</sup> segments, anterolateral angle reaching 0.3 length of 2<sup>nd</sup> segment, 2<sup>nd</sup> segment distinctly longer than 3<sup>rd</sup> segment. Stylocerite reaching 0.7–0.8 length of basal segment of antennular peduncle. Scaphocerite  $3.5 \times$  as long as wide, with extension of the distolateral spine reaching end of antennular peduncle.

Mandible with incisor process ending in irregular teeth; molar process truncated. Maxillule lower lacinia broadly rounded; upper lacinia elongate, with a row of 30 distinct spiniform setae on inner margin; palp short. Maxilla distal endite subdivided; palp short; scaphognathite tapering posteriorly with some long, curved setae at posterior end. 1<sup>st</sup> maxilliped with stout palp. 2<sup>nd</sup> maxilliped typical of genus, endopod with fused dactylus and propodal segments. 3<sup>rd</sup> maxilliped reaching to end of antennular peduncle, with ultimate segment slightly longer than penultimate segment.

First four pereopods with epipod. 1<sup>st</sup> pereopod reaching slightly beyond distal end of basal segment of antennular peduncle; merus  $1.8$ – $2.1 \times$  as long as broad, as long as carpus; carpus excavated anteriorly, shorter than chela,  $1.2$ – $1.5 \times$  as long as high; chela  $2.0$ – $2.1 \times$  as long as broad; fingers slightly longer than palm. 2<sup>nd</sup> pereopod reaching end of antennular peduncle; merus shorter than carpus,  $3.6$ – $4.1 \times$  as long as broad; carpus  $1.1$ – $1.2 \times$  as long as chela,  $3.9$ – $4.3 \times$  as long as high; chela  $2.1$ – $2.3 \times$  as long as broad; fingers  $1.3$ – $1.4 \times$  as long as palm. 3<sup>rd</sup> pereopod reaching beyond end of antennular peduncle by dactylus; merus stout; propodus straight in females, slightly incurved in males,  $2.7$ – $3.0 \times$  as long as dactylus (terminal claw included),  $7.2$ – $7.5 \times$



**Figure 1.** *Neocaridina fonticulata* sp. n.: **A** carapace and cephalic appendages, lateral view **B** telson, dorsal view **C** preanal carina, lateral view **D** right scaphocerite and antenna, ventral view **E** right mandible **F** right maxillula **G** right maxilla **H** right 1<sup>st</sup> maxilliped **I** right 2<sup>nd</sup> maxilliped **J** right 3<sup>rd</sup> maxilliped. Scale bars: 1.5 mm (**A**); 0.5 mm (**B**, **E**-**J**); 1 mm (**C**, **D**) (male, cl 3.0 mm, paratype, ZRC 2018.1013).

as long as broad, numerous spinules on posterior margin; dactylus terminating in two claws, 4–6 accessory spines on flexor margin, strongly incurved in males. 4<sup>th</sup> pereiopod similar to 3<sup>rd</sup> pereiopod in form and length. 5<sup>th</sup> pereiopod reaching to end of 2<sup>nd</sup> segment of antennular peduncle, propodus 8.0–9.5 × as long as broad, 2.7–2.8 × as long as dactylus, dactylus 2.9–3.4 × as long as wide (spinules included), terminating in one claw, with 46–54 spinules on flexor margin.

Endopod of male 1<sup>st</sup> pleopod extending to 0.8 × exopod length, inflated at distal ¾, pyriform, 1.7 × as long as wide, with tiny spinules on distal margin of dorsal surface, appendix interna at base of inflated part, short. Appendix masculina of male 2<sup>nd</sup> pleopod cylindrical, reaching to about 0.7 length of endopod, inner and distal surface densely lined with long, stout spines, appendix interna reaching to 0.6 length of appendix masculina.

Uropodal diaeresis with 13–14 movable spinules.

Eggs 1.10 × 0.68 to 1.20 × 0.75 mm in diameter.

**Colour in life.** Body colour varying from translucent to light blue, with darker red-brown spots on dorsal surface and lighter red-brown spots on lateral surface of carapace; pleon usually with several dark red-brown vertical stripes on lower lateral surface, and white star-shaped pigment scattered on whole body (Figure 4A–D). Appendages mostly transparent.

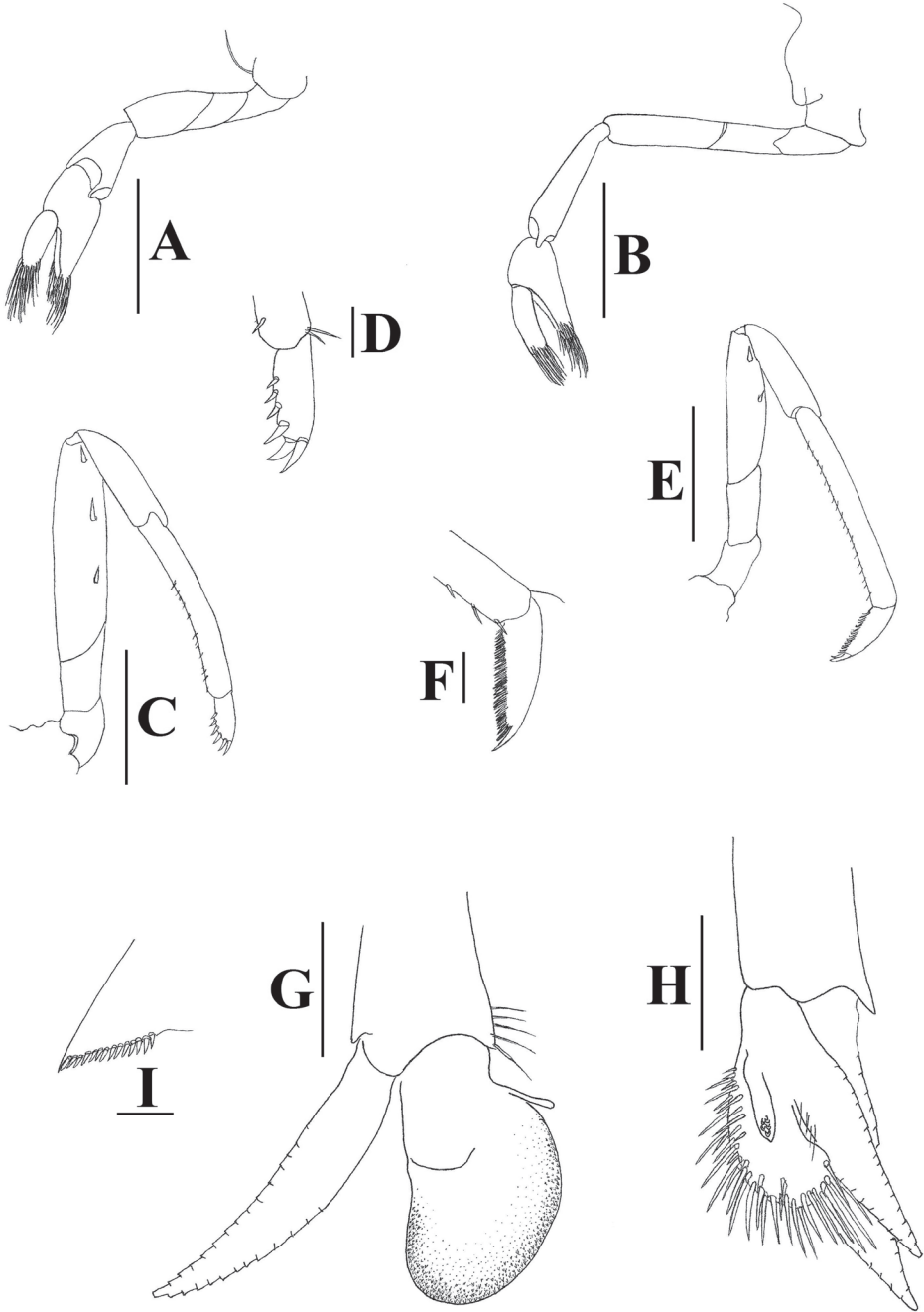
**Etymology.** *Neocaridina fonticulata* is named after its known habitat, from the Latin root, *fonticulus*, for little spring.

**Ecological notes.** Specimens of the new species were collected from leaf litter layer of a small stream (Figure 4E, F) next to a spring outlet at a limestone hill. The collection site consists of concretized substrate and banks, representing the headwater of the stream. The water flow is slow, cool temperature (about 25 °C), neutral (pH 7.06–7.16), and with moderately high dissolved oxygen (7.33–7.70 mg/L). The freshwater crabs, *Candidiopotamon rathbuni* (De Man, 1914) and *Geothelphusa ferruginea* Shy, Ng & Yu, 1994, were found to be sympatric with this new species. Oviparous females were found in July.

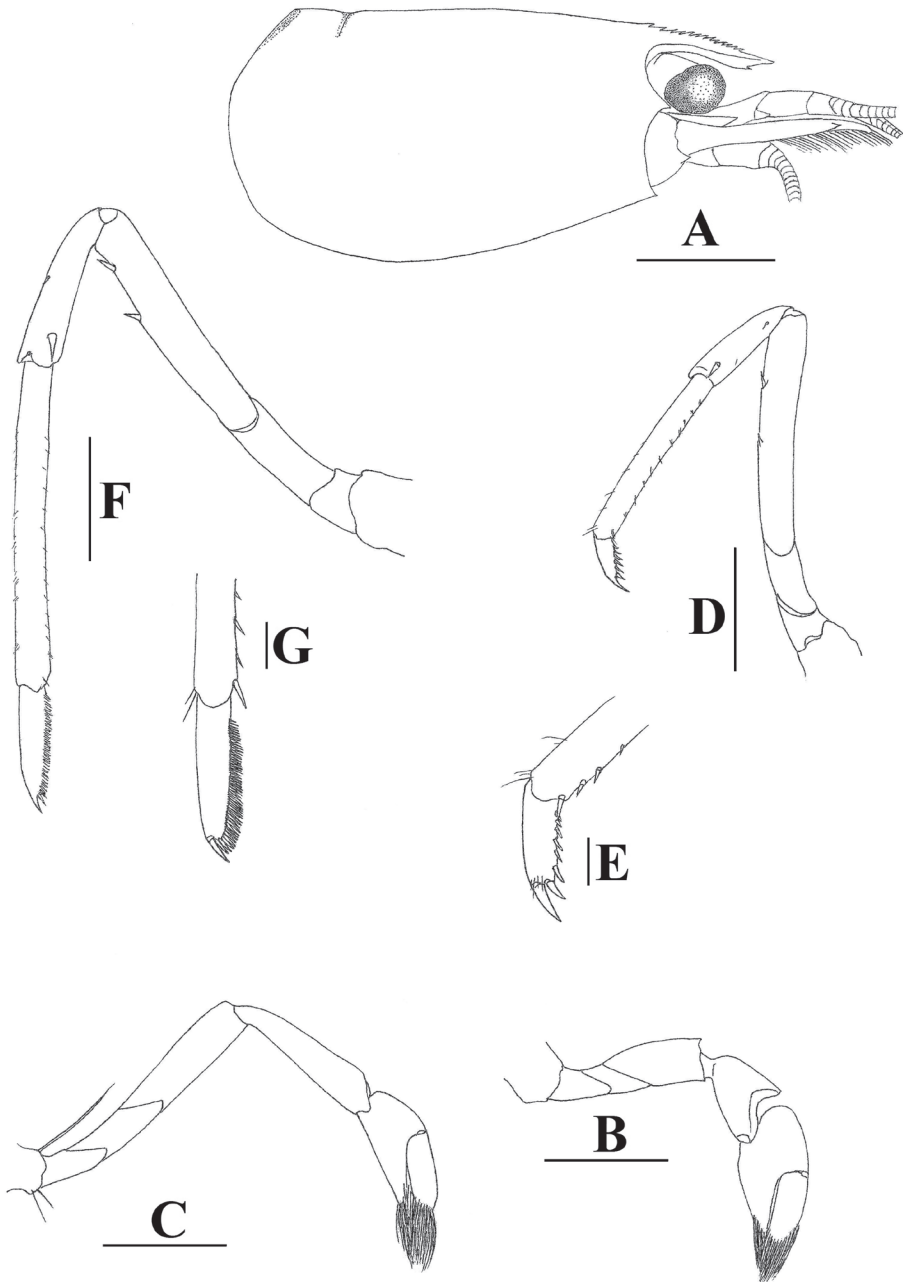
**Distribution.** Presently known only from Shedding, Kenting, southern Taiwan.

**Remarks.** With the short rostrum, *Neocaridina fonticulata* sp. n. is morphologically most similar to the insular Chinese species *Neocaridina zhoushanensis* Cai, 1996, originally described as a subspecies of *N. denticulata*, from Zhoushan Islands of Zhejiang Province. It can be differentiated by the more slender chela of the 1<sup>st</sup> pereiopod (2.0–2.1 × as long as wide in the new species vs. 1.6–1.7 × in *N. zhoushanensis*; cf. Figures 2A, 3B vs. fig. 10B in Cai 1996); the sexually dimorphic 3<sup>rd</sup> pereiopods (vs. no sexual dimorphism in *N. zhoushanensis*); the slender dactylus of the 3<sup>rd</sup> pereiopods (2.9–3.4 × as long as wide (spinules included) vs. 2.8 × in *N. zhoushanensis*; cf. Figures 2C, 3D vs. fig. 10E in Cai 1996), the spination of the dactylus of 5<sup>th</sup> pereiopods (with 46–54 spinules on flexor margin vs. 35–43 in *N. zhoushanensis*); and the shorter appendix interna on the male 2<sup>nd</sup> pleopod, which reaches to 0.6 length of appendix masculina (vs. 0.7 in *N. zhoushanensis*; cf. Figure 2H vs. fig. 9F in Cai 1996).





**Figure 2.** *Neocaridina fonticulata* sp. n.: pereopods in lateral view. **A** right 1<sup>st</sup> pereopod **B** right 2<sup>nd</sup> pereopod **C** right 3<sup>rd</sup> pereopod **D** same, dactylus **E** right 5<sup>th</sup> pereopod **F** same, dactylus **G** right male 1<sup>st</sup> pleopod, front view **H** right male 2<sup>nd</sup> pleopod, internal view **I** diaeresis of left uropodal exopod. Scale bars: 1 mm (**A–C, E**); 0.2 mm (**D, F**); 0.5 mm (**G, H**); 0.2 mm (**I**) (male, cl 3.0 mm, paratype, ZRC 2018.1013).



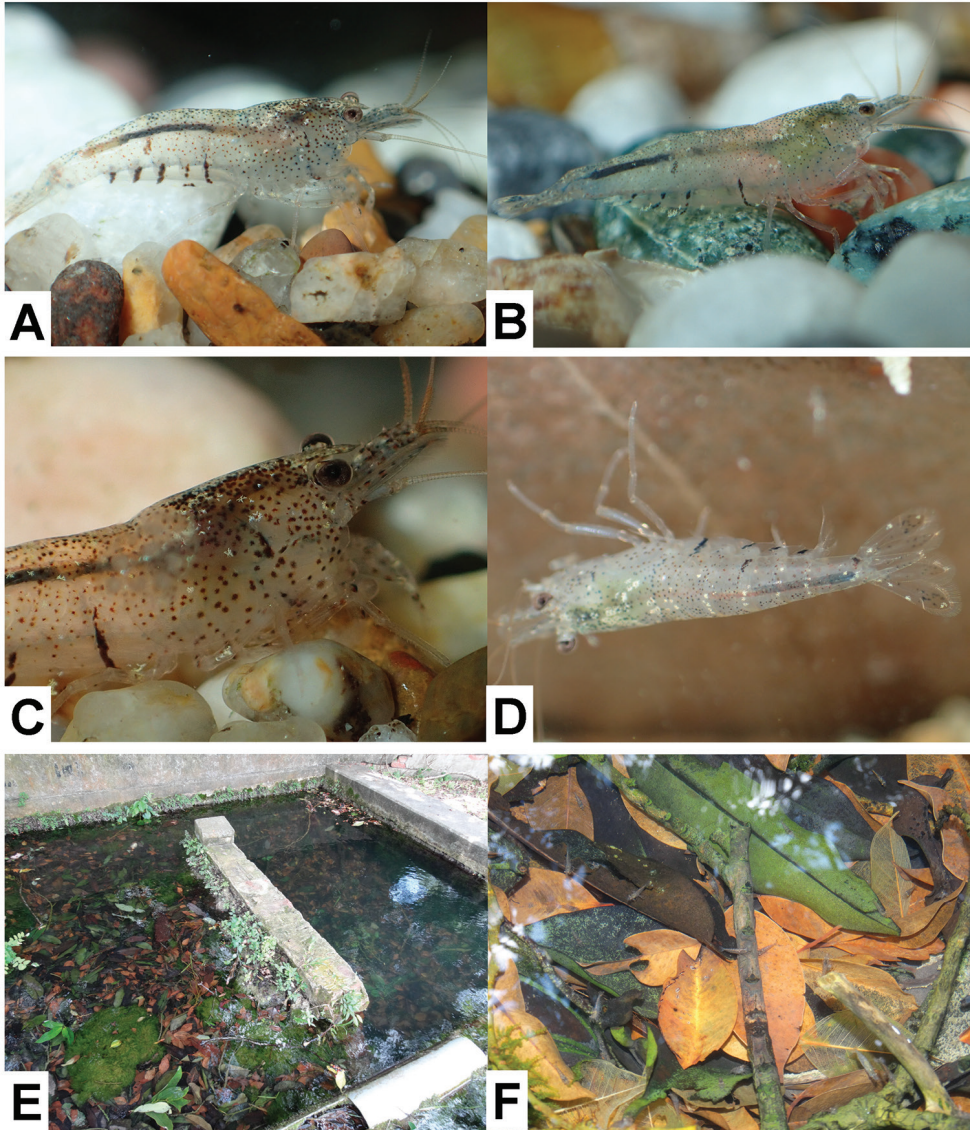
**Figure 3.** *Neocaridina fonticulata* sp. n.: **A** carapace and cephalic appendages, lateral view **B** right 1<sup>st</sup> pereopod **C** right 2<sup>nd</sup> pereopod **D** left 3<sup>rd</sup> pereopod **E** same, dactylus **F** left 5<sup>th</sup> pereopod **G** same, dactylus. Scale bars: 1.5 mm (**A**); 1 mm (**B–D, F**); 0.2 mm (**E, G**) (female, cl 3.8 mm, paratype, ZRC 2018.1013).

With the relatively short rostrum, *Neocaridina fonticulata* sp. n. morphologically resembles two Taiwanese species, *N. saccam* Shih & Cai, 2007 and *N. ketagalan* Shih & Cai, 2007. It differs from *N. saccam* (cf. Shih and Cai 2007) by the shorter rostrum (falling slightly short of or reaching slightly beyond the end of the 1<sup>st</sup> segment of the antennular peduncle vs. reaching the midlength of the 2<sup>nd</sup> segment of the antennular peduncle or nearly reaching beyond it in *N. saccam*; cf. Figures 1A, 3A vs. figs 2A, 4A in Shih and Cai 2007); the more slender merus of the 1<sup>st</sup> pereopod ( $1.8\text{--}2.0 \times$  as long as high vs.  $1.4\text{--}1.7 \times$  in *N. saccam*; Figures 2A, 3B vs. figs 3A, 4B in Shih and Cai 2007); the more slender carpus of the 2<sup>nd</sup> pereopod ( $3.9\text{--}4.3 \times$  as long as high vs.  $3.6\text{--}3.8 \times$  in *N. saccam*; Figures 2B, 3C vs. figs 3B, 4C in Shih and Cai 2007); the more slender male 1<sup>st</sup> pleopod ( $1.7 \times$  as long as broad vs.  $1.4 \times$  in *N. saccam*; Figure 2G vs. fig. 3G in Shih and Cai 2007); and the shorter endopod of the male 1<sup>st</sup> pleopod ( $0.8 \times$  length of exopod vs.  $0.9 \times$  in *N. saccam*; Figure 2G vs. fig. 3G in Shih and Cai 2007).

*Neocaridina fonticulata* sp. n. can be separated from *N. ketagalan* (cf. Shih and Cai 2007) by its shorter rostrum (reaching from slightly short of to slightly beyond end of 1<sup>st</sup> segment of antennular peduncle vs. reaching the middle or end of the 2<sup>nd</sup> segment of the antennular peduncle; Figures 1A, 3A vs. figs 5A, 6A in Shih and Cai 2007). It also differs from *N. ketagalan* (cf. Shih and Cai 2007) by the slender male 1<sup>st</sup> pleopod ( $1.7 \times$  as long as broad vs.  $1.4 \times$  in *N. ketagalan*; Figure 2G vs. fig. 5J in Shih and Cai 2007); the male 2<sup>nd</sup> pleopod appendix masculina being half the endopod length (vs.  $0.7 \times$  in *N. ketagalan*; Figure 2H vs. fig. 5K in Shih and Cai 2007); and the appendix interna of the male 2<sup>nd</sup> pleopod being relatively longer, reaching to  $0.7 \times$  length of appendix masculine (vs.  $0.6 \times$  in *N. ketagalan*; Figure 2H vs. fig. 5K in Shih and Cai 2007).

With its relatively short rostrum, *Neocaridina fonticulata* sp. n. morphologically also resembles the recently described Japanese species *Neocaridina ikiensis* Shih, Cai, Niwa & Nakahara, 2017. It can be differentiated from the latter by its shorter rostrum (reaching from slightly short of to slightly beyond the end of the 1<sup>st</sup> segment of antennular peduncle vs. reaching slightly short of to distinctly beyond the end of the 2<sup>nd</sup> segment of antennular peduncle; cf. Figures 1A, 3A vs. figs 2A, 4A in Shih et al. 2017). The propodus and dactylus of the 3<sup>rd</sup> pereopod of the new species displays sexual dimorphism (vs. no sexual dimorphism in *N. ikiensis*); the male 2<sup>nd</sup> pleopod appendix masculina is  $0.7 \times$  endopod length (vs.  $0.5 \times$  in *N. ikiensis*; Figure 2H vs. fig. 3I in Shih et al. 2017); and the appendix interna of the male 2<sup>nd</sup> pleopod is relatively shorter (reaching to  $0.6$  length of appendix masculina vs.  $0.8$  in *N. ikiensis*; Figure 2H vs. fig. 3J in Shih et al. 2017).

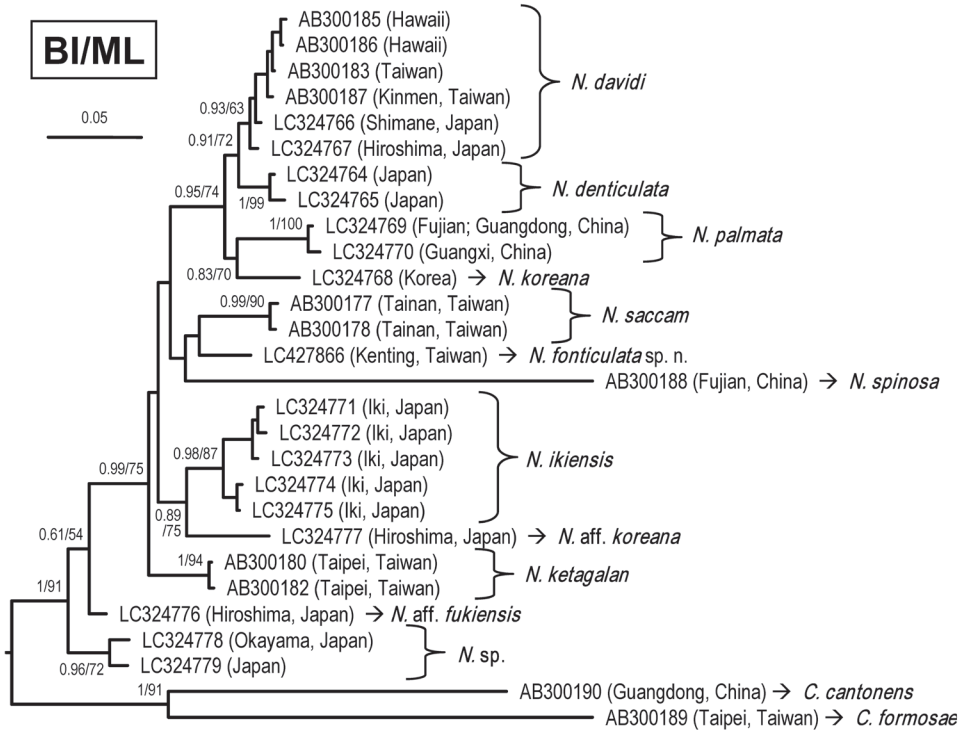
With the relatively slender endopod of the male 1<sup>st</sup> pleopod, the new species is similar to *N. koreana* Kubo, 1938. It can be separated from the latter by the relatively shorter rostrum, which mostly reaches to or slightly beyond the end of the 1<sup>st</sup> segment of antennular peduncle vs. almost reaching to or slightly beyond antennular peduncle in *N. koreana* (cf. Kubo 1938); and the fewer ventral rostral teeth (2–6 teeth, mode 2–4 vs. 4–6, average 5.6 in *N. koreana* (cf. Kubo 1938)).



**Figure 4.** Live colouration of *Neocaridina fonticulata* sp. n. (A–D) and its habitat in Kenting, southern Taiwan (E–F). Specimens were collected from the type locality on 1 July 2015 and kept in aquarium for observation and photography.

### DNA analyses and discussion

A total of four specimens from Shedding, Kenting, were used in the molecular phylogenetic analysis. A 658-bp segment of COI was amplified, resulting in one haplotype (accession number LC427866). Based on the COI haplotypes, the phylogenetic tree was reconstructed using BI analysis, with the support values from the BI and ML analyses



**Figure 5.** Bayesian inference (BI) tree of species of *Neocaridina* from East Asia and outgroups based on COI gene. Support values at the nodes represent posterior probability and bootstrap values for BI and maximum likelihood (ML), respectively.

shown in Figure 5. Specimens assigned to *Neocaridina fonticulata* sp. n. formed a clade distinct from other species. The pairwise nucleotide divergences with the K2P distance and bp differences of haplotypes are shown in Table 1. The minimum K2P interspecific divergences between *N. fonticulata* sp. n. and *N. ketagalan* and *N. saccam* are 5.42% and 5.43% respectively, which are close to or larger than the values between species of *N. davidi*, *N. denticulata*, *N. koreana*, and *N. palmata* (from 2.17% to 5.56%; Table 1). Consequently, the establishment of the new taxon is warranted.

The discovery of the new species increases the number of *Neocaridina* species in Taiwan to four, i.e., *N. davidi*, *N. saccam*, *N. ketagalan*, and *N. fonticulata* sp. n. (Shih and Cai 2007, Shih et al. 2017). While the common species, *N. davidi*, is distributed in both western and eastern sides of Taiwan Island, as well as the offshore islands, Penghu, Siao-liouciou and Kinmen (Shih and Cai 2007, Shih et al. 2017; unpublished data), the distributional range of the other three endemic species is narrower. *Neocaridina ketagalan* is distributed in northern Taiwan, *N. saccam* is limited in southwestern Taiwan, and *N. fonticulata* sp. n. is presently known only from Kenting. Previous molecular studies on aquatic organisms, including the freshwater crab *Candidiopotamon rathbuni* (De Man, 1914) and frog *Hylarana latouchii* (Boulenger, 1899) (= *Sylvirana latouchii*)

**Table 1.** Matrix of percentage pairwise nucleotide divergences (lower left) and mean number of differences (upper right) based on COI within and between some species of *Neocaridina* from East Asia. Values of range are shown in parentheses.

	Intraspecific		Interspecific						
	Nucleotide divergence	Mean nucleotide difference	<i>N. davidi</i>	<i>N. denticulata</i>	<i>N. koreana</i>	<i>N. palmata</i>	<i>N. fonticulata</i> sp. n.	<i>N. ketagalan</i>	<i>N. saccam</i>
<i>N. davidi</i>	0.67 (0–1.54)	4.39 (0–10)		17.75 (14–22)	29.88 (28–32)	30.63 (28–34)	36.5 (35–39)	44.46 (41–48)	45.67 (42–50)
<i>N. denticulata</i>	0.46 (0–0.77)	3 (0–5)	2.77 (2.17–3.46)		31.5 (30–33)	33.17 (31–36)	38.5 (36–41)	48.83 (46–52)	48.83 (47–51)
<i>N. koreana</i>	0 (0)	0 (0)	4.73 (4.42–5.07)	5 (4.75–5.25)		35.67 (35–37)	46 (46–46)	46.33 (46–47)	52.33 (52–53)
<i>N. palmata</i>	0.41 (0–0.61)	2.67 (0–4)	4.83 (4.4–5.39)	5.26 (4.9–5.73)	5.67 (5.56–5.89)		47 (47)	48.33 (48–49)	53 (53–53)
<i>N. fonticulata</i> sp. n.	0 (0)	0 (0)	5.82 (5.57–6.24)	6.16 (5.74–6.59)	7.41 (7.41)	7.56 (7.56–7.56)		34.67 (34–35)	34.33 (34–35)
<i>N. ketagalan</i>	0.1 (0–0.15)	0.67 (0–1)	7.17 (6.58–7.78)	7.94 (7.44–8.5)	7.49 (7.44–7.61)	7.82 (7.76–7.93)	5.53 (5.42–5.58)		38 (37–39)
<i>N. saccam</i>	0.31 (0–0.46)	2 (0–3)	7.39 (6.75–8.14)	7.94 (7.62–8.32)	8.51 (8.45–8.63)	8.62 (8.62–8.62)	5.49 (5.43–5.6)	6.08 (5.91–6.25)	

(Shih et al. 2006, Jang-Liaw et al. 2008), have shown the populations in Hengchun Peninsula to be closely related to the populations in eastern Taiwan due to the weak isolation effect of the lower mountains. It is expected that *Neocaridina fonticulata* sp. n. will be found in eastern Taiwan as well.

## Acknowledgements

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# A survey of parasitoids from Greece with new associations

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## Abstract

We report 22 parasitoid species from Greece that have emerged from their hosts belonging to Blattodea, Coleoptera, Hymenoptera and Lepidoptera, including 12 Braconidae, one Eulophidae, one Evaniidae, seven Ichneumonidae, and one Tachinidae. Nine parasitoids are reported for the first time in Greece, i.e., three Ichneumonidae: *Campoplex difformis* (Gmelin, 1790), *Gelis albipalpus* (Thomson, 1884), and *Lysibia tenax* Townes, 1983; five Braconidae: *Charmon cruentatus* Haliday, 1833, *Dendrosoter protuberans* (Nees, 1834), *Dolichogenidea longipalpis* (Reinhard, 1880), *Ecpshylus silesiacus* (Ratzeburg, 1848), and *Spathius curvicaudis* Ratzeburg, 1844; and one Eulophidae: *Melittobia acasta* (Walker, 1839). Nine of the 23 recorded parasitoid-host associations are new. These findings are discussed in relation to the overall related parasitoid-host associations in the target area, as well as the potential of parasitoid use in the biological control of pests.

## Keywords

Balkans, field pests, natural enemies, urban pests

## Introduction

Parasitoids, especially those belonging to Hymenoptera, are important elements of agroecosystems (Godfray 1994). Faunistic surveys of parasitoids constitute the major baselines upon which further applicative studies are based (Tomanović et al. 2014; Petrović et al. 2019). Many pest species, especially lepidopterans, are prone to increase their abundance if conditions are favorable and inflict significant hazards in agriculture and forestry (Elkinton and Liebhold 1990; Devetak et al. 2014). As many parasitoids are able to attack several host species or to specialize on a certain host species, their population dynamics depends on their hosts' abundance, and thus, they are of major importance in controlling pests (Hassell 1980; Hassell and May 1986; Barbosa 1998; Gagić et al. 2016). Systematic investigations of parasitoid fauna in Greece, except for Aphidiinae (Hymenoptera: Braconidae) (Kavallieratos et al. 2004, 2006, 2008, 2013, 2016), have scarcely been conducted. In fact, some surveys of the parasitoid spectrum in Greece in various host species, with the exception of Aphidiinae, have been initiated only during the last years. For example, Kolarov (2007) catalogued the Ichneumonidae (Hymenoptera) of Greece, Papp (2007) presented a list of Braconidae, and Zeegers (2017) provided information on some Tachinidae (Diptera). Other studies are mostly related to particular subgroups of parasitoids. For instance, Tsankov et al. (1999) reported egg parasitoids of *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae), Anagnou Veroniki et al. (2006) studied the parasitization of *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae), Žikić et al. (2017) revealed the parasitoids of two serious pests in forestry, *Lymantria dispar* (L., 1758) (Lepidoptera: Erebididae) and *Malacosoma neustria* (L., 1758) (Lepidoptera: Lasiocampidae), and Alissandrakis et al. (2018) investigated the parasitoid complex that is related to *Prays oleae* (Bernard, 1788) (Lepidoptera: Praydidae). Considering the lack of information on the spectrum of parasitoids of noxious insects in Greece, the objective of our study was to conduct a survey and shed light on the associations between hymenopterous or dipterous parasitoids and their field or urban hosts in the country.

## Materials and methods

The samples were collected in several localities on the Greek mainland (central Greece, Epirus, Macedonia, Peloponnese, Thrace) and the island of Crete (Fig. 1). The insect material was collected either with or without visible signs of parasitism. Insect specimens were collected from field or urban environments, and then separately placed in plastic containers covered with nylon mesh, transferred to the laboratory and reared at 25 °C until parasitoid emergence. When host insects were found on plants, voucher samples of the plants were kept in herbariums and later identified by Prof. Bojan Zlatković (Department of Biology and Ecology, University of Niš). To collect specimens of *Ptosima undecimmaculata* (Herbst, 1784) (Coleoptera: Buprestidae) and



**Figure 1.** Investigated areas marked on the map of Greece: **1** Almyros (Thessaly) **2** Agios Ioannis (Thessaly) **3** Agrioleukes (Thessaly) **4** Arogi (Thrace) **5** Athens (central Greece) **6** Grammos (Epirus) **7** Nemea (Peloponnese) **8** Tatoi (central Greece) **9** Thessaloniki (Macedonia) **10** Voutes (Crete).

*Scolytus rugulosus* (Müller, 1818) (Coleoptera: Curculionidae), infested wood was cut into small pieces. Then, larvae were very carefully removed, put in plastic containers and kept in the same conditions as above. As soon as the adult parasitoids were emerged, they were captured using an aspirator and killed into vials containing 70% ethyl alcohol. They were then slide-mounted for detailed examination. Specimens for slides were washed in distilled water, boiled in 10% KOH for 2 minutes, rewashed, and then placed in a drop of Faure-Berlese medium (Krantz 1978) for dissection or whole mounting. External morphology was studied using an Olympus SZX9 (zoom ratio 9; total magnification 12.6–114.0×) (Olympus Corporation, Tokyo, Japan) or a Zeiss Discovery V8 (zoom ratio 8; total magnification 10.0–80.0×) (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) stereomicroscopes. The identification of

parasitoids was based on Tobias and Jakimavicius (1986), Tschorsnig and Herting (1994), Broad (2011), while the host identifications were conducted by Prof. Josef J. De Freina (Museum Witt, Munich, Germany) on the basis of larval morphology. The material was deposited in the insect collection of the Laboratory of Agricultural Zoology of Entomology, Agricultural University of Athens, Greece, the Laboratory of Entomology, Department of Agriculture, Technological Educational Institute of Crete, Greece, and the Faculty of Sciences and Mathematics, Department of Biology and Ecology, University of Niš, Serbia. Nomenclature of hosts is based on Goulet and Huber (1993). In the following results, new records of parasitoids for Greece are marked with an asterisk (\*) in front of each parasitoid name while new parasitoid-host associations are indicated with a dagger (†) in front of each host name. Dates indicate the time when the hosts were collected.

## Results

The identifications of adult parasitoids which emerged from their hosts, revealed 22 species belonging to 5 families. The most numerous were those of the family Braconidae (12 species) followed by Ichneumonidae (7 species). Twenty-three parasitoid-host associations, nine of which were not previously known, are listed in detail below. Nine parasitoid species are recorded for the first time from Greece.

### Family Braconidae

#### Subfamily Brachistinae

##### *Triaspis thoracica* (Curtis, 1860)

**Material examined.** 13♀, 18♂, Macedonia, Thessaloniki (40°37'58.07"N, 22°57'28.47"E), 01 March 2016, leg. D. Koveos. Host: *Bruchus pisorum* (L., 1758) (Coleoptera: Chrysomelidae) on *Vicia sativa*; 31♀, 13♂, Macedonia, Thessaloniki (40°37'58.07"N, 22°57'28.47"E), 01 March 2016, leg. D. Koveos. Host: *Bruchus pisorum* (L., 1758) (Coleoptera: Chrysomelidae) on *Vicia peregrina*.

#### Subfamily Charmontinae

##### \**Charmon cruentatus* Haliday, 1833

**Material examined.** 2♀, Thessaly, Almyros (39°10'34.39"N, 22°45'17.46"E), 11 May 2016, leg. S. Stanković. Host: †*Tortrix viridana* L., 1758 (Lepidoptera: Tortricidae) on *Quercus pubescens*.

### Subfamily Cheloninae

#### *Chelonus (Microchelonus) sulcatus* Jurine, 1807

**Material examined.** 2♀, 1♂, Macedonia, Thessaloniki (40°37'55.03"N, 22°57'25.57"E), 15 July 2002, leg. N. G. Kavallieratos. Host: *Prays citri* (Millière, 1873) (Lepidoptera: Praydidae) on *Citrus sinensis*.

### Subfamily Doryctinae

#### \**Dendrosoter protuberans* (Nees, 1834)

**Material examined.** 1♀, 3♂, Crete, Heraklion, Voutes (35°15'57.86"N, 25°3'17.11"E), 24 April 2017, leg. E. Alissandrakis. Host: †*Prosimia undecimmaculata* (Herbst, 1784) (Coleoptera: Buprestidae) on *Prunus domestica*.

#### \**Ecpbylus silesiacus* (Ratzeburg, 1848)

**Material examined.** 2♂, 2♀, Crete, Heraklion, Voutes (35°15'55.58"N, 25°3'42.82"E), 23 May 2017, leg. E. Alissandrakis. Host: *Scolytus rugulosus* (Müller, 1818) (Coleoptera: Curculionidae) on *Prunus domestica*.

#### \**Spathius curvicaudis* Ratzeburg, 1844

**Material examined.** 2♂, 5♀, Crete, Heraklion, Voutes (35°16'8.13"N, 25°3'49.67"E), 16–22 May 2017, leg. E. Alissandrakis. Host: †*Scolytus rugulosus* (Müller, 1818) (Coleoptera: Curculionidae) on *Prunus domestica*.

### Subfamily Microgastrinae

#### *Cotesia spuria* (Wesmael, 1837)

**Material examined.** 13♀, 5♂, central Greece, Dekeleia, Tatoi (38°11'59.02"N, 23°47'40.44"E), 07 May 2016, leg. N. G. Kavallieratos, V. Žikić. Host: *Diloba caeruleocephala* (L., 1758) (Lepidoptera: Noctuidae) on *Pyrus spinosa*.

#### *Cotesia zygaeenarum* Marshall, 1885

**Material examined.** 7♀, 4♂, Peloponnese, Nemea (37°50'14.26"N, 22°38'37.33"E), 24 May 2016, leg. A. Nahirnić. Host: *Zygaena lonicerae* (Scheven, 1777) (Lepidoptera: Zygaenidae) on *Tetragonolobus purpureus*.

***Diolcogaster alvearia* (F., 1798)**

**Material examined.** 20♀, 7♂, Thrace, Arogi (40°57'20.77"N, 25°10'12.08"E), 05 June 2005, leg. V. Žikić. Host: *Peribatodes rhomboidaria* (Denis & Schiffermüller, 1775) (Lepidoptera: Geometridae) on *Malus pumila*; 31♀, 8♂, Athens (37°58'54.65"N, 23°44'49.09"E), 07 May 2016, leg. V. Žikić. Host: *Peribatodes rhomboidaria* (Denis & Schiffermüller, 1775) (Lepidoptera: Geometridae) on *Lonicera pileata*.

***Dolichogenidea candidata* (Haliday, 1834)**

**Material examined.** 4♀, Thessaly, Mt Pelion, Agios Ioannis (39°24'58.81"E, 23°9'34.27"E), 10 August 2017, leg. V. Žikić. Host: *Choreutis nemorana* (Hübner, 1799) (Lepidoptera: Choreutidae) on *Ficus carica*.

**\**Dolichogenidea longipalpis* (Reinhard, 1880)**

**Material examined.** 3♀, 1♂, Epirus, Mt Grammos (40°21'5.95"N, 20°46'43.45"E), 17 July 2003, leg. N. G. Kavallieratos. Host: †*Dablica* sp. Enderlein, 1912 (Lepidoptera: Psychidae) on rock.

***Glyptapanteles vitripennis* (Curtis, 1830)**

**Material examined.** 3♂, central Greece, Dekeleia, Tatoi (38°9'46.13"N, 23°47'39.34"E), 07 May 2016, leg. N. G. Kavallieratos, V. Žikić. Host: *Diloba caeruleocephala* (L., 1758) (Lepidoptera: Noctuidae) on *Pyrus spinosa*.

**Family Eulophidae****Subfamily Tetrastichinae****\**Melittobia acasta* (Walker, 1839)**

**Material examined.** 5♀, central Greece, Dekeleia, Tatoi (38°12'2.17"N, 23°39'53.51"E), 07 May 2016, leg. V. Žikić, N. G. Kavallieratos. Host: †*Parocneria terebinthi* (Freyer, 1838) (Lepidoptera: Erebidae) on *Pistacia terebinthus*.

**Family Evaniidae*****Prosevania fuscipes* (Illiger, 1807)**

**Material examined.** 3♀, 1♂, Thrace, Arogi (40°57'18.70"N, 25°10'6.79"E), 05 August 2016, leg. V. Žikić. Host: *Blatta orientalis* L., 1758 (Blattodea: Blattidae).

**Family Ichneumonidae**  
**Subfamily Banchinae**

***Lissonota culiciformis* Gravenhorst, 1829**

**Material examined.** 4♂, central Greece, Dekeleia, Tatoi (38°10'2.26"N, 23°48'15.65"E), 07 May 2016, leg. N. G. Kavallieratos, V. Žikić. Host: *Malacosoma neustria* (L., 1758) (Lepidoptera: Lasiocampidae) on *Quercus coccifera*.

**Subfamily Campopleginae**

**\**Campoplex difformis* (Gmelin, 1790)**

**Material examined.** 5♀, central Greece, Dekeleia, Tatoi (38°9'53.71"N, 23°49'11.51"E), 07 May 2016, leg. N. G. Kavallieratos, V. Žikić. Host: †*Pammene oxycedrana* (Millière, 1876) (Lepidoptera: Tortricidae) on *Arbutus unedo*; 1♀, central Greece, Dekeleia, Tatoi (38°10'2.98"N, 23°49'40.93"E), 07 May 2016, leg. N. G. Kavallieratos, V. Žikić. Host: *Archips rosana* (L., 1758) (Lepidoptera: Tortricidae) on *Pyrus spinosa*.

**Subfamily Cryptinae**

**\**Gelis albipalpus* (Thomson, 1884)**

**Material examined.** 6♀, Thessaly, Mt Pelion, Agrioleukes (39°23'13.74"N, 23°5'1.00"E), 11 May 2016, leg. S. Stanković, V. Žikić. Host: †*Cotesia neustriiae* (Tobias, 1986) (Hymenoptera: Braconidae) parasitizing *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae) feeding on *Quercus coccifera*.

***Gelis areator* (Panzer, 1804)**

**Material examined.** 3♀, 1♂, Thessaly, Mt Pelion, Agrioleukes (39°23'15.16"N, 23°5'1.36"E), 11 May 2016, leg. S. Stanković, V. Žikić. Host: †*Cotesia neustriiae* (Tobias, 1986) parasitizing *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae) feeding on *Quercus coccifera*.

***Gelis ilicicola* (Seyrig, 1927)**

**Material examined.** 4♀, Thessaly, Mt Pelion, Agrioleukes (39°23'16.90"N, 23°5'3.91"E), 11 May 2016, leg. S. Stanković, V. Žikić. Host: *Cotesia neustriiae* (Tobias, 1986) parasitizing *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidae) feeding on *Quercus coccifera*.

**\**Lysibia tenax* Townes, 1983**

**Material examined.** 12♀, 9♂, central Greece, Dekeleia, Tatoi (38°10'53.42"N, 23°46'50.76"E), 07 May 2016, leg. N. G. Kavallieratos, V. Žikić. Host: †*Cotesia neustriæ* (Tobias, 1986) parasitizing *Lymantria dispar* (L., 1758) (Lepidoptera: Erebidæ) feeding on *Quercus coccifera* L.

**Subfamily Pimplinae*****Itoplectis tunetana* (Schmiedeknecht, 1914)**

**Material examined.** 6♀, Thessaly, Mt Pelion, Agios Ioannis (39°25'9.74"N, 23°9'21.43"E), 10 August 2017, leg. V. Žikić. Host: *Choreutis nemorana* (Hübner, 1799) (Lepidoptera: Choreutidae) on *Ficus carica*.

**Family Tachinidae****Subfamily Exoristinae*****Exorista segregata* (Rondani, 1859)**

**Material examined.** 4♂, central Greece, Dekeleia, Tatoi (38°9'23.16"N, 23°45'3.40"E), 07 May 2016, leg. N. G. Kavallieratos, V. Žikić. Host: *Parocneria terebinthi* (Freyer, 1838) (Lepidoptera: Erebidæ) on *Pistacia terebinthus*.

**Discussion**

Our findings revealed that the subfamily Charmontinae is represented by *C. cruentatus*, which is a solitary parasitoid of several microlepidopterans (van Achterberg 1979; Yu et al. 2012) and is recorded in Greece for the first time. The only identified species of the subfamily Cheloninae, *C. sulcatus*, is a parasitoid of several microlepidopterous insects (Aydogdu and Beyarslan 2006). It is an important natural enemy of *Prays citri* (Millière, 1873) (Lepidoptera: Praydidae), a pest of citrus that is common in the Mediterranean region (Moreno et al. 1990). Most likely, this moth has been introduced in the Mediterranean region from Africa with the import of citrus propagating material (de Carvalho and Aguiar 1997). The evaluation of *C. sulcatus* as a biological control agent against *P. citri* could be of interest, given that this parasitoid species is widely spread in the Palaearctic (Aydogdu and Beyarslan 2006).

One of the largest braconid subfamily, Microgastrinae, is specialized on the parasitization of caterpillars (Shaw and Huddleston 1991). Both identified species of *Cotesia* Cameron (*C. spuria* and *C. zyaenarum*) are gregarious endoparasitoids of cater-



pillars (Žikić et al. 2013; Gadallah et al. 2015). *Cotesia spuria* is a polyphagous and cosmopolitan species that inhabits whole Palaearctic (Yu et al. 2012; Gadallah et al. 2015). During our study we identified *C. spuria* parasitizing caterpillars of *D. caeruleocephala*, which is recognized as a pest in orchards of the family Rosaceae (Bolu and Özgen 2007). *Cotesia zyaenarum* is an oligophagous parasitoid of the genus *Zygaena* F. and several other lepidopterous genera (Gadallah et al. 2015). Another gregarious parasitoid identified in this study, *D. alvearia*, has a very narrow host range which includes 9 taxa of coleopterous, hymenopterous and lepidopterous insects commonly found in Europe (Gadallah et al. 2015). The controversial genus *Dolichogenidea* Viereck, which is hardly separated from *Apanteles* Foerster (sensu stricto) (Fernández Triana et al. 2014), predominantly contains both solitary and gregarious endoparasitoids of microlepidoptera (i.e., Gracillariidae, Plutellidae, Pyralidae, Tortricidae, Yponomeutidae) (Medvedev 1989; Jankowska and Wiech 2006). *Glyptapanteles vitripennis* is a gregarious parasitoid with broad host range that includes major pests in forest and agricultural ecosystems, e.g., *L. dispar*, *M. neustria*, *Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae) (Yu et al. 2012). Interestingly, this species emerged from *D. caeruleocephala* caterpillars under no evident superparasitism because some other caterpillars were parasitized by *C. spuria*.

The subfamily Doryctinae includes species that parasitize wood-feeding coleopterans, including Curculionidae (Scolytinae), Bostrychidae, Buprestidae and Cerambycidae (Farahani et al. 2014). *Ptosima undecimmaculata* is identified for the first time as a host of *D. protuberans* in our study. Larvae of *P. undecimmaculata* live inside wood of dead trees and living branches of different species of *Prunus* spp. for 2–3 years (Sakalian 2003). Moreover, with the exception of *Dolichomitus tuberculatus* (Geoffroy, 1785) (Hymenoptera: Ichneumonidae), parasitoids of this species have not been previously recorded (Aliyev and Maharramova 2009). *Dendrosoter protuberans* is an oligophagous ectoparasitoid recorded from numerous hosts, mainly Curculionidae, e.g., *Hylesinus* F., *Leperisinus* Reitter, *Phloeosinus* Chapuis, *Phloeotribus* Latreille, *Scolytus* Geoffroy, *Tomicus* Latreille, and also from few Buprestidae, Cerambycidae and Chrysomelidae (Wegensteiner et al. 2015; Beyarslan 2017). This parasitoid species is a very important natural enemy of *Scolytus multistriatus* (Marsham, 1802) (Coleoptera: Curculionidae) that seriously attacks elm trees in Europe (Manojlović et al. 2003). The genus *Ptosima* Solier is solely represented in Europe by *P. undecimmaculata* with two subspecies; *Ptosima undecimmaculata* ssp. *metallescens* Bily, 1982 (Coleoptera: Buprestidae) which is only found in Crete, and *Ptosima undecimmaculata* ssp. *undecimmaculata* (Herbst, 1784) (Coleoptera: Buprestidae) which is dispersed in the rest of Greece and Europe (van Achterberg 2013). Besides *D. protuberans*, two more species of the genus *Dendrosoter* Wesmael have been reported in Europe, i.e., *Dendrosoter curtisii* (Ratzeburg, 1848) (Coleoptera: Buprestidae) and *Dendrosoter middendorffii* (Ratzeburg, 1848) (Coleoptera: Buprestidae) (van Achterberg 2013), which have been supplemented by *E. silesiacus* and *S. curvicaudis* as new members of the Greek fauna recorded in our study. The fact that *S. curvicaudis* is recorded for the first time from larvae of *S. rugulosus* in cut woods of *P. domestica* may open a new path towards the biological control

of this serious scolytid pest of fruit and nut trees (Seybold et al. 2016), whose chemical control is not effective when *S. rugulosus* is inside wood (Fox 2015).

With few exceptions, *T. thoracica* is a specialized parasitoid of the genus *Bruchus* L., which includes various species of economic importance for stored legumes (Parker 1957; Reddy et al. 2018). This species belongs to the subfamily Brachistinae that includes endoparasitoids of various beetles (Belokobylskij 1998). Several species oviposit inside eggs and emerge from larvae of their hosts, becoming therefore egg-larval parasitoids (Shaw and Huddleston 1991). *Triaspis thoracica* acts that way leading to successful biological control of the noxious *B. pisorum* (Clausen 1954; Nikolova 2016).

Out of the five ensign wasp species inhabiting Europe, four are recorded in Greece, these are: *Evania appendigaster* (L., 1758), *P. fuscipes*, *Prosevania incerta* (Kieffer, 1904) and *Zeuxevania splendidula* (Costa, 1884) (van Achterberg 2013). These wasps are specialized parasitoids of oothecae of Blattodea (Cameron 1957; Carlson 1979). *Prosevania fuscipes* has been recorded in several European countries, but most likely it occurs in all countries where its host, *B. orientalis*, is present (Ceianu 1986). The only identified member of the family Eulophidae, *M. acasta*, is a gregarious ectoparasitoid of insects belonging to various orders (Browne 1922) (i.e., Hymenoptera, Diptera, Lepidoptera and Coleoptera); it was reared from *P. terebinthi*, which constitutes a new host record.

We also identified seven parasitoid species that belong to the largest hymenopterous family, the Ichneumonidae. *Itopectis tunetana* is primary endoparasitoid of lepidopterous larvae or pupae, while *C. difformis* and *L. culiciformis* emerge from lepidopterous larvae (Georgiev 2000; Talebi et al. 2005; Yu et al. 2012; Žikić et al. 2017). *Lysibia tenax* is a specialized hyperparasitoid of the genus *Cotesia*, while *C. neustriae* is a newly recorded host for this species. The remaining three ichneumonids belong to genus *Gelis* Thunberg and behave either as primary parasitoids or hyperparasitoids; this type of strategy is usual for most of the members of this genus (Žikić et al. 2017). As a secondary parasitoid, *G. albipalpus* has been recorded only from *Apanteles murinanae* Čapek & Zwölfer, 1957 (Hymenoptera: Braconidae) (Čapek and Zwölfer 1957), and therefore, *C. neustriae* is listed for the first time as a host. The polyphagous *G. aerator*, having a host range that exceeds 180 species (Yu et al. 2012), was found for first time parasitizing also *C. neustriae*. Out of about 25 braconid species on the list of hosts of *G. aerator*, there are 12 species of the genus *Cotesia* (Yu et al. 2012). Prior to the revision by Schwarz (2016) of the macropterous females of genus *Gelis* from the Western Palaearctic, *G. ilicicola* was often mixed with related species and thus most records are not reliable. However, Schwarz (2016) reported that specimens of *G. ilicicola* reared from small lepidopterans (Coleophoridae), neuropterans (Chrysopidae) and hymenopterans (Braconidae: Microgastrinae), suggesting that this species is a parasitoid of a wide range of small cocoons and cocoon-like structures. This hypothesis posits additional investigation provided that the ability of a parasitoid to attack the immobile life stages of host species may have some advantages for its use in biological control. For example, in several cases, larvae choose a site to pupate other than the original feeding one, where they are parasitized by pupal parasitoids (Hawkins 1994; Cancino et al. 2012). In this way, pupal parasitoids restrict the suitable locations for insects' development (Machtinger et al. 2015).

The only identified species of the family Tachinidae in our study is *E. segregata*. This is a common parasitoid of numerous lepidopterous species (Mückstein et al. 2007). Until now, it has been reared from over 50 species belonging to the Erebidae, such as *L. dispar*, and the Lasiocampidae, such as *M. neustria*, but also from many belonging to the Noc-tuidae, Thaumetopoeidae and Zygaenidae (Tschorsnig 2017, Žikić et al. 2017). *Exorista segregata* has a relatively wide geographical range, and it has been recorded in most European countries but also in the North Africa, Anatolia and the USA (Tschorsnig 1997).

Our findings shed light on a greatly overlooked issue, which is the fauna of parasitoids occurring in Greece. The several new recorded parasitoid species in Greece and the previously unknown parasitoid-host associations indicate the high level of biodiversity that exists in the investigated ecosystems and potentially triggers natural biological control of several harmful insect species. The recorded elevated richness of beneficial insects makes us hypothesize that additional natural enemies may occur in the same or similar ecosystems, a premise that should be further investigated and also confirmed with additional field surveys. Furthermore, a common practice of pests' management deals with their identification and the application of chemical compounds without considering the role of the existing beneficial fauna (Mehle and Trdan 2012). Our study clearly shows that the overlooked intermediate stage of identification of parasitoids is also crucial, as it can bring to light the high level of activity of these species against pests. More carefully designed pest management strategies would be modelled towards the conservation of the local parasitoid species.

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# Taxonomic review of the Asian *Trogloneta* species (Araneae, Mysmenidae)

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## Abstract

Five *Trogloneta* species from Southwest China and Japan are reviewed that two new combinations and a new synonymy are proposed in the current paper: *T. nojimai* (Ono, 2010), **comb. n.** is transferred from *Mysmena*, *T. yunnanense* (Song & Zhu, 1994), **comb. n.** (= *T. denticocleari* Lin & Li, 2008, **syn. n.**) is transferred from *Pholcomma* of the Theridiidae, *T. speciosum* Lin & Li, 2008, *T. uncata* Lin & Li, 2013, and *T. yuensis* Lin & Li, 2013. The female of *T. yuensis* is described for the first time. An identification key and diagnoses are provided for these species, as well as new photographs or illustrations of the genital organs and habitus of *T. yuensis* and *T. yunnanense*.

## Keywords

China, identification key, Japan, mysmenids, new combination, new synonymy, taxonomy

## Introduction

The genus *Trogloneta* Simon, 1922 (= *Troglonata*, lapsus calami) was erected by Simon (1922) on the basis of an interesting cave spider from France which originally thought to be a member of the *Mysmeneae* group, and placed in the family Theridiidae Sundevall, 1833. It was later transferred to Symphytognathidae Hickman, 1931 by Gertsch (1960), and placed in Mysmenidae Petrunkevitch, 1928 by Forster and Platnick

(1977). Brescovit and Lopardo (2008) reviewed a taxonomic history and proposed a syncretic diagnostic for *Trogloneta*. Recent phylogenetic study indicates that *Trogloneta* is sister to the clade comprising *Maymena* Gertsch, 1960 plus Mysmenopsinae (Lopardo and Hormiga 2015), and a consistent diagnosis is also presented for this genus.

Currently, *Trogloneta* consists of eleven described species (World Spider Catalog 2018), and these members are mainly distributed in Europe, China, Brazil, USA, Madeira, and Canary Island, and prefer to living in cryptic microhabitats such as deciduous layer (e.g., *T. cantareira*, *T. cariacica*, and *T. mourai* in Brescovit and Lopardo 2008), or forest canopy (e.g., *T. speciosum* in Lin and Li 2008) or even in dark caves (e.g., *T. granulum* in Simon 1922, “*T. denticocleari*” and *T. uncata* in Lin and Li 2008, 2013b).

In this paper we provide a brief revision of *Trogloneta* species from Asia. The female of *T. yuensis* Lin & Li, 2013 is described for the first time. An identification key is also provided for these Asian *Trogloneta* species.

## Materials and methods

Specimens were collected by hand and preserved in 95% ethanol. They were examined using a Leica M205 C stereomicroscope. Further details were studied under an Olympus BX43 compound microscope. The epigynes were removed and treated with lactic acid before being photographed. Photographs were taken with a Canon EOS 60D wide zoom digital camera (8.5 megapixels) mounted on an Olympus BX 43 compound microscope. The images were montaged using Helicon Focus 3.10 (Khmelik et al. 2006) image stacking software.

All measurements are in millimetres. Leg measurements are given as follow: total length (femur, patella, tibia, metatarsus, and tarsus). Abbreviations in figures or text are as follows:

<b>AA</b>	apical apophysis	<b>E</b>	embolus
<b>Acc</b>	accessory gland	<b>Et</b>	embolic tip
<b>AL</b>	apical lobe	<b>FD</b>	fertilisation ducts
<b>ALE</b>	anterior lateral eyes	<b>Pa</b>	patella
<b>AME</b>	anterior median eyes	<b>PC</b>	paracymbium
<b>At</b>	atrium	<b>PLE</b>	posterior lateral eyes
<b>CD</b>	copulatory ducts	<b>PME</b>	posterior median eyes
<b>CO</b>	copulatory opening	<b>S</b>	spermathecae
<b>Co</b>	conductor	<b>SD</b>	spermatid duct
<b>Cy</b>	cymbium	<b>Sp</b>	scape
<b>CyC</b>	cymbial conductor	<b>St</b>	subtegulum
<b>CyF</b>	cymbial fold	<b>T</b>	tegulum
<b>CyFs</b>	setae on cymbial fold	<b>TA</b>	tegular apophysis
<b>CyP</b>	cymbial process	<b>Ti</b>	tibia

Abbreviations of specimen depository institutions:

<b>IZCAS</b>	Institute of Zoology, Chinese Academy of Sciences in Beijing, China
<b>NHMSU</b>	Natural History Museum of Sichuan University in Chengdu, China
<b>NSMT</b>	Department of Zoology, National Museum of Nature and Science in Tokyo, Japan

## Taxonomy

### *Trogloneta* Simon, 1922

**Type species.** *Trogloneta granulum* Simon, 1922.

**Diagnosis.** *Trogloneta* differs from other mysmenid genera by the following combination of features: AME (or absent) smaller than ALE, all eyes gathered at apex of carapace; one femoral spot on leg I on both sexes (none on leg II); carapace height dimorphism (male carapace higher than female); anterior booklungs reduced; males with shorter, but stout and straight setae comprising the tarsal prolateral row on leg I; male palp huge (at least as big as half carapace), embolus tubular, its tip simple; cymbium with intricate decorations, and cymbial terminal acting as conductor; tegulum broad, usually having a apophysis; epigynal area elevated ventrally, with a scape, accessory glands on valve, and smooth uniform proximal copulatory ducts of increased diameter (Brescovit and Lopardo 2008, Lopardo and Hormiga 2015).

**Composition.** *T. canariensis* Wunderlich, 1987, *T. cantareira* Brescovit & Lopardo, 2008, *T. cariacica* Brescovit & Lopardo, 2008, *T. granulum* Simon, 1922, *T. madeirensis* Wunderlich, 1987, *T. mourai* Brescovit & Lopardo, 2008, *T. nojimai* (Ono, 2010), comb. n., *T. paradoxa* Gertsch, 1960, *T. speciosum* Lin & Li, 2008, *T. uncata* Lin & Li, 2013, *T. yuensis* Lin & Li, 2013, *T. yunnanense* (Song & Zhu, 1994), comb. n. (= *T. denticocleari* Lin & Li, 2008, syn. n.).

**Distribution.** China (Chongqing, Guizhou, Hunan, Yunnan), Japan (Honshu), Europe (Austria, Czech Republic, France, Germany, Italy, Poland, Slovakia), USA (Utah, Oregon, California), Brazil (Minas Gerais, Rio de Janeiro, San Paulo, Santa Catarina, Espirito Santo, Parana), Canary Island, Madeira.

### Key to Asian species of *Trogloneta* Simon, 1922

1	Abdomen subglobose (Figs 1A, B, 4A–C, 9A–D) .....	2
–	Abdomen pointed dorsally or posteriorly (Figs 2A, B, F, G, 6A, B, E, F) ....	6
2	Males.....	3
–	Females.....	5
3	Embolus stubby, distal tip falcate (Fig. 5A–D) .....	<i>T. uncata</i>
–	Embolus long, distal tip spiculate (Figs 1E, 10B, D) .....	4

- 4 Cymbium strongly modified, with a huge cymbial process (Fig. 10A, C, D)....  
..... *T. yunnanense*
- Cymbium moderately modified, cymbial process absent (Fig. 1E, F) .....  
..... *T. nojimai*
- 5 Epigyne with a short scape, spermathecae globular (Fig. 11C, D) .....  
..... *T. yunnanense*
- Epigyne with a long scape, spermathecae oviform (Fig. 1G, H) ..... *T. nojimai*
- 6 AME absent, embolus straight distally and epigynal scape short (Figs 2A, F,  
3C–G) ..... *T. speciosum*
- AME present, embolus hooked distally and epigynal scape long (Figs 6A, B,  
7B, 8D) ..... *T. yuensis*

***Trogloneta nojimai* (Ono, 2010), comb. n.**

Figure 1 (as modified from Ono, 2010)

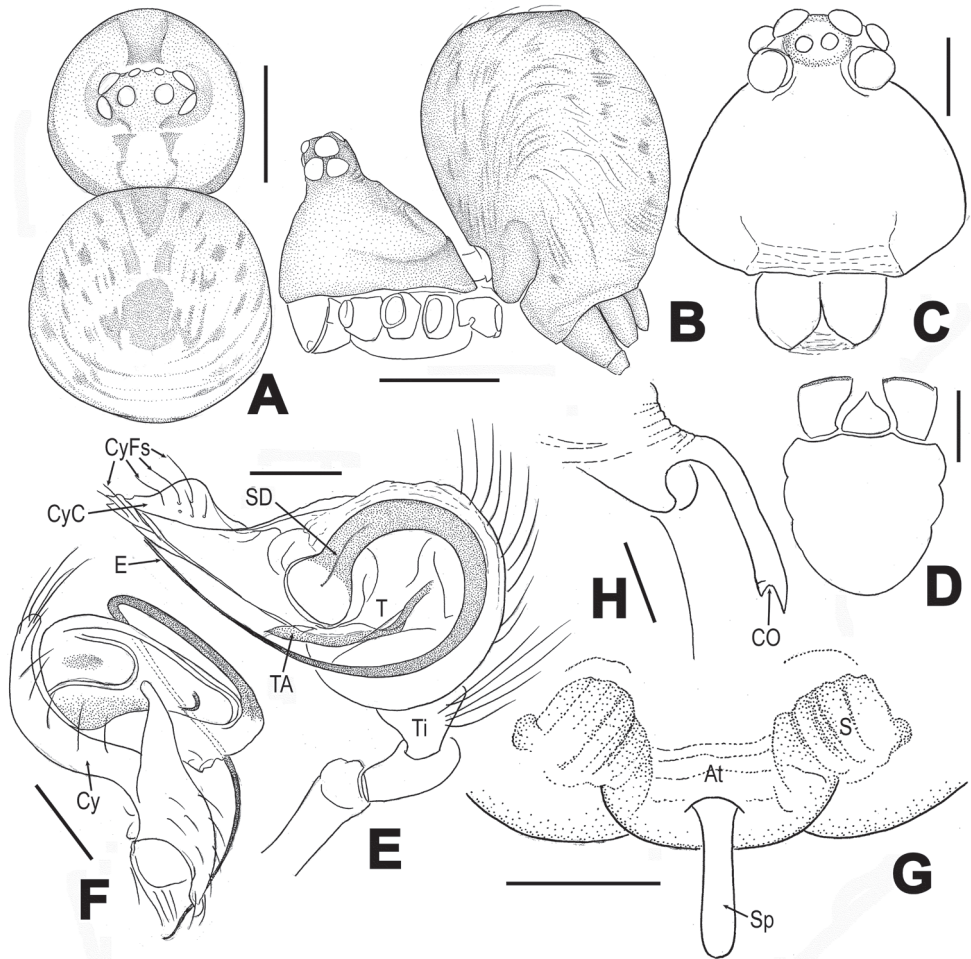
*Mysmena nojimai* Ono, 2010: 2, figs 1–8.

**Type material.** *Holotype*: ♂ (NSMT-Ar 8515) from JAPAN: Honshu, Okayama Prefecture, Tsuyama-shi, Kamo-cho, Uno, alt. ca. 100 m, 12.VI.2009, K. Nojima leg. *Paratypes*: 1♀ (NSMT-Ar 8516) from JAPAN: Honshu, Okayama Prefecture, Okayama-shi, Kita-ku, Awai, alt. 100–200 m, 6.VI.2009, K. Nojima leg.; 2♀ (NSMT-Ar 8568) from JAPAN: Honshu, Aichi Prefecture, Toyota-shi, Sakaue-cho, Mt. Rokushosan, alt. ca. 400 m, 18.X.2009, K. Ogata leg. Not examined.

**Diagnosis.** *Trogloneta nojimai* can be distinguished from other congeners but except of *T. yuensis* by the globular abdomen in both sexes (Figure 1A, B), the palp with a long embolus and a distinctly extended cymbial conductor (Figure 1E), and a long and narrow scape in the epigyne (Figure 1G, H). It seems more similar to *T. yuensis* in share with the structure of palp and the configuration of epigyne, but differs from the latter by the abdomen without a dorsal-posteriorly pointed tubercle in both sexes (Figure 1A, B vs. Figure 6A, B, E, F), a straight embolic end and lack of cymbial process (Figure 1E, F vs. Figure 7A–D), and by the oval spermathecae (Figure 1G vs. Figure 8C, D).

**Taxonomic justification.** Although the type material of this species has not been examined for this study, the shape of palpal bulb, the configuration of epigyne, the patterns of eyes arrangement, and the distinctly elevated, conical carapace in male leave little doubt that it should be a member of the genus *Trogloneta*, but not *Mysmena*. The original illustrations of palp and epigyne of *T. nojimai* by (Ono 2010: figs 1–8) are rather simple and show many important similarities in comparison with those of *T. yuensis* (Figs 7A–D, 8A–D), as one of *Trogloneta* species. Therefore, we propose it as a new combination, *Trogloneta nojimai* (Ono, 2010), comb. n., transferring it from *Mysmena*.

**Distribution.** Japan (Honshu).



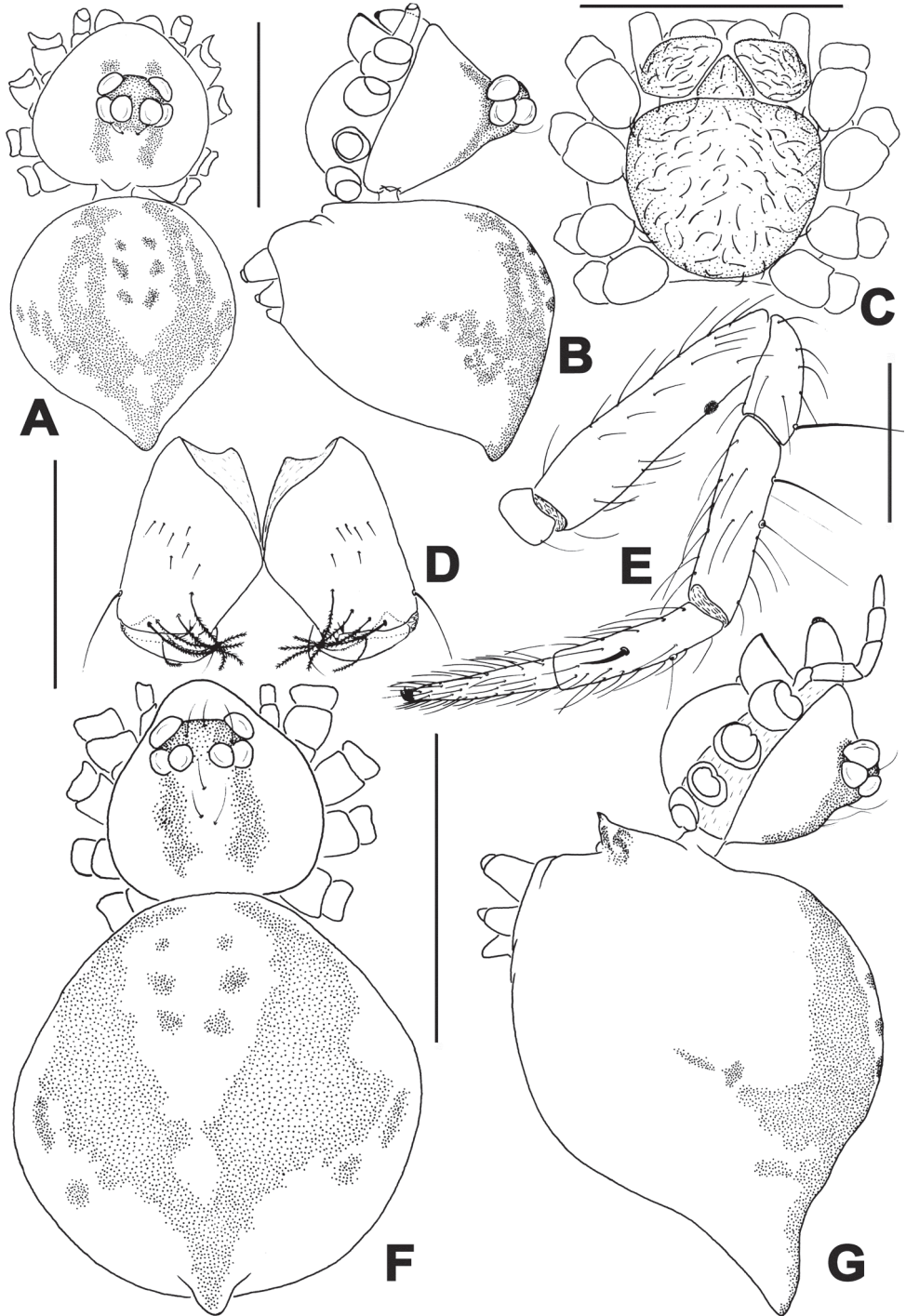
**Figure 1.** *Trogloneta nojimai* (Ono, 2010), comb. n., male holotype (**A–F**) and female paratype (**G, H**) (cited from Ono, 2010, slightly modified). **A, B** habitus (appendages omitted) **C** Prosoma **D** Endites, labium and sternum **E, F** Left palp **G, H** Epigyne **A, F** dorsal **B, H** lateral **C** anterior **D, G** ventral **E** retrolateral. Abbreviations: At atrium; CO copulatory opening; Cy cymbium; CyC cymbial conductor; CyFs setae on cymbial fold; E embolus; S spermathecae; SD spermatic duct; Sp scape; T tegulum; TA tegular apophysis; Ti tibia. Scale bars: 0.25 mm (**A, B**); 0.10 mm (**C–H**).

***Trogloneta speciosum* Lin & Li, 2008**

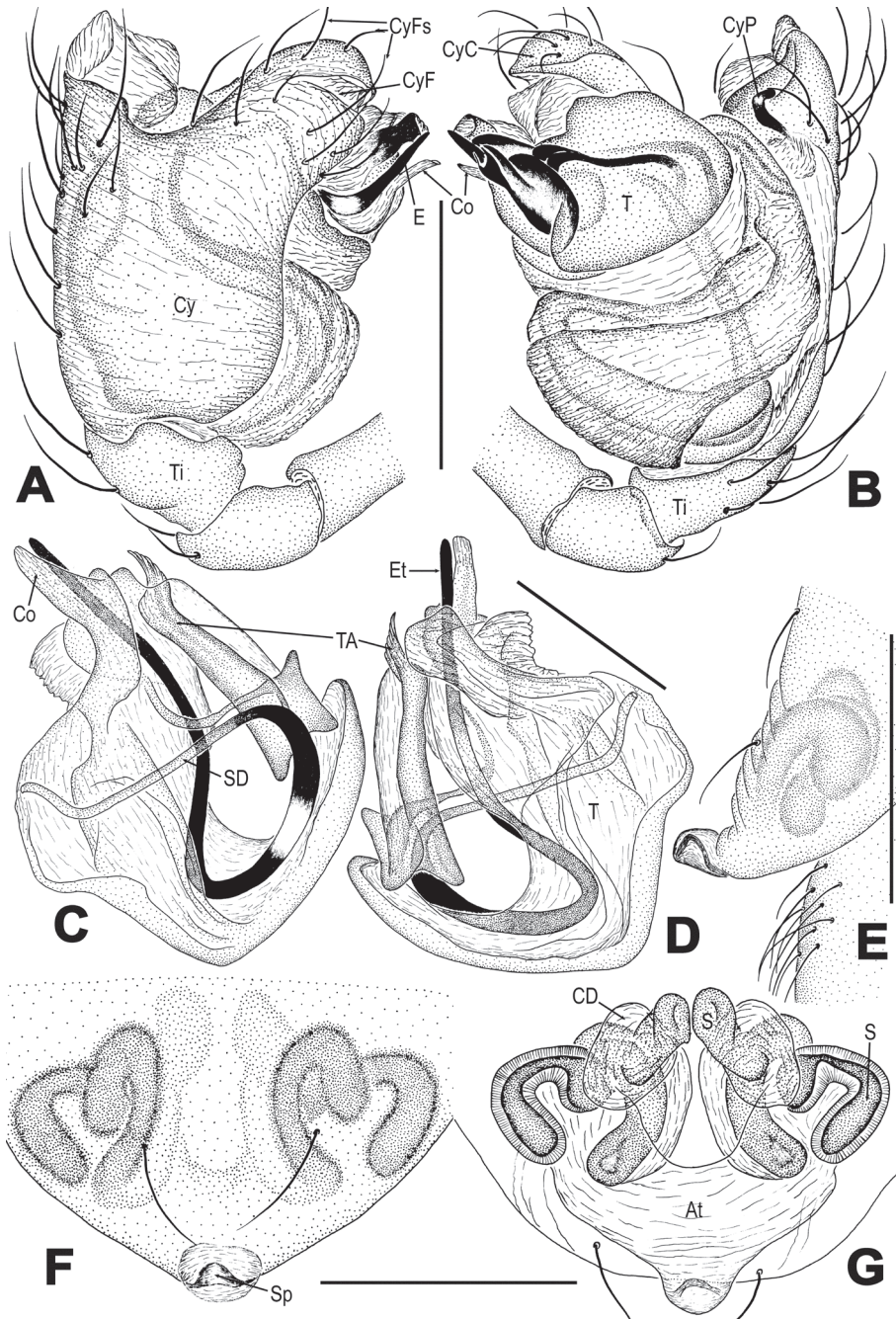
Figures 2, 3

*Trogloneta speciosum* Lin & Li, 2008: 514, figs 18A–E, 19A–I.

**Type material.** *Holotype*: ♂ (IZCAS), *paratypes*: 1♂, 3♀ (IZCAS) from CHINA: Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun



**Figure 2.** *Trogloneta speciosum* Lin & Li, 2008, male (A–E) and female (F–G). **A, B** Habitus **C** Prosoma (appendages omitted) **D** Chelicerae **E** Leg I **F, G** Habitus. **A, F** dorsal **B, G** lateral **C** ventral **D** posterior **E** prolateral. Scale bars: 0.50 mm (A–D, F, G); 0.20 mm (E).



**Figure 3.** *Trogloneta speciosum* Lin & Li, 2008, male (**A–D**) and female (**E–G**). **A, B** Left palp **C, D** Embolic division **E, F** Epigyne **G** Vulva. **A** prolateral **B** retrolateral **C, G** dorsal **D, F** ventral **E** lateral. Abbreviations: At atrium; CD copulatory ducts; CO copulatory opening; Co conductor; Cy cymbium; CyC cymbial conductor; CyF cymbial fold; CyFs setae on cymbial fold; CyP cymbial process; E embolus; Et embolic tip; S spermathecae, SD spermatheca; Sp scape; T tegulum, TA tegular apophysis; Ti tibia. Scale bars: 0.20 mm (**A, B, E–G**); 0.10 mm (**C, D**).

Nature Reserve, Primary tropical seasonal rainforest (21°57.420'N, 101°13.020'E; alt. 744±15 m), 30.VII.2007, G. Zheng leg. Examined.

**Diagnosis.** This species differs from all species of *Trogloneta* by the absence of anterior median eyes and the posteriorly pointed abdomen in both sexes (Figure 2A, B, F, G).

**Remarks.** This is the only species of *Trogloneta* spider ever found living in the tropical rainforest canopy, and is also a relatively rare six-eyed mysmenid species. We have tried to collect it again in the type locality, hoping to obtain some samples for molecular study but unfortunately, more material was not found.

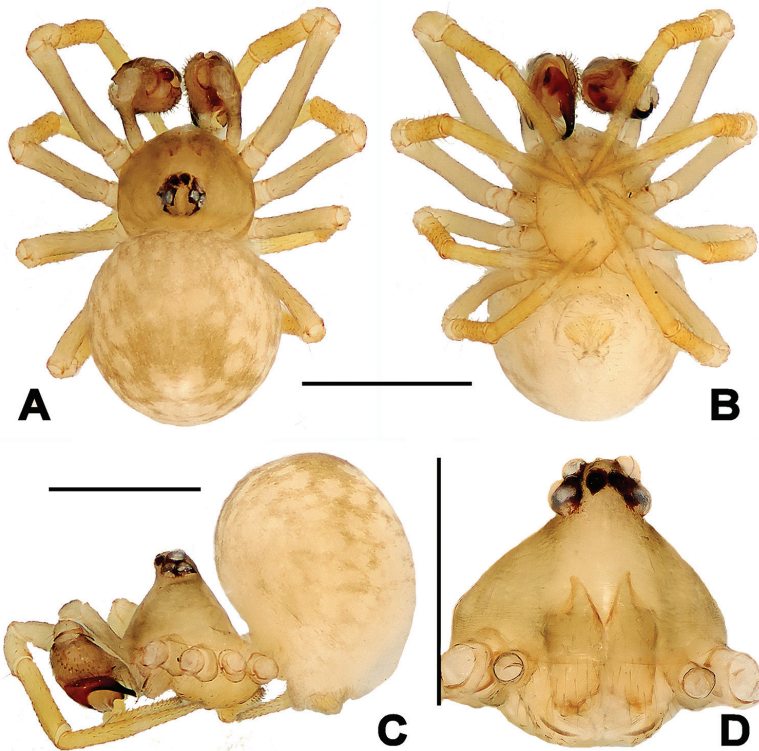
**Distribution.** China (Yunnan).

### *Trogloneta uncata* Lin & Li, 2013

Figures 4, 5

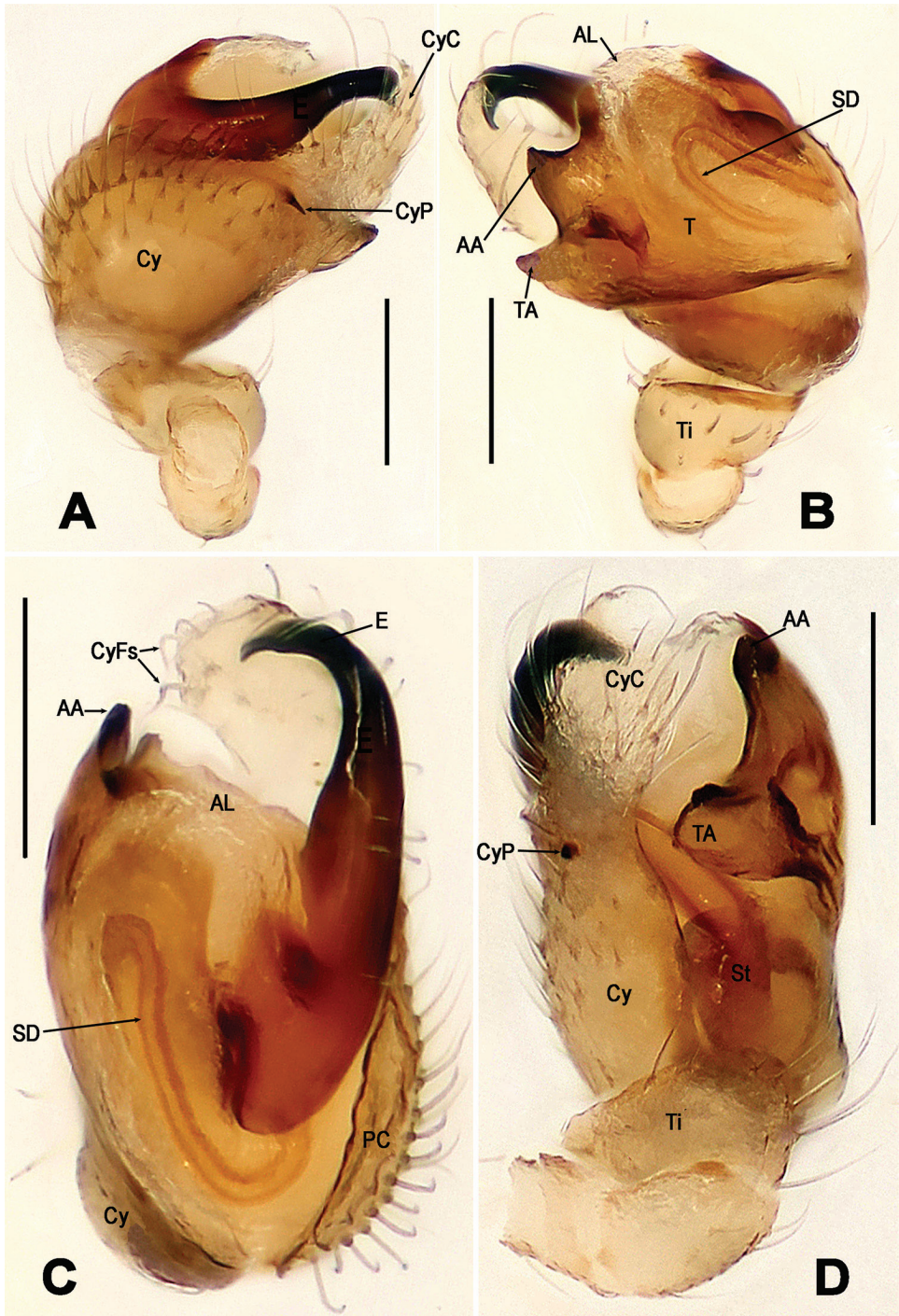
*Trogloneta uncata* Lin & Li, 2013b: 476, figs 23A–G, 24A–D, 25A–D.

**Type material.** Holotype ♂ (IZCAS) from CHINA: Yunnan Province, Nanjian County, Xiaowan Town, Huilong Mt., Banpoyan Cave (24°56.012'N, 100°18.866'E; alt. 1990 m), 25.VI.2010, C. Wang, Q. Zhao and L. Lin leg. Examined.



**Figure 4.** *Trogloneta uncata* Lin & Li, 2013, male (A–D). A–C Habitus D Prosoma (appendages omitted) A dorsal B ventral C lateral D anterior. Scale bars: 0.50 mm.





**Figure 5.** *Trogloneta uncata* Lin & Li, 2013, male left palpe (A–D). **A** prolateral **B** retrolateral **C** apical **D** ventral. Abbreviations: AA apical apophysis; AL apical lobe; Cy cymbium; CyC cymbial conductor; CyFs setae on cymbial fold; CyP cymbial process; E embolus; TA tegular apophysis; PC paracymbium; SD spermatic duct; St subtegulum; T tegulum; Ti tibia. Scale bars: 0.10 mm.

**Diagnosis.** *Trogloneta uncata* can be distinguished from Brazilian *Trogloneta* spp. (Brescovit and Lopardo 2008) by the globular abdomen, lack of pointed tubercle posteriorly; from the American *T. paradoxa* (see Gertsch 1960: figs 12, 16) and the Chinese *T. speciosum* (Figure 3A, B), *T. yuensis* (Figure 7A, B), and *T. yunnanense* (Figure 10A, B) by the presence of a spur-shaped cymbial process, and the stout embolus with a falcate tip (Figure 5A–D); from the European *T. canariensis*, the type species *T. granulum*, and *T. madeirensis* from Madeira Is. (see Wunderlich 1987: figs 371, 376, 383) by a stout, hooked embolus and having a cymbial process in the palpal bulb (Figure 5B–D). Female unknown.

**Distribution.** China (Yunnan).

### *Trogloneta yuensis* Lin & Li, 2013

Figures 6–8

*Trogloneta yuensis* Lin & Li, 2013a: 43, figs 8A–E, 9A–B, 10A–F, 11A–B, 12A–E.

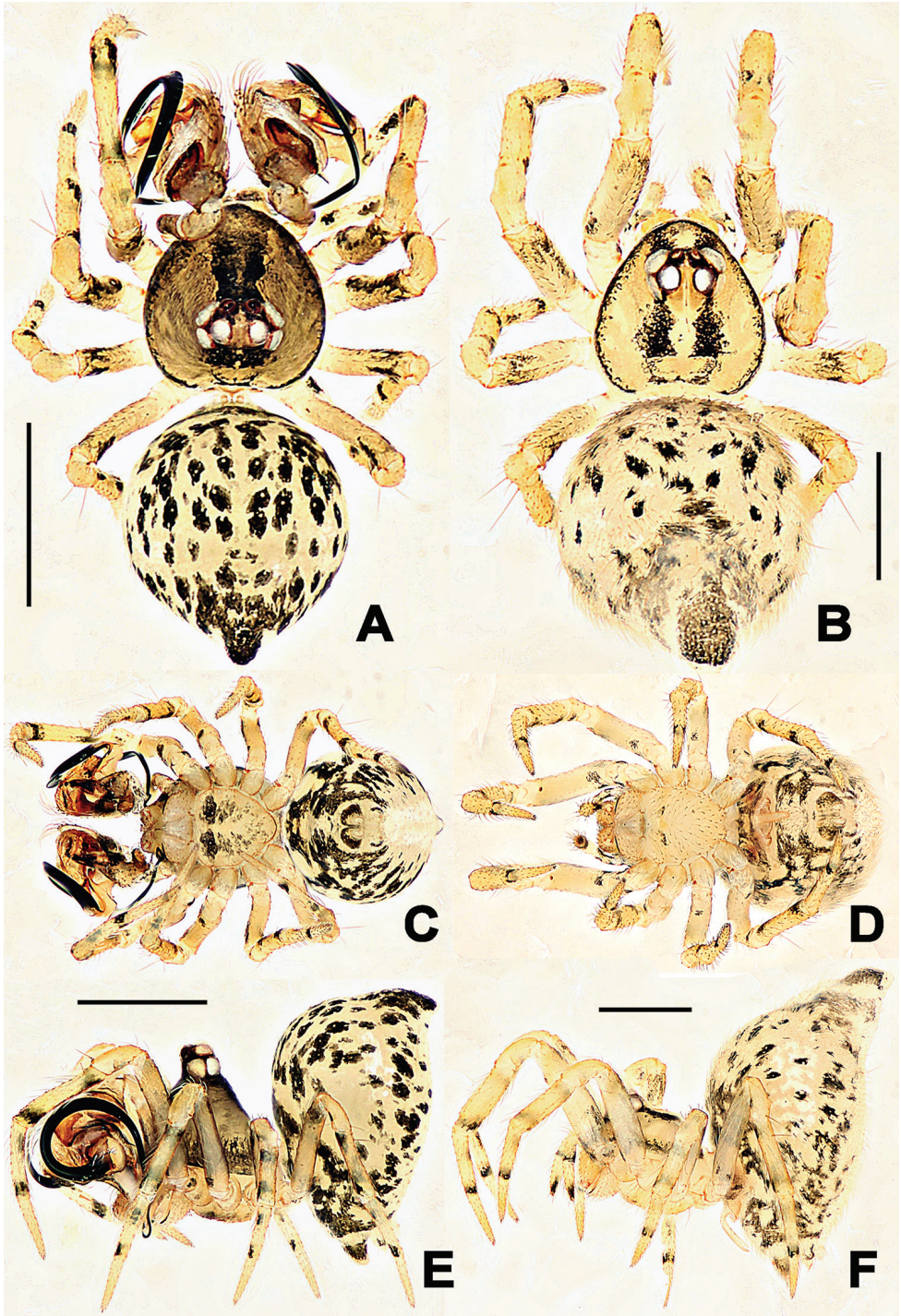
**Type material.** Holotype ♂ (NHMSU) from CHINA: Chongqing City, Beibei District, Jinyun Mt., Guankou (29°50.261'N, 106°23.811'E; alt. 531 m), 5-IV-2010, by sieving, Z. Zhang leg. Examined.

**Other material.** 5♂, 6♀ (NHMSU) from CHINA: Hunan Province, Changsha City, Yuelu District, Yuelu Mt. Parkland, (112°56.526'E, 28°11.211'N; alt. 163 m), 19-IV-2018, by sieving, G. Zhou leg.

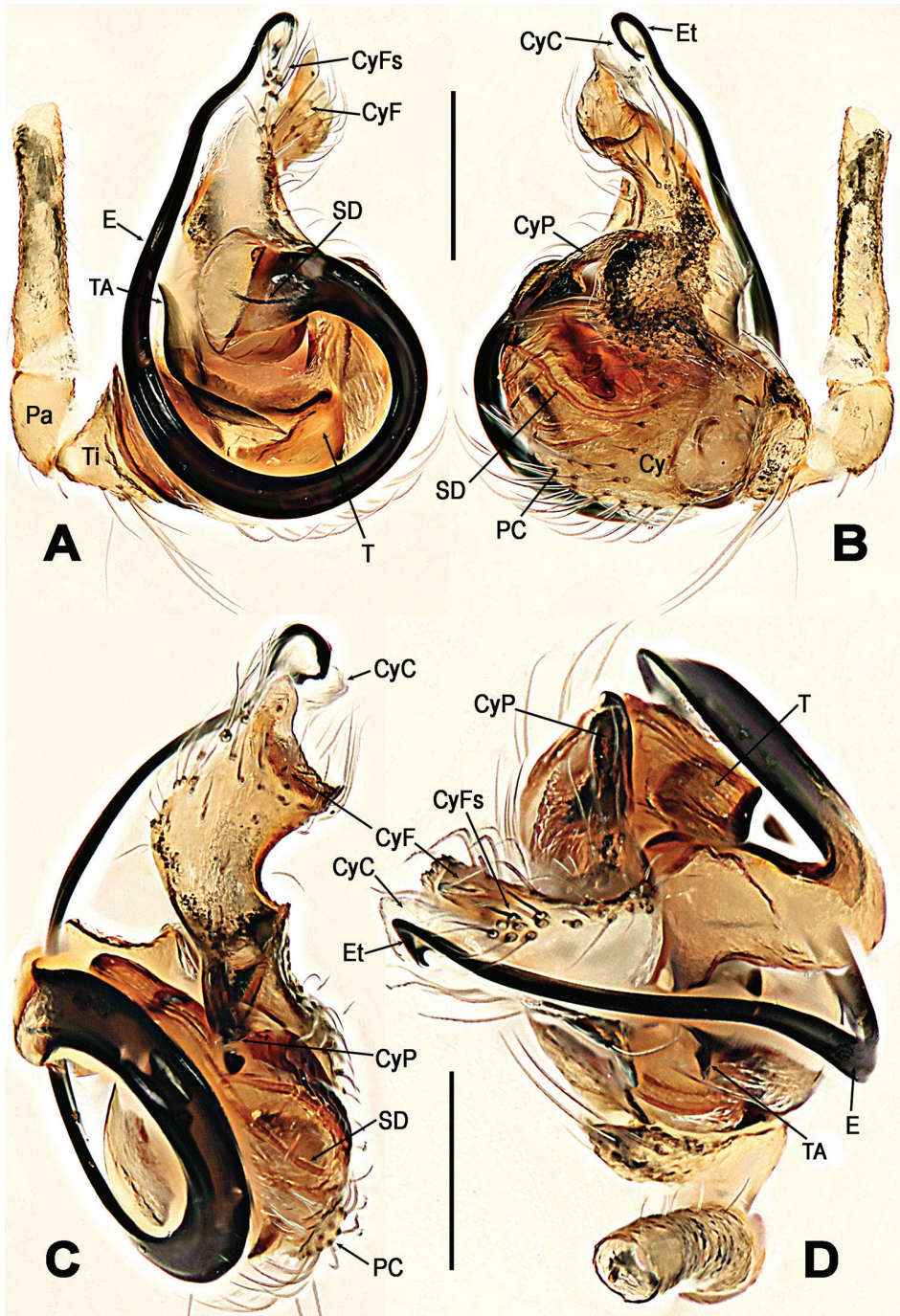
**Diagnosis.** *Trogloneta yuensis* differs from most congeners except the Brazilian *Trogloneta* species and *T. speciosum* by the presence of pointed tubercle on abdomen dorso-posteriorly (Figure 6A, B, E, F). It differs from Brazilian *Trogloneta* spp. (see Brescovit and Lopardo 2008: figs 1D–J, 2C–I, 2K–N) by the presence a large cymbial process of palp and a long epigynal scape (Figs 7A–C, 8A–D); from *T. speciosum* by the larger body size, distinctly stretched cymbial conductor, and long epigynal scape (Figs 7A–C, 8A–D). It is most similar to *T. nojimai* in the male palpal structure and the epigynal configuration, but can be distinguished from the latter by the pointed abdomen dorso-posteriorly, a hooked embolic end, a large cymbial process, and the clavate spermethecae (Figs 7A–C, 8A–D).

**New morphological data. Male. Measurements:** total length 1.08. Prosoma 0.46 long, 0.45 wide, 0.60 high. Clypeus 0.32 high. Sternum 0.31 long, 0.30 wide. Opisthosoma 0.57 long, 0.55 wide, 0.98 high. Length of legs: I 1.45 (0.44, 0.17, 0.33, 0.29, 0.22); II 1.17 (0.39, 0.16, 0.23, 0.23, 0.16); III 1.00 (0.30, 0.13, 0.22, 0.18, 0.17); IV 1.18 (0.37, 0.15, 0.26, 0.22, 0.18). Legs formula: I-II-IV-III.

**Somatic characters** (Figure 6A, C, E). **Coloration:** carapace black centrally and marginally, thoracic region dim yellow. Clypeus black. Sternum pale yellow, with a pair of shoulder dark speckles. Abdomen pale yellow, modified by irregular dark spots. **Prosoma:** carapace sub-round dorsally, conical laterally. Cephalic pars sharply raised, slope forward and backward. Ocular area at apex. AME black, others white. AME



**Figure 6.** *Trogloneta yuensis* Lin & Li, 2013, male habitus (**A, C, E**) and female habitus (**B, D, F**). **A, B** dorsal **C–D** ventral **E–F** lateral. Scale bars: 0.50 mm.



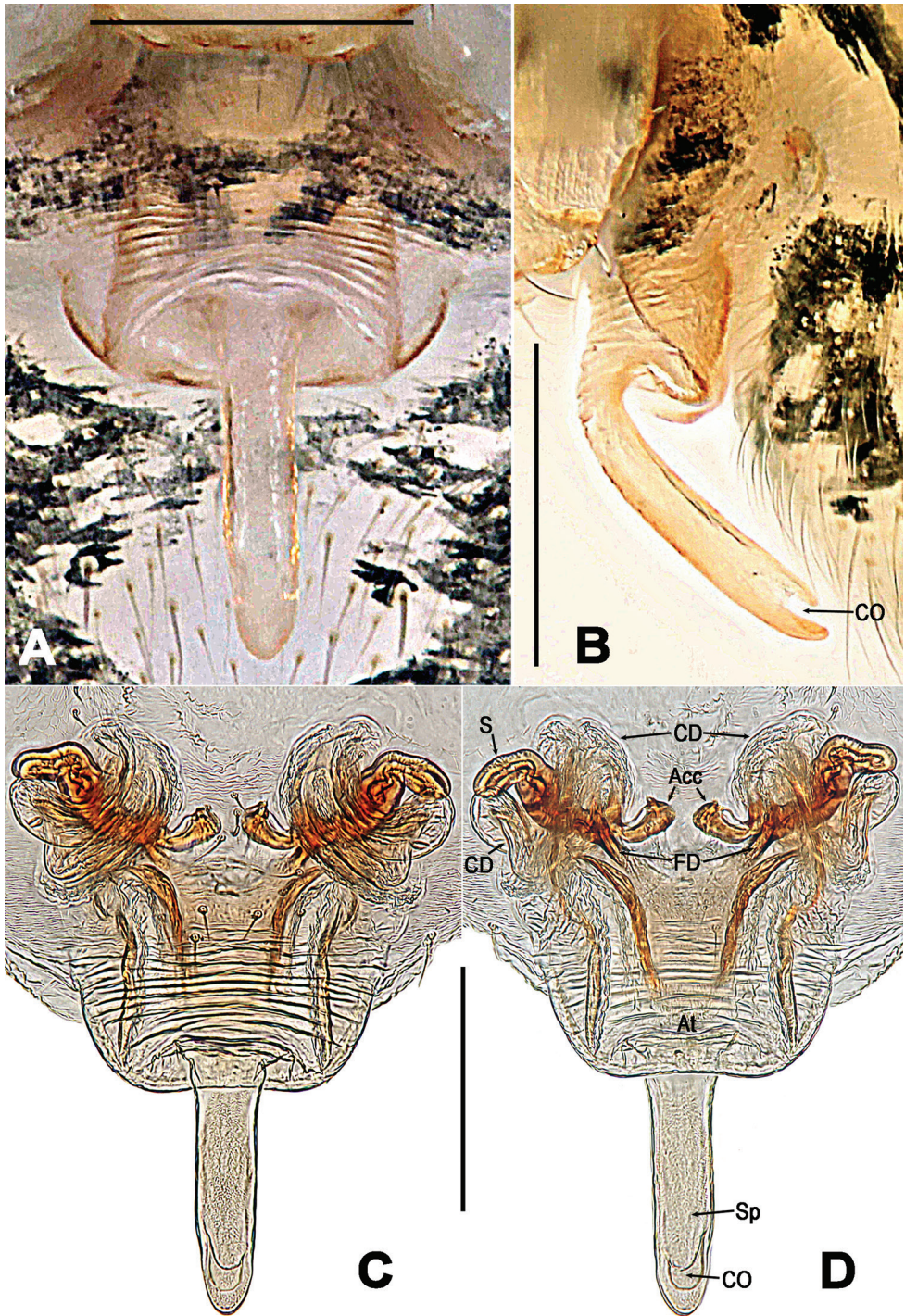
**Figure 7.** *Trogloneta yuensis* Lin & Li, 2013, male left palp (A–D). **A** prolateral **B** retrolateral **C** apical **D** ventral. Abbreviations: Cy cymbium; CyC cymbial conductor; CyF cymbial fold; CyFs setae on cymbial fold; CyP cymbial process; E embolus; Et embolic tip; TA tegular apophysis; Pa patella; PC paracymbium; SD spermatid duct; T tegulum; Ti tibia. Scale bars: 0.20 mm.

smallest, ALE largest. ALE>PLE>PME>AME. ARE and PRE distinctly procurved. Chelicerae pale yellow, shorter than endites, fang furrow with two promarginal and a single retromarginal teeth. Labium pale, triangular, no fused to sternum. Sternum cordiform, truncated posteriorly. **Legs:** each segments pale yellow, except tarsi, other segments modified by grey rings distally. A subdistal sclerotized femoral spot on leg I ventrally, but none on leg II. A clasping macroseta on metatarsus I submesial pro-laterally. A dorsal seta on each patella distally and tibia proximally. Three trichobothria on tibiae I, II, and IV respectively, but four on tibia III. All metatarsi and tarsi lack trichobothrium. **Opisthosoma:** elliptic dorsally, triangular laterally, with a tubercle dorso-posteriorly. Spinnerets at lowest position, the anterior ones black, and the posterior ones pale. Colulus small, tongue-shaped. Anal tubercle pale. **Palp** (Figure 7A–D): larger than half carapace, strongly sclerotized. Femur 2.5× as long as patella. Patella short. Tibia wider than long, conical, bears a dorsal trichobothrium and a few long setae ventro-marginally. Cymbium large, paracymbium flattened, bears dense long setae. A long cymbial process (aquiline distally, constricted proximally) arisen from inner side subdistal margin of cymbium. Cymbial fold distinct, with long setae. Cymbial conductor extended distally, membranous, attaching with a cluster of setae. Tegulum smooth. Spermatic duct long, visible through subtegulum. A long, finger-like apophysis derives from the junction between tegulum and subtegulum. Embolus long, arched, gradually tapering to form a hooked end. Embolic end with accessory membrane, hidden behind cymbial conductor.

**Female. Measurements:** Total length 1.32. Prosoma 0.52 long, 0.49 wide, 0.46 high. Clypeus 0.15 high. Sternum 0.34 long, 0.33 wide. Opisthosoma 0.92 long, 0.88 wide, 1.13 high. Length of legs: I 1.64 (0.53, 0.19, 0.36, 0.31, 0.25); II 1.39 (0.44, 0.18, 0.30, 0.25, 0.22); III 1.15 (0.35, 0.15, 0.25, 0.21, 0.19); IV 1.32 (0.42, 0.17, 0.28, 0.24, 0.21). **Somatic characters** (Figure 6B, D, F) as in male, but larger size and lighter colour than male, ocular area more anterior than in male. **Epigyne** (Figure 8A–D): Epigynal area elevated ventrally. A long and narrow scape curving basally, copulatory opening at scape distally (Figure 8B). Epigynal plate transversely rugose (Figure 8A, C). Inner vulval structure peculiar (Figure 8D). Copulatory ducts long and bell-shaped proximally (Figure 8D), most duct areas translucent and coiled around the spermathecae (Figure 8C, D). Atrium broad, subquadrate. Spermathecae clavate, curved. Fertilisation ducts stem from the spermathecae baso-dorsally (Figure 8D). Inside accessory gland connected with the base of spermathecae ventrally (Figure 8C).

**Remarks.** The species is original described on the basis of only a male specimen from Jinyun Mt. of Chongqing City that was donated by Prof Zhisheng Zhang (Southwest University in Chongqing, China) (Lin and Li 2013a). Since then, we have conducted two supplementary collections in the type locality, and not obtained female samples. During 2018, when Mr Guchun Zhou (Hunan Normal University in Changsha, China) was investigating the spider diversity of Yuelu Mt in Changsha City, a large number of samples of this species were obtained by sieving the surface deciduous layers. He presented us with some material for this study.

**Distribution.** China (Chongqing, Hunan).



**Figure 8.** *Trogloneta yuensis* Lin & Li, 2013, female epigyne (**A, B**) and vulva (**C, D** lactic acid-treated). **A, C** ventral **B** lateral **D** dorsal. Abbreviations: Acc accessory gland; At atrium; CD copulatory ducts; CO copulatory opening; FD fertilisation ducts; S spermathecae; Sp scape. Scale bars: 0.20 mm.

***Trogloneta yunnanense* (Song & Zhu, 1994), comb. n.**

Figures 9–11

*Pholcomma yunnanense* Song & Zhu, 1994: 38, fig. 4A–C; Song, Zhu & Chen, 1999: 127, fig. 66A–B; Li & Lin, 2016: 320.

*Trogloneta denticocleari* Lin & Li, 2008: 513, figs 16A–E, 17A–E. **Syn. n.**

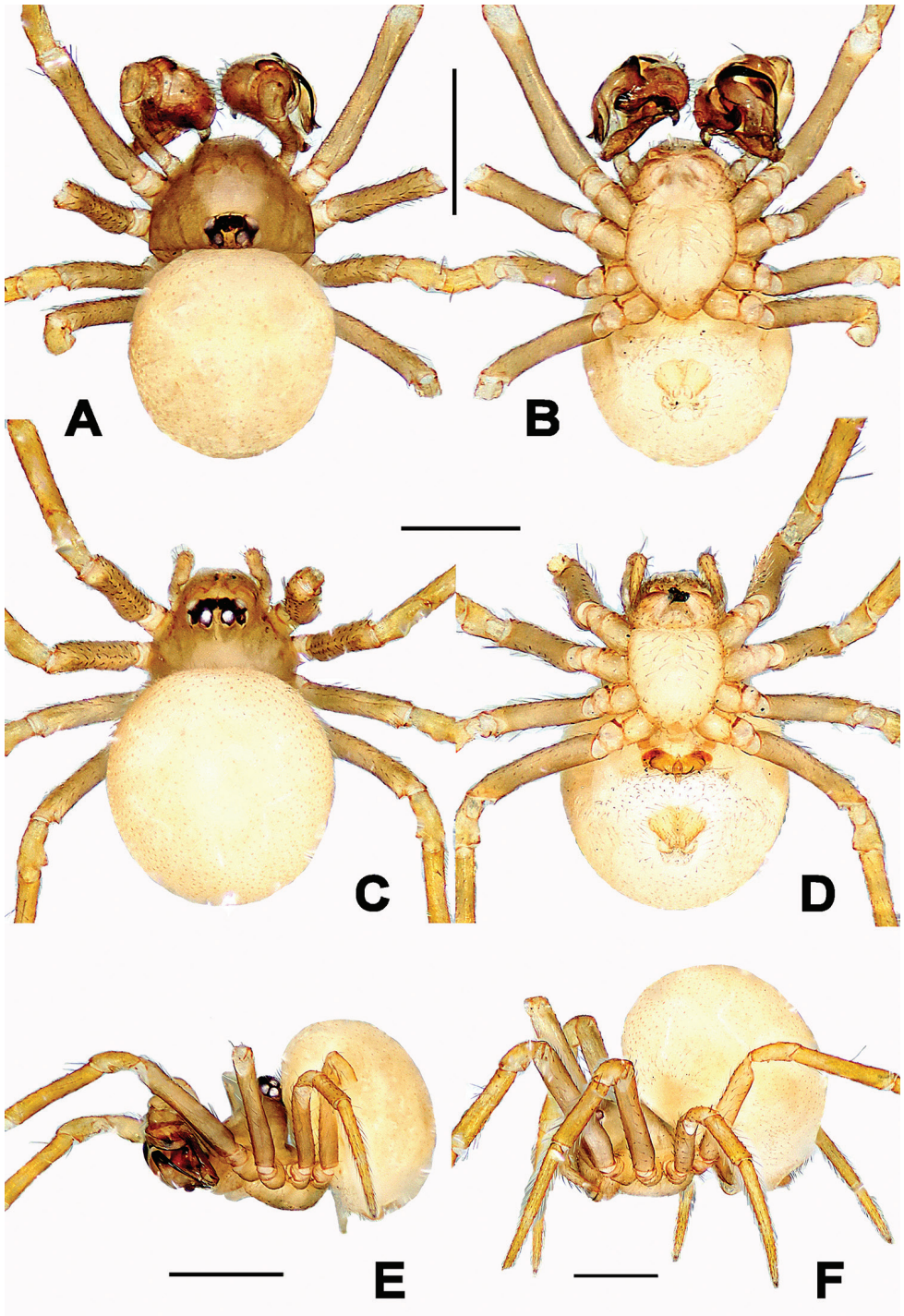
**Type material.** Holotype ♀ (of *Pholcomma yunnanense*) (IZCAS) from CHINA: Yunnan Province, Gejiu City, Feixia Cave, 4-IV-1992, by hand, D. Song leg. Examined.

**Other material.** (Types of *T. denticocleari*): Holotype ♂, and paratypes 6♂, 29♀ (IZCAS) from CHINA: Yunnan Province, Kunming City, Panlong District, Xiaohe Town, Yanzidong Cave (25°11.280'N, 102°48.420'E; T. 9 °C; H. 90%; alt. 2,042 m), 4-IV-2007, Y. Lin and J. Liu leg.; 7♂, 11♀ (IZCAS) from CHINA: Yunnan Province, Yiliang County, Jiuxiang Town, Baiyan Cave (25°09.060'N, 103°24.060'E; T. 12 °C; H. 90%; alt. 1,875 m), 9-IV-2007, Y. Lin and J. Liu leg.; 5♂, 7♀ (IZCAS) from CHINA: Guizhou Province, Dafang County, Yangchangba Town, Longdong Village, Qianxudong Cave (27°05.940'N, 105°40.260'E; T. 15 °C; H. 95%; alt. 1,486±14 m), 17-V-2004, Y. Tong and Y. Lin leg.; 2♂, 8♀ (IZCAS) from CHINA: Guizhou Province, Dafang County, Wen'ge Town, Sanhe Village, Yelaodadong Cave (27°10.920'N, 105°28.260'E; T. 10 °C; H. 90%; alt. 1,438 m), 3-V-2007, Y. Lin and J. Liu leg.; 4♂, 5♀ (IZCAS) from CHINA: Guizhou Province, Panxian County, Zhudong Town, Shilipin Village, Shilidadong Cave (25°37.560'N, 104°45.000'E; T. 13 °C; H. 80%; alt. 1,680 m), 15-IV-2007, Y. Lin and J. Liu leg.

**Supplementary material.** 2♂, 18♀ (NHMSU) from CHINA: Yunnan Province, Chuxiong City, Wuning County, Cat street, Xianren Cave (25°27.931'N, 102°10.437'E; alt. 2,066 m), 18-IV-2010, C. Wang, Z. Zhao and L. Lin leg.; 3♂, 1♀ (NHMSU) from CHINA: Guizhou Province, Zunyi City, Shenxi Town, Longjiang Village, Juzizu, Guanniu Cave (27°36.745'N, 106°58.091'E; alt. 814 m), 20-III-2011, Z. Chen and Z. Zha leg.

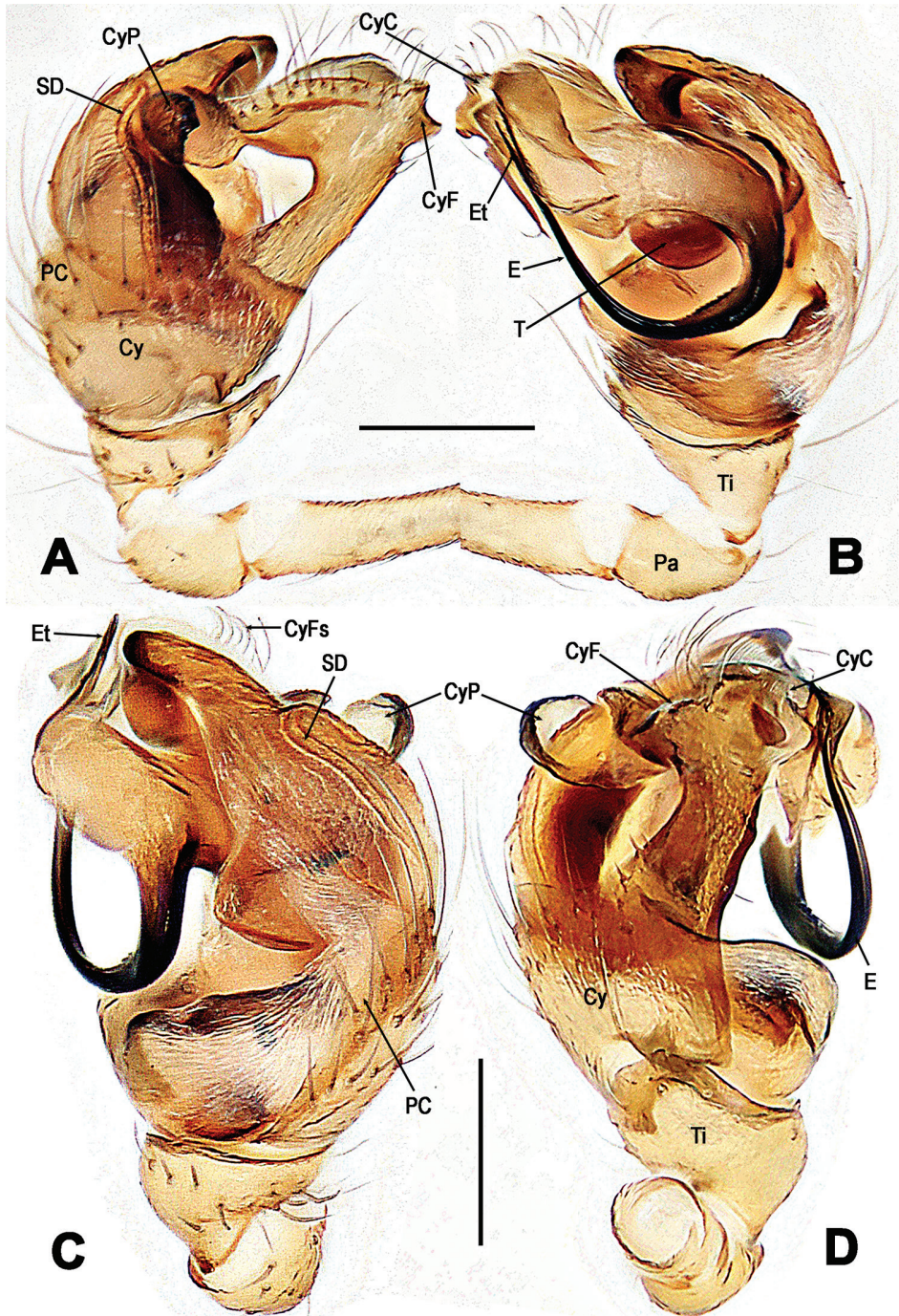
**Diagnosis.** *Trogloneta yunnanense* can be distinguished from those *Trogloneta* species with a pointed abdominal tubercle (*T. cantareira*, *T. cariacica*, and *T. mourai* in Brescovit and Lopardo 2008: figs 1A–C, 2A, B, and 2J; and *T. speciosum* in Figure 2A, B, F, G and *T. yuensis* in Figure 6A, B, E, F) by the globular abdomen (Figure 9A–F). It differs from *T. canariensis* and *T. madeirensis* (see Wunderlich 1987: figs 375–380, 382–387) by the long embolus, huge cymbial process, a recurved scape, and the unique broad epigynal plate (Figure 10A, B, 11A, B). It differs from the type species *T. granulum* (see Lopardo and Hormiga 2015: figs 66A–E, 67A, 128F, 131E) and *T. paradoxa* (see Gertsch 1960: figs 12, 15, 16) by a well-developed, spoon-shaped cymbial process and a protruded, pentagonal epigynal plate (Figs 10A, C, D, 11A, B). It differs from *T. uncata* in Figure 5A–D by the long embolus with a straight, tapering distal end (Figure 10B vs. 5A, C) and a huge cymbial process (Figure 10A vs. 5A, D).

**Taxonomic justification.** The type material of *Pholcomma yunnanense* has been examined as well as its related literatures in this study, the habitus features, the body size, the shape of protruded epigyne with an inflexed space, the broad epigynal plate, the configura-

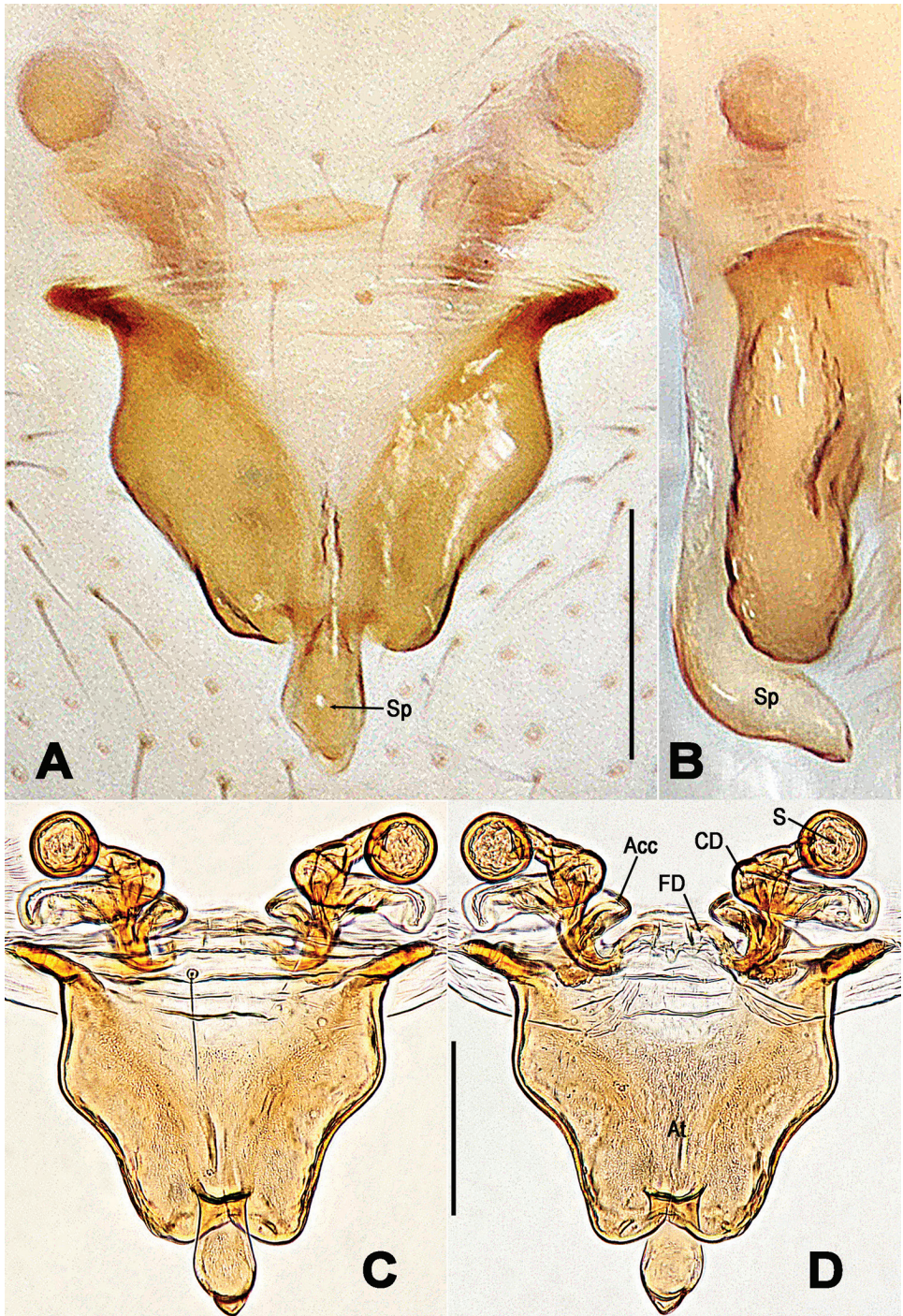


**Figure 9.** *Trogloneta yunnanense* (Song & Zhu, 1994) comb. n., male habitus (**A, B, E**) and female habitus (**C, D, F**). **A, C** dorsal **B, D** ventral **E, F** lateral. Scale bars: 0.50 mm.





**Figure 10.** *Trogloneta yunnanense* (Song & Zhu, 1994) comb. n., male left palp (**A–D**). **A** prolateral **B** retrolateral **C** dorsal **D** ventral. Abbreviations: Cy cymbium; CyC cymbial conductor; CyF cymbial fold; CyFs setae on cymbial fold; CyP cymbial process; E embolus; Et embolic tip; TA regular apophysis; Pa patella; PC paracymbium; SD spermatheca; T tegulum; Ti tibia. Scale bars: 0.20 mm.



**Figure 11.** *Trogloneta yunnanense* (Song & Zhu, 1994) comb. n., female epigyne (**A, B**) and vulva (**C, D**, lactic acid-treated). **A, C** ventral **B** lateral **D** dorsal. Abbreviations: Acc accessory gland; At atrium; CD copulatory ducts; FD fertilization ducts; S spermathecae; Sp scape. Scale bars: 0.20 mm.

tion of vulva with a pair of far apart, globular spermathecae depicted in the type and original illustrations (Song and Zhu 1994: figs 4A–C) leave little doubt that the identification is correct. The original illustrations of epigyne and vulva of *Pholcomma yunnanense* by Song and Zhu (1994) are rather simple and show some differences in comparison with those of *Trogloneta denticocleari* (Lin & Li, 2008: fig. 17A, D–E), but we have examined the type of *Pholcomma yunnanense* and the plentiful specimens of *Trogloneta denticocleari*. Therefore, we consider these subtle differences as being intraspecific variation. To further confirm our judgments using DNA barcoding, a survey at the type locality was specially conducted in August 2018. Unfortunately, the habitat of type locality had become so dry that no samples were obtained. Nevertheless, there are valid reasons to think they are synonyms and *T. yunnanense* (Song & Zhu, 1994), comb. n. is proposed and transferred from the genus *Pholcomma* of Theridiidae, as well as *T. denticocleari* proposed as a new synonym.

**Distribution.** China (Guizhou, Yunnan).

## Discussion

After this study, the genus *Trogloneta* contains twelve nominal mysmenid species. Among its members, three species live in caves (*T. yunnanense*, *T. granulum*, and *T. uncata*), and the other nine are found in surface leaf litter (*T. canariensis*, *T. cantareira*, *T. cariacica*, *T. madeirensis*, *T. mourai*, *T. nojimai*, *T. paradoxa*, and *T. yuensis*) or in forest canopy (*T. speciosum*). Although the genus *Trogloneta* is widely distributed in Europe, Asia, North to South America, and in parts of the Atlantic islands, its many members are clearly endemic species according to the original literature. However, there are two exceptions, and they are *T. granulum* and *T. yunnanense*. The former as the type species of this genus was first found in caves of France, and later reported to be widespread on the surface Beech forest floor and in caves of many European countries, such as Austria, Czech Republic, France, Germany, Italy, Poland, and Slovakia. The latter as a new combination proposed in the current paper is also widely distributed in the isolated limestone caves in Southwest China. So far it has not yet been found on the surface. We know that the caves are relatively closed and isolated habitats: why they have such a distribution pattern, how do they get into caves to eventually colonise them, how does the isolation mechanism of the population work, and other questions are worth further study.

## Acknowledgments

The manuscript benefitted greatly from comments by Lara Lopardo (Lufthansa, Germany) and Hirotsugu Ono (Tokyo, Japan). We are especially grateful to Jeremy A Miller (Leiden, Netherlands), the subject editor of this manuscript, for his editorial efforts. Thanks to Mr Guchun Zhou for donating part specimen materials of *Trogloneta yuensis*. This study was supported by the National Natural Science Foundation of China (NSFC-31772410, 31750002).

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# Revision of the genus *Cephalobyrrhus* of Japan and Taiwan (Coleoptera, Limnichidae)

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## Abstract

Japanese and Taiwanese species of the genus *Cephalobyrrhus* are revised. A new species, *C. amami* **sp. n.**, is described from Amami-Ōshima, the Ryukyus, Japan. This species is clearly distinguished from the other known species by the short and pointed median lobe and long phallobase. Two previously known species from Japan and Taiwan, *C. latus* and *C. japonicus*, are redescribed.

## Keywords

Byrrhoidea, Cephalobyrrhinae, key, new species, taxonomy

## Introduction

The family Limnichidae Erichson, 1846, contains four subfamilies, and is represented by approximately 400 species in 37 genera (Spangler et al. 2001; Hernando and Ribera 2005). Of these, Cephalobyrrhinae Champion, 1925 is a small subfamily consisting of four genera: *Throscinus* LeConte, 1874 (seven species) from New World; *Cephalobyrrhus* Pic, 1923 (15 species) and *Parathroscinus* Wooldridge, 1984 (five species) mainly from the Oriental Region; and *Erichia* Reitter, 1895 (= *Jaechobyrrhinus* Pütz, 1991) (one extant species) from Turkey (Jäch and Pütz 2001; Hernando and Ribera 2005; Yu et al. 2018). The most speciose genus, *Cephalobyrrhus*, was revised by Pütz (1998), and is distributed in Japan, China, India, and Nepal (Pütz 1998). However, numer-

ous undescribed species still remain. One previously known species is recorded from each, Japan and Taiwan, but two species were revised based on limited specimens (Pütz 1998) and further study was needed.

In this paper, the Japanese and Taiwanese species of the genus are reviewed, and the description of a new species is provided.

## Materials and methods

The materials examined in this paper are deposited in the following institutions:

- EUMJ** Ehime University Museum, Matsuyama, Japan  
**TARI** Taiwan Agricultural Research Institute, Taichung, Taiwan  
**SEHU** Systematic Entomology, Hokkaido University, Sapporo, Japan  
**NMW** Naturhistorisches Museum, Vienna

General observations and dissections were made under a Leica MZ95 stereomicroscope. Microstructures of the dissected parts in pure glycerin were studied under an Olympus BH-2 compound microscope. After observation, the dissected parts were mounted on the same card with the specimen. Photographs were taken under the Leica MZ95 and combined in Helicon Focus ver. 4.70.5 Pro (Helicon Soft Limited).

Morphological terminology follows Hernando and Ribera (2005). Morphological abbreviations used in the measurements are as follows:

- EL** length of elytra in suture;  
**EW** maximum width of elytra;  
**PL** length of pronotum in median line;  
**PW** maximum width of pronotum;  
**TL** total length (PL+EL).

The average is given in parentheses after the range.

## Taxonomy

### *Cephalobyrrhus* Pic, 1923

*Cephalobyrrhus*: Pic 1923: 4. Champion 1925: 174; Wooldridge 1977: 29; 1984: 121; Pütz 1998: 342.

**Type species.** *Cephalobyrrhus latus* Pic, 1923: 4

**Diagnosis.** Body medium size in the family, ca 2.2–4.5 mm, oblong, weakly convex dorsally, closely covered with short setae in dorsal surface. Eyes large; distance be-

tween their inner margins approximately equal to the diameter of one eye. Mandibles (Fig. 2B) slender, with pointed tridentate apex. Maxillae (Fig. 2C) rounded in apical part of galea and lacinia, with 4-segmented palpi. Labial palpi (Fig. 2D) 3-segmented, with rhomboid terminal palpomere. Pronotum bisinuate in posterior margin, gently tapering anteriorly. Hind wings (Fig. 2A) fully developed, 2.5 times as long as wide;  $AA_{3+4}$  present;  $MP_3$  and  $MP_4$  not connected with  $AA_{3+4}$ . Elytra elongate, bisinuate in basal margin, with obvious irregular zigzag markings consisting of adpressed long silver setae. Metacoxae transverse. Legs relatively long; hind tibiae exteriorly smooth; tarsal formula 5-5-5. Sexual dimorphism indistinct.

**Remarks.** This genus is similar to *Erichia* Reitter, 1895 (= *Jaechobyrrhinus* Pütz, 1991), in general appearance, but differs from the latter in the following characteristics (Pütz 1991): body oblong, gently tapering posteriorly (elongate and subparallel-sided in *Erichia*); pronotum rounded in anterior and posterior corners (pointed in *Erichia*), posterior margin bisinuate (with two rounded extensions in *Erichia*). Most species of this genus have similar external features and intraspecific variation, and cannot be reliably identified without examination of the male genitalia (Pütz 1998).

### Key to males of the species of the genus *Cephalobyrrhus* of Japan and Taiwan

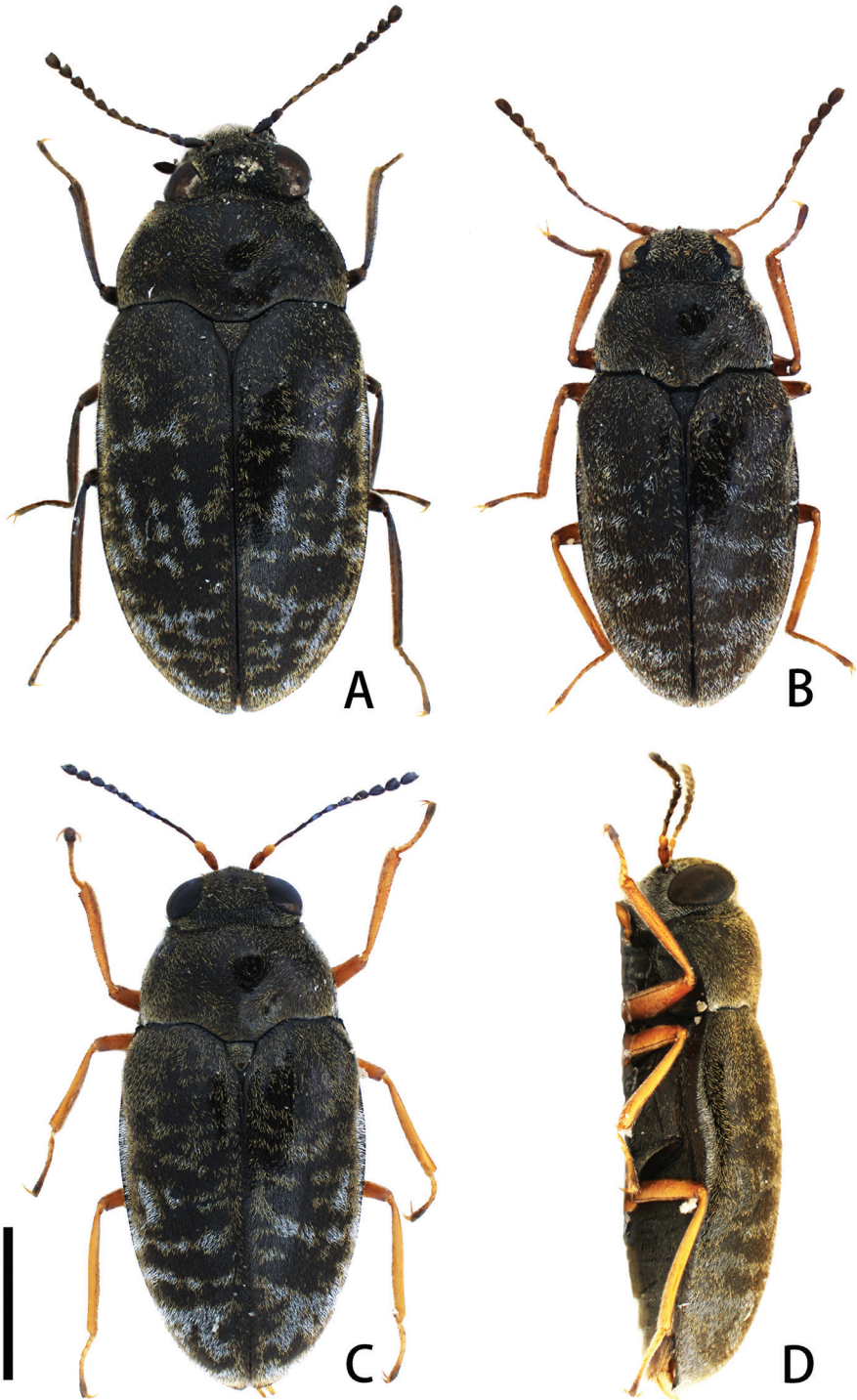
- 1 Phallobase long, 3.3 times as long as parameres; median lobe pointed at apex. Distribution: Japan (Amami-Ōshima) ..... ***C. amami* sp. n.**
- Phallobase moderate in length, 2.3 times as long as parameres; median lobe obtuse at apex ..... **2**
- 2 Phallobase slightly tapering basally; parameres obtuse at apices; median lobe widened in basal 1/3. Distribution: Taiwan..... ***C. latus***
- Phallobase subparallel-sided; parameres curved interiorly and rather pointed at apices; median lobe straight in sides. Distribution: Japan (Honshu to Kyushu) ..... ***C. japonicus***

### *Cephalobyrrhus latus* Pic, 1923

Figs 1A, 2E, 3A–G

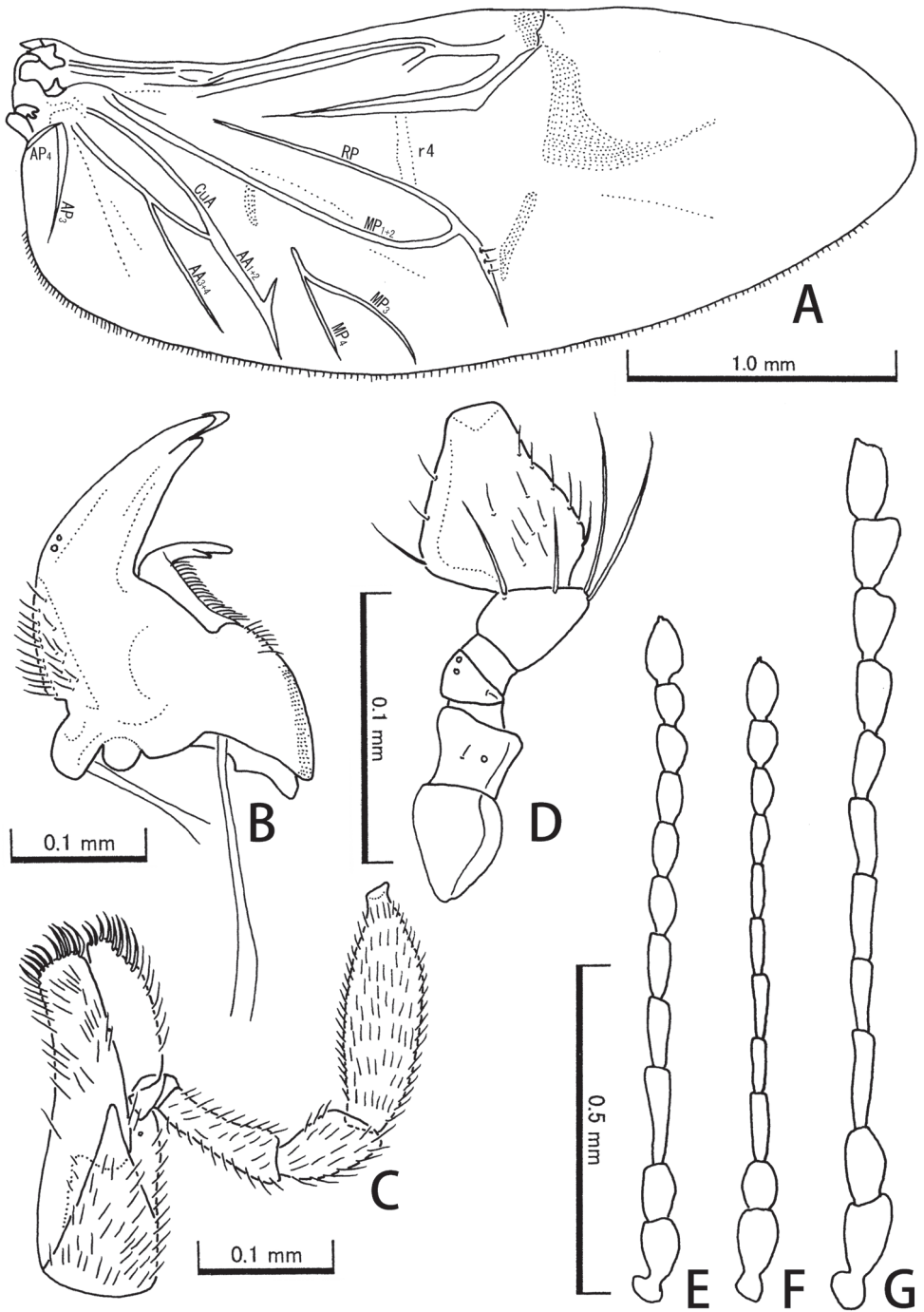
*Cephalobyrrhus latus*: Pic 1923: 4; Champion 1925: 174; Satô 1965: 123; Pütz 1991: 132; 1998: 357.

**Specimens examined.** 1 ex. (SEHU), Alishan Chiayi, 4–5. VII. 1975, H Takizawa leg.; 1 ex. (SEHU), Fenchihu, Nantou, 11–12.VII.1981, H Takizawa leg.; 4 exs. (EUMJ), same loc., 7.VII.1961, S Ueno leg.; 2 exs. (EUMJ), Fushan, Taipei Hsien, 12.IV.1999, Y-Y Lien leg.; 5 exs. (TARI), same loc., 30.III.2012, C-F Lee leg.; 1 ex. (EUMJ), Hsileng, Taoyuan Hsien, 3.V.1981, S Tsuyuki leg.; 1 ex. (EUMJ), Kuanshan, Yakou, Taichung Hsien, 2,600 m, 11.VI.1989, M Satô leg.; 4 exs. (EUMJ), Liyuan



**Figure 1.** Dorsal (A–C) and lateral (D) habitus of *Cephalobyrrhus* spp. from Japan and Taiwan. **A** *Cephalobyrrhus latus* Pic, 1923 **B** *C. japonicus* Champion, 1925 **C, D** *C. amami* sp. n. (holotype). Scale bar: 1.0 mm.





**Figure 2.** Hind wing (A), mandible (B), maxilla (C), labial palpus (D), and male antennae (E–G) of *Cephalobyrrhus* spp. **A–D, F** *C. japonicus* Champion, 1925 **E** *C. latus* Pic, 1923 **G** *C. amami* sp. n. (paratype).

(1800–1900 m), Haiduan, Taichung Hsien, 5.VI.2013, K Sonaka leg.; 1 ex. (EUMJ), Mt. Lala, Taoyuan Hsien, 30.IV.1979, S Tsuyuki leg.; 1 ex. (EUMJ), same loc., 2.V.1981, S Tsuyuki leg.; 1 ex. (TARI), Nantou, Tatachia, 6–12.V.2008, C-S Tung leg.; 17 exs. (EUMJ), Nihonmatsu-Hokuko, Byoritsu-ken, 10.IV.1967, T Shirozu leg.; 1 ex. (EUMJ), Oiwake, 24.VI.1961, T Shirozu leg.; 25 exs. (EUMJ), Paolai, Kaosung Hsien, 11.VI.1989, M Satô leg.; 3 exs. (EUMJ), Sihling-Sicun, Taoyuan Hsien, 19.IV.2007, S-T Hisamatsu leg.; 1 ex. (EUMJ), Sungkang-Meifeng, Nantou Hsien, 19.V.1969, S Hisamatsu leg.; 5 exs. (EUMJ), Tsuifeng, Nantou Hsien, 18.VI.1989, M Satô leg.; 4 exs. (SEHU), Tungpu Chiayi, 14–17.VII.1976, H Takizawa leg.; 4 exs. (EUMJ), Wulai, Taipei Hsien, 4.V.1968, Y Watanabe leg.; 4 exs. (EUMJ), same loc., 17.V.1972, M Sakai leg.

**Description.** For full description see Pütz (1998). Antennae (Fig. 2E) long, approximate ratio of each antennomere ( $n = 1$ ) as 2.20 : 1.40 : 2.60 : 1.80 : 1.80 : 1.60 : 1.40 : 1.40 : 1.20 : 1.00 : 1.80. PW/PL 1.57–2.10 (1.79); EL/EW 1.46–1.71 (1.55); EL/PL 3.00–4.29 (3.47); EW/PW 1.17–1.33 (1.24); TL/EW 1.92–2.13 (2.00). Legs reddish brown, but frequently infuscate.

Male. Sternite VIII (Fig. 3D) membranous, transverse. Sternite IX (Fig. 3E) with long and slender lateral struts. Aedeagus 0.6 mm; phallobase slightly tapering basally, with straight basal projection; parameres relatively wide, obtuse at apices, slightly arcuate in lateral sides, 0.42 times as long as phallobase; median lobe widened in basal 1/3, obtuse at apex, 0.88 times as long as parameres.

Female. Spiculum ventral (Fig. 3F) long, as long as ovipositor. Ovipositor (Fig. 3G) well sclerotized, long; coxite gently pointed at apices; approximate ratio of coxite and baculus ( $n = 1$ ) as 1.0 : 5.0.

Measurements of adults (unsexed;  $n = 20$ ). TL 2.22–3.70 (3.04) mm; PW 0.88–1.50 (1.22) mm; PL 0.42–0.85 (0.69) mm; EL 1.80–2.85 (2.36) mm; EW 1.05–1.85 (1.52) mm.

**Distribution.** Taiwan.

**Remarks.** The male genitalia of the lectotype shown by Pütz (1998) were broken in the basal part.

**Biological notes.** This species is common in Taiwan, and lives in a natural forest of a mountainous area. Immature stages are unknown.

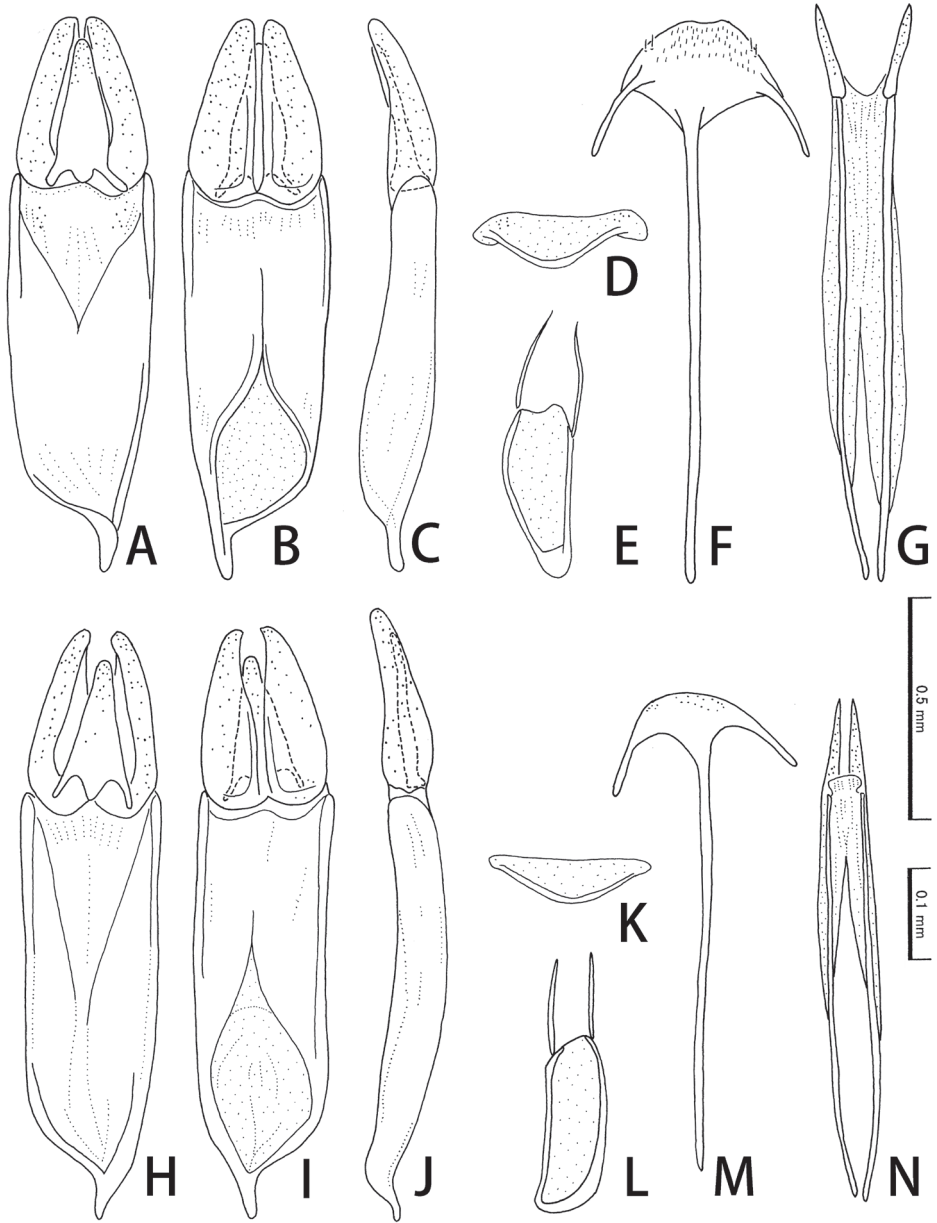
### *Cephalobyrrhus japonicus* Champion, 1925

Figs 1B, 2A–D, F, 3H–N, 5A–B

Japanese name: Oome-hoso-chibidoromushi

*Cephalobyrrhus japonicus*: Champion 1925: 176; Satô 1966: 62; Pütz 1991: 132; 1998: 352.

**Specimens examined.** [Honshu] Tokyo Met. 15 exs. (SEHU), Nippara, 24.VI.1969, H Takizawa leg.; 26 exs. (SEHU), Takao-san, 20.VI.1969, H Takizawa leg. Chiba Pref. 3 exs. (EUMJ), Orikisawa, Kimitsu-shi, 12.VI.2013, A Saito leg. Kanagawa



**Figure 3.** Male (**A–E, H–L**) and female (**F, G, M, N**) genitalia of *Cephalobyrrhus* spp. **A–G** *Cephalobyrrhus latus* Pic, 1923 **H–N** *C. japonicus* Champion, 1925 **A–C, H–J** Aedeagus in ventral (**A, H**), dorsal (**B, I**), and lateral (**C, J**) aspects **D, K** sternite VIII **E, L** sternite IX **F, M** spiculum ventral **G, N** ovipositor. Scale bars: 0.1 mm (**A–C, H–J**); 0.5 mm (**D–G, K–N**).

Pref. 3 exs. (EUMJ), Miyanoshita, 1.VI.1895; 1 ex. (EUMJ), Yokohama. Yamana-shi Pref. 3 exs. (EUMJ), Koganezawa-rindo, 29.VI.1975, S Tsuyuki leg.; Nagano Pref. 1 ex. (EUMJ), Shimajima, 22.VII.1967, M Tomokuni leg. Aichi Pref. 2 exs.

(EUMJ), Hongu-san, 15.VI.1991, M Satô leg. Mie Pref. 1 ex. (EUMJ), Hirakura, Misugi-mura, 14.VII.1993, N Kanie leg.; 1 ex. (EUMJ), same loc., 19.VI.1955, Z Naruse leg.; 1 ex. (EUMJ), same loc., 24.VIII.1956, M Satô leg. Gifu Pref. 1 ex. (EUMJ), Suhara, 24.V.1955, K Ohbayashi leg.; 1 ex. (EUMJ), Uchibami-dani, Itadori-mura, 1.VII.1973, Y Horii leg.; 4 exs. (EUMJ), Wara-gawa, 24.VIII.1967, M Satô leg. Tottori Pref. 1 ex. (EUMJ), Daisenji, Daisen, 8.VII.1971, S. Hisamatsu leg. [Shikoku] Ehime Pref. 1 ex. (EUMJ), Fukumi-gawa, 20.VI.1986, S Hisamatsu leg.; 1 ex. (EUMJ), Ishizuchi-san, 16–17.VII.1977, M Tomokuni leg.; 1 ex. (EUMJ), Koguchi, 26.VII.1958, M Miyatake leg.; 1 ex. (EUMJ), Komenono, Matsuyama-shi, 4.VII.1978, Y Seiyama leg.; 2 exs. (EUMJ), same loc., 14.VI.1978, Y Seiyama leg.; 1 ex. (EUMJ), same loc., 4.VII.1974, Y Notsu leg.; 1 ex. (EUMJ), same loc., 13.VI.1976, Y Notsu leg.; 1 ex. (EUMJ), same loc., 23.VI.1977, A Oda leg.; 1 ex. (EUMJ), same loc., 28.VI.1970, M Sakai leg.; 1 ex. (EUMJ), Matsubagawa, 8.VII.1961, M Miyatake leg.; 1 ex. (EUMJ), Myojingamori, 26.VI.1966, S Hisamatsu leg.; 3 exs. (EUMJ), Oda-cho, 10.VII.1983, E Yamamoto leg.; 1 ex. (EUMJ), Odamiyama, 21.VII.1993, M Sakai leg.; 1 ex. (EUMJ), same loc., 27.VI.1972, M Sakai leg.; 1 ex. (EUMJ), same loc., 16.VIII.1972, M Sakai leg.; 2 exs. (EUMJ), same loc., 1–2.VII.1995, N Ohbayashi leg.; 1 ex. (EUMJ), same loc., 18.V.1986, E Yamamoto leg.; 1 ex. (EUMJ), same loc., 19.VII.1993, K Okada leg.; 14 exs. (EUMJ), Ohnogahara, 4.VII.1977, A Oda leg.; 2 exs. (EUMJ), same loc., 11.VII.1965, N Ohbayashi leg.; 3 exs. (EUMJ), same loc., 28.V.1980, A Sakai leg.; 4 exs. (EUMJ), same loc., 6.VII.1979, Y Seiyama leg.; 1 ex. (EUMJ), same loc., 14.VII.1981, M Kotani leg.; 3 exs. (EUMJ), same loc., 14.VII.1981, K Sasagawa leg.; 17 exs. (EUMJ), Omogokei, 26.VI.1951, M Miyatake leg.; 3 exs. (EUMJ), same loc., 15–17.VI.1956, M Miyatake leg.; 6 exs. (EUMJ), same loc., 12–13.VI.1954, S Hisamatsu leg.; 3 exs. (EUMJ), same loc., 19.VI.1955, M Miyatake leg.; 2 exs. (EUMJ), same loc., 26.VI.1955, S Hisamatsu leg.; 6 exs. (EUMJ), same loc., 2.VII.1978, A Oda leg.; 8 exs. (EUMJ), same loc., 29.VI.1963, M Miyatake leg.; 7 exs. (EUMJ), same loc., 21.VII.1979, M Satô leg.; 3 exs. (EUMJ), same loc., 17.VII.1977, A Oda leg.; 1 ex. (EUMJ), same loc., 20.VI.1981, K Sasagawa leg.; 1 ex. (EUMJ), same loc., 14.VII.1981, K Sasagawa leg.; 4 exs. (EUMJ), same loc., 18.VI.1986, T Nagata leg.; 1 ex. (EUMJ), same loc., 12.VI.1959, M Miyatake leg.; 23 exs. (EUMJ), Oonaru, Omogo, 13–14.VI.1998, T. Kan et al. leg.; 2 exs. (EUMJ), Saragamine, 26–27.VI.1959, M Satô leg.; 1 ex. (EUMJ), Shiraitono-taki, 30.VI.1978, I Amano leg.; 3 exs. (EUMJ), Shiratsue, 10–11.VI.1972, M Sakai leg.; 1 ex. (EUMJ), same loc., 19.VI.1966, S Hisamatsu leg.; 1 ex. (EUMJ), Sugitate, Matsuyama-shi, 14.VI.1955, Y Wake leg.; 1 ex. (EUMJ), Wakayama, Kumakogen-cho, 28.VI.2009, K Hashimoto leg. Kagawa Pref. 1 ex. (EUMJ), Daisenzan, Kotonami-cho, 9.VI.2002, Y Kamite leg. Tokushima Pref. 1 ex. (EUMJ), Mimune, Higashi-iya, 2–3.VIII.1969, S Kinoshita leg.; 2 exs. (EUMJ), Takashiro-yama, 17.VII.1988, M Sakai leg.; 1 ex. (EUMJ), same loc., 18.VII.1978, A Oda leg.; 1 ex. (EUMJ), same loc., 18.VII.1978, M Tomokuni leg.; 9 exs. (EUMJ), Tsurugi, 11–12.VII.1976, S Hisamatsu et al. leg. Kochi Pref. 11

exs. (EUMJ), Kage, 11.VI.1971, M Tomokuni leg.; 5 exs. (EUMJ), Kamioriwatari, Yusu-hara, 14–15.VI.1997, M Sakai leg.; 5 exs. (EUMJ), Kuroson, 21.VI.1999, M Sakai leg.; 1 ex. (EUMJ), same loc., 15.VII.1953, E Edashige leg.; 3 exs. (EUMJ), Shimooriwatari, Yusu-hara, 14–15.VI.1997, N Ohbayashi leg.; 15 exs. (EUMJ), Teba-ko-yama, 9–11.VI.1960, M Miyatake leg. [Kyushu] Fukuoka Pref. 3 exs. (EUMJ), Hikosan, 7.VII.1957, M. Miyatake leg.; 5 exs. (EUMJ), same loc., 12.VII.2002, J Ogawa leg.; 1 ex. (EUMJ), Ukiha-cho, 18.VII.1956, N Gyotoku leg. Nagasaki Pref. 1 ex. (EUMJ), Sasuna, Kamiagata, 22.VI.2002, T Kurihara leg.; 1 ex. (EUMJ), same loc., 16.VII.2000, J Ogawa leg. Kumamoto Pref. 2 exs. (EUMJ), Hagi, Izumi-mura, 4.V.1988, K Ishida leg.; 1 ex. (EUMJ), same loc., 3.V.1988, K Ishida leg.; 5 exs. (EUMJ), Ichibusa, Mizukami-mura, 8.VI.1967, S Hisamatsu leg.; 4 exs. (EUMJ), same loc., 11.VI.1972, S Hisamatsu leg.; 1 ex. (EUMJ), Nabenodaira, Takamori, 18.VII.1968, S Kinoshita leg. Oita Pref. 2 exs. (EUMJ), Kozubaru, 9–11.VII.1968, S Kinoshita leg.; 2 exs. (EUMJ), Kurodake, Naoiri-gun, 16.VI.1979, S Nagai leg.; 4 exs. (EUMJ), Kyusuikei, Kusu-gun, 16.VI.1979, S Nagai leg. Miyazaki Pref. 1 ex. (EUMJ), Ebino-kogen, Ebino-cho, 14.VII.2002, J Ogawa leg.; 1 ex. (EUMJ), Shirai-wa-yama, Higashi-usuki, 19.VII.1968, S Kinoshita leg.; 27 exs. (EUMJ), Sobu-san, 6.VII.1980, Y Seiyama leg. Kagoshima Pref. 4 exs. (SEHU), Yakushima, 7.VI.1969, K Kushigemati leg.

**Description.** Adults. For full description see Pütz (1991). Antennae (Fig. 2F) long; approximate ratio of each antennomere ( $n = 1$ ) as 1.85 : 1.08 : 1.38 : 1.08 : 1.38 : 1.23 : 1.23 : 1.08 : 1.00 : 1.00 : 1.38. PW/PL 1.51–1.90 (1.68); EL/EW 1.43–1.68 (1.56); EL/PL 2.85–3.76 (3.23); EW/PW 1.15–1.30 (1.23); TL/EW 1.94–2.15 (2.04).

Male. Sternite VIII (Fig. 3K) membranous, transverse. Sternite IX (Fig. 3L) with long lateral struts. Aedeagus about 0.6 mm; phallobase subparallel-sided, with straight basal projection; parameres relatively wide, curved interiorly and rather pointed at apices, 0.42 times as long as phallobase; median lobe straight in sides, obtuse at apex, 0.78 times as long as parameres.

Female. Spiculum ventral (Fig. 3M) long, as long as ovipositor. Ovipositor (Fig. 3N) well sclerotized, long; coxite sharply pointed at apices; approximate ratio of coxite and baculus ( $n = 1$ ) as 1.0 : 3.9.

Measurements of adults (unsexed;  $n = 20$ ). TL 2.38–4.02 (3.04) mm; PW 0.95–1.65 (1.21) mm; PL 0.50–1.00 (0.72) mm; EL 1.88–3.02 (2.32) mm; EW 1.20–1.95 (1.49) mm.

**Distribution.** Japan (Honshu, Shikoku, Kyushu, Tsushima, Yakushima).

**Remarks.** This record is the first of this species from Tsushima.

**Biological notes.** This species lives in natural forests (particularly in the Japanese beech tree zone, above ca. 1,000 m elevation) near a small stream (Fig. 5A, B). The adults were observed on the surface of rocks and fallen rotten wood during the day-time, and were sometimes attracted to light. Overwintering probably occurs in the larval stage. Immature stages are unknown.

***Cephalobyrrhus amami* sp. n.**

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Figs 1C–D, 2G, 4, 5C–D

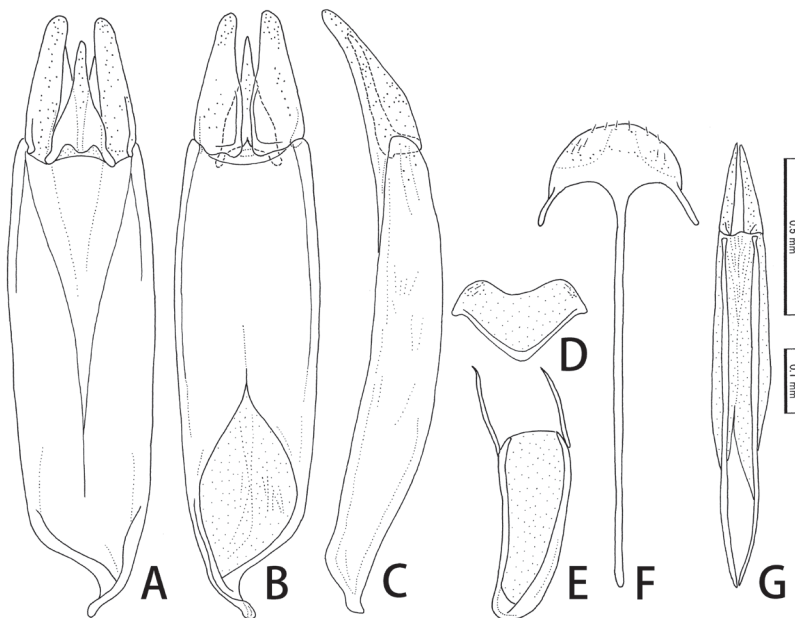
Japanese name: Amami-oome-hoso-chibidoromushi

**Type series.** Holotype (EUMJ): Male, [AM6] Akatsuchi-yama, Uken-son, Amami-Ōshima, Kagoshima Pref., Japan, 28.151853, 129.195539, ca. 107 m, 23.IV.2017, H Yoshitomi leg. **Paratypes** (EUMJ, NMW): 13 males & 2 females, same data as for holotype.

**Diagnosis.** The new species *C. amami* sp. n. has a short and pointed median lobe and a long phallobase.

**Description.** Adults. Body oblong, convex dorsally, shiny, densely covered with short golden setae. Coloration of body black; antennomeres I–II, maxillae, labial palpi and legs pale brown, but infuscate in apical part of tarsomeres V.

Head densely punctate, convex anteriorly between antennal insertions. Antennae (Fig. 2G) long, reaching at base of elytra; approximate ratio of each antennomere ( $n = 1$ ) as 1.78 : 1.22 : 2.00 : 1.11 : 1.33 : 1.11 : 1.00 : 1.11 : 1.11 : 1.00 : 1.22. Pronotum punctate as in head; PW/PL 1.64–1.97 (1.81). Scutellar shield triangular. Elytra oblong, widest at middle, gently arcuate in lateral margins; irregular markings consisting of adpressed setae relatively distinct; EL/EW 1.47–1.56 (1.52); EL/PL 3.06–3.79 (3.41); EW/PW 1.21–1.27 (1.24); TL/EW 1.88–2.01 (1.96).



**Figure 4.** Male (A–E) and female (F, G) genitalia of *Cephalobyrrhus amami* sp. n. A–C Aedeagus in ventral (A), dorsal (B), and lateral (C) aspects D sternite VIII E sternite IX F spiculum ventral G ovipositor. Scale bars: 0.1 mm (A–C); 0.5 mm (D–G).

Male. Sternite VIII (Fig. 4D) membranous, slightly transverse. Sternite IX (Fig. 4E) with long lateral struts. Aedeagus long, 0.9 mm; phallobase long, widest at the middle, then weakly tapering posteriorly and anteriorly, with curved nasal projection; parameres relatively slender, weakly pointed at apices, 0.30 times as long as phallobase; median lobe slender, straightly tapering apically, pointed at apex, 0.89 times as long as parameres.

Female. Spiculum ventral (Fig. 4F) long, a little longer than ovipositor. Ovipositor (Fig. 4G) well sclerotized, long; coxite sharply pointed at apices; approximate ratio of coxite and baculus ( $n = 1$ ) as 1.0 : 3.8.

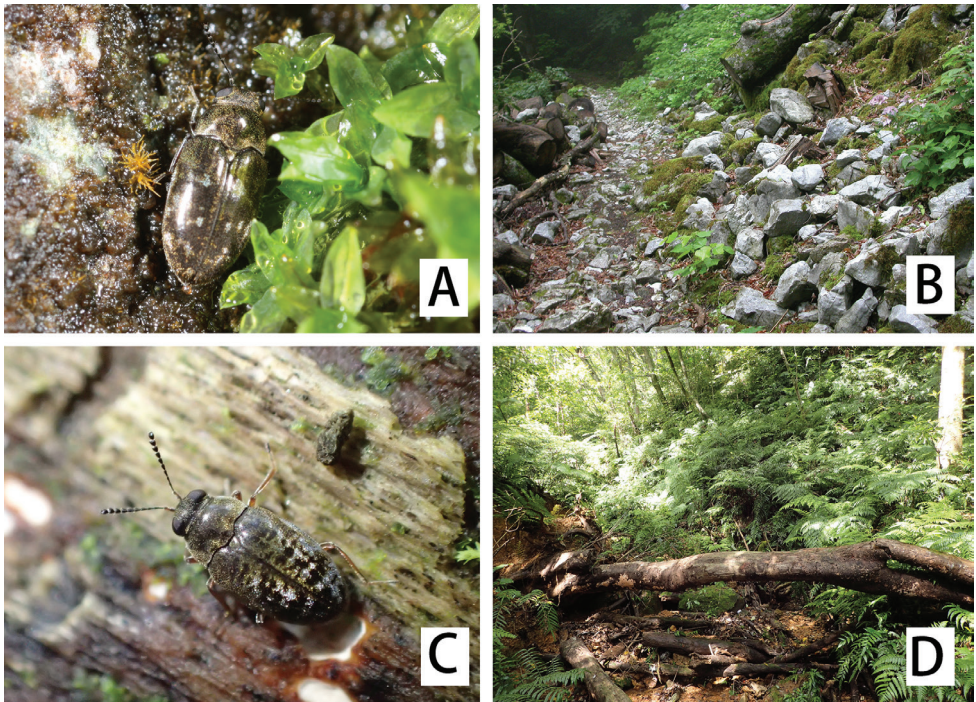
Measurements of adults (unsexed;  $n = 20$ ). TL 3.15–3.65 (3.36) mm; PW 1.30–1.50 (1.38) mm; PL 0.70–0.90 (0.76) mm; EL 2.45–2.75 (2.59) mm; EW 1.60–1.83 (1.71) mm.

**Distribution.** Amami-Ōshima.

**Remarks.** This species is clearly distinguished from the other two known species in the region by the short and pointed median lobe and long phallobase.

**Etymology.** The species is named after the type locality.

**Biological notes.** This species lives in a natural forest near a small stream (Fig. 5C, D). The type series was collected from the surface of fallen rotten wood during the daytime. Immature stages are unknown.



**Figure 5.** Habitat and habits of *Cephalobyrrhus* spp. in nature. **A** *C. japonicus* Champion, 1925 and its habitat **B** Keyaki-daira, Ehime Pref. **C** *C. amami* sp. n. and its type locality **D** Akatsuchi-yama.

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# Beta diversity patterns of fish and conservation implications in the Luoxiao Mountains, China

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## Abstract

The Luoxiao Mountains play an important role in maintaining and supplementing the fish diversity of the Yangtze River Basin, which is also a biodiversity hotspot in China. However, fish biodiversity has declined rapidly in this area as the result of human activities and the consequent environmental changes. Beta diversity was a key concept for understanding the ecosystem function and biodiversity conservation. Beta diversity patterns are evaluated and important information provided for protection and management of fish biodiversity in the Luoxiao Mountains. The results showed that the spatial turnover component was the main contributor to beta diversity of Hemiramphidae, Amblycipitidae, Catostomidae, Clariidae, Balitoridae and Percichthyidae in the Luoxiao Mountains, which indicated that a number of protected areas would be necessary to conserve fish biodiversity and that these families would need conservation measures. Most protected areas are currently limited to some regions; therefore, in order to protect fish diversity, conservation efforts must target an increase in the number of protected areas which should be spread across each of the regions.

## Keywords

beta diversity, commercial fishes, Luoxiao Mountains, protected areas

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## Introduction

Biodiversity patterns and their formation mechanisms have been one of the hot issues, and it is also an important foundation for conservation (Kennedy and Norman 2005; Sutherland et al. 2009). Biodiversity is important for the future sustainability of freshwater natural resources (Hiddink et al. 2008). While it is axiomatic that biodiversity is essential for sustainable productive fisheries there is surprisingly little supporting evidence (Dulvy et al. 2000; Hilborn et al. 2003). Freshwater fishes are among the most diverse assemblages on Earth, which provide important economic value (e.g., nutrition) and valuable ecosystem services (e.g., natural water filtration; Naylor et al. 2000; Cressey 2009; De Silva 2012). However, due to dam construction, overfishing (commercial fish fishing), pollution, deforestation, and other human activities, fish numbers have declined rapidly in global terms (Fu et al. 2003; Arthington et al. 2016; Liu et al. 2017) and they are thus one of the most threatened assemblages.

Beta diversity is an important tool for conservation planning (Anderson et al. 2006); knowledge on beta diversity patterns can aid the decision on the number of protected areas needed and their sizes (Margules and Pressey 2000; Wiersma and Urban 2005). Beta diversity can be decomposed into species turnover (species replacement) and nestedness (richness difference; Baselga 2010; Carvalho et al. 2012). The species turnover component (species replacement) is the replacement of some species by others leading to a low number of shared species among two communities where turnover is high (Baselga 2010). In addition, the nestedness component (richness difference components) represents the differences between two communities only in terms of species richness, with the poorer community as a subset of the richer one (Baselga 2010). According to the percentage of spatial turnover and nestedness components in total beta diversity, different conservation strategies can be selected. If species turnover is the main component of beta diversity, a larger number of protected areas would be necessary to conserve regional biodiversity (Baselga 2010; Carvalho et al. 2012). If the nestedness is the main component of beta diversity, one large protected area comprising a high species richness could be sufficient (Baselga 2010; Carvalho et al. 2012).

The Luoxiao Mountains range is located in the southeast of China's mainland and has a long history and complex environmental factors (Liao et al. 2014; Wei et al. 2015). The northern part of the mountains is connected with the Yangtze River, and the southern part is connected with the Nanling Mountain (Gong et al. 2016). It is the most important ecotone and fragile zone in the third step of eastern China, and is an important channel for the migration and diffusion of terrestrial organisms in the Northern Hemisphere (Liao et al. 2014; Gong et al. 2016). In addition, the Luoxiao Mountains is also a biodiversity hotspot in China (Liao et al. 2014; Gong et al. 2016). At the same time, as being the watershed of the Poyang Lake Basin and the Dongting Lake Basin in the middle reaches of the Yangtze River, the Luoxiao Mountains are a refuge to many endemic and endangered fishes (Liao et al. 2014; Gong et al. 2016). Therefore, fish resources of the Luoxiao Mountains play an important role in maintaining and supplementing the aquatic biodiversity of the Yangtze River Basin. However, due to dam construction, overfishing, pollution,

deforestation, and other human activities, fish diversity declined rapidly in this region. Here, we aim to evaluate beta diversity patterns and to provide useful information for the protection and management of fish biodiversity in the Luoxiao Mountains.

## **Material and methods**

### **Study area**

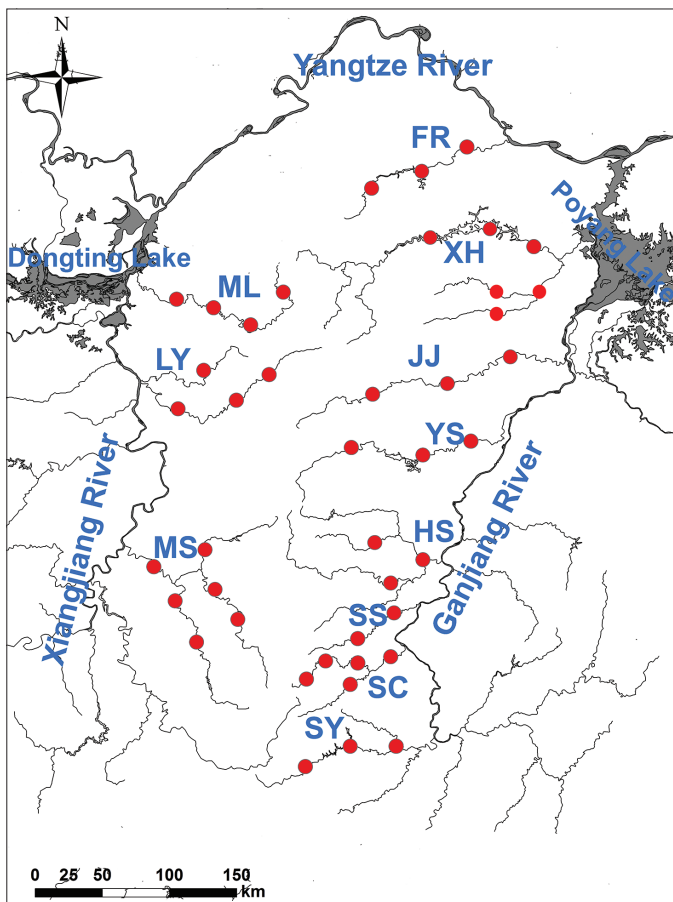
The Luoxiao Mountains (25°32'–29°28'N, 113°09'–114°26'E) are a large system of mountain ranges, located in the southeast of China's mainland with an overall north-south trend, stretching across Hubei, Hunan, and Jiangxi provinces. It consists of Mufu Mountain, Jiuling Mountain, Wugong Mountain, Zhuguang Mountain, and others. The total length of the Luoxiao Mountains is 400 km and altitude ranges are 82–2120 m. Lingfeng Peak (2122 m) is one of the highest mountains in the southeastern Eurasia. Its average precipitation range is 1341–1943 mm and forest coverage in the watershed reaches 90% (Table 1). The tributaries of the Ganjiang River from the eastern stream of the Luoxiao Mountains flow into Poyang Lake. The tributaries of Xiangjiang River from the western stream of it flow into the Dongting Lake. The Fushui River alone flows into the Yangtze River.

### **Sampling methods**

Sampling sites were selected by considering habitats, variations, and anthropogenic activities in the Luoxiao Mountains. Fish samples were collected from April 2014 to 2017 in eleven streams of the Luoxiao Mountains. We selected eleven streams (42 sampling sites) (Figure 1), including the (1) Fu River (sampling code is FR; three sampling sites), Xiuhe River (sampling code is XH; six sampling sites), Jinjiang River (sampling code is JJ; three sampling sites), Yuanshui River (sampling code is YS; three sampling sites), Heshui River (sampling code is HS; three sampling sites), Shushui River (sampling code is SS; two sampling sites), Suichuan River (sampling code is SC; five sampling sites), Shangyou River (sampling code is SY; three sampling sites), Miluo River (sampling code is ML; four sampling sites), Liuyang River (sampling code is LY; four sampling sites), Mishui River (sampling code is MS; six sampling sites). We collected the fish catch from professional fishermen who captured fish using fully standardized five gillnet clusters, each consisting of six gillnets of 50–80 m in length 4–10 m in height (mesh size = 1.0–10.0 cm) in the Luoxiao Mountains rivers. In addition, we assumed similar capture efficiencies from gillnet samples at each site. At the same time, we surveyed and collected fish in the township markets along the river which enhanced the species checklists at each section. All fish specimens were identified according to Chen (1998), Chu et al. (1999), and Yue (2000), and the scientific name was corrected according to Fishbase (<http://www.fishbase.org/search.php>). The division of endangered categories of fish was decided according to Jiang et al. (2016) and IUCN (2017).

**Table 1.** Hydrology and environmental characteristics of the streams of Luoxiao Mountains. JJ: Jinjiang River; YS: Yuanshui River; HS: Heshui River; SS: Shushui River; SC: Suichuan River; SY: Shangyou River; MS: Mishui River; ML: Miluo River; FS: Fushui River; XH: Xiuhe River; LY: Liuyang River.

Stream	Latitude	Longitude	Length (km)	Area (km <sup>2</sup> )	Average gradient (%)	Average precipitation (mm)	Average temperature (°C)	Annual average runoff (×10 <sup>8</sup> m <sup>3</sup> )	Average Altitude (m)
LY	28°24'–28°46'	112°99'–114°04'	222	4665	0.57	1598	17.3	39.41	252
MS	27°16'–26°25'	112°88'–113°99'	296	10305	1.01	1483	18.1	76.03	352
ML	28°86'–29°02'	112°93'–114°05'	253	5543	0.46	1400	17.6	43.04	250
FS	29°49'–29°86'	114°40'–115°45'	196	5250	0.79	1275	16.6	43.5	613
XH	28°31'–29°12'	114°14'–116°01'	419	14700	0.48	1663	16.7	135.1	676
SY	25°37'–25°49'	113°43'–114°49'	204	4647	0.70	1570	18.8	33	615
SC	26°11'–26°30'	113°56'–114°44'	176	2882	2.36	1640	16.9	27.1	971
SS	126°29'–26°47'	14°04'–114°50'	152	1301	2.14	1630	16.7	11.3	1610
HS	27°04'–27°24'	114°01'–114°59'	256	9103	0.59	1580	17.8	27.4	747
YS	27°27'–28°04'	114°10'–115°29'	279	6262	0.34	1678	17.2	29.6	391
JJ	27°57'–28°25'	114°01'–115°49'	307	7886	0.26	1679	17.6	70	391



**Figure 1.** Map showing the sampling location of the streams of the Luoxiao Mountains.

## Data analysis

Beta diversity is represented by the difference in species composition between different communities, which was determined by species turnover (species replacement) and nestedness (richness difference; Baselga 2010; Carvalho et al. 2012). In order to quantify the effects of two processes, Baselga (2010) systematically proposed the beta diversity decomposition method (BAS frameworks) based on the Sørensen index ( $\beta_{\text{sor}}$ ), which was decomposed into species spatial turnover components ( $\beta_{\text{sim}}$ ) and nestedness components ( $\beta_{\text{sne}}$ ). Podani and Schmera (2011) and Carvalho et al. (2012) proposed the beta diversity decomposition method (POD frameworks) based on the Jaccard index ( $\beta_{\text{jac}}$ ), which was decomposed into species replacement components ( $\beta_{-3}$ ) and richness difference components ( $\beta_{\text{rich}}$ ). Here, we analyzed the fish biodiversity based on both the BAS and POD frameworks. BAS frameworks (Sørensen index):

$$\beta_{\text{sor}} = \frac{b + c}{2a + b + c}$$

$$\beta_{\text{sim}} = \frac{\min(b, c)}{a + \min(b, c)}$$

$$\beta_{\text{sne}} = \frac{|b - c|}{2a + b + c} \times \frac{a}{a + \min(b, c)}$$

POD frameworks (Jaccard index):

$$\beta_{\text{jac}} = \frac{b + c}{a + b + c}$$

$$\beta_{-3} = \frac{2\min(b, c)}{a + b + c}$$

$$\beta_{\text{rich}} = \frac{|b - c|}{a + b + c}$$

where  $a$  was the number of shared species among two streams, and  $b$  and  $c$  were the number of species only present in the first and second stream, respectively. Sørensen and Jaccard indices ranged from 0 to 1, representing respectively no species and all species in common among the two streams (Appendix 1).

A principal component analysis (PCA) was performed separately based on Sørensen and Jaccard indices to visualize patterns of fish assemblages among the Luoxiao Mountains rivers (Legendre and Legendre 2012). PCA results were then analyzed using R 3.2.0 version (R Development Core Team 2014) and using the “ade4” package (Dray and Dufour 2007).

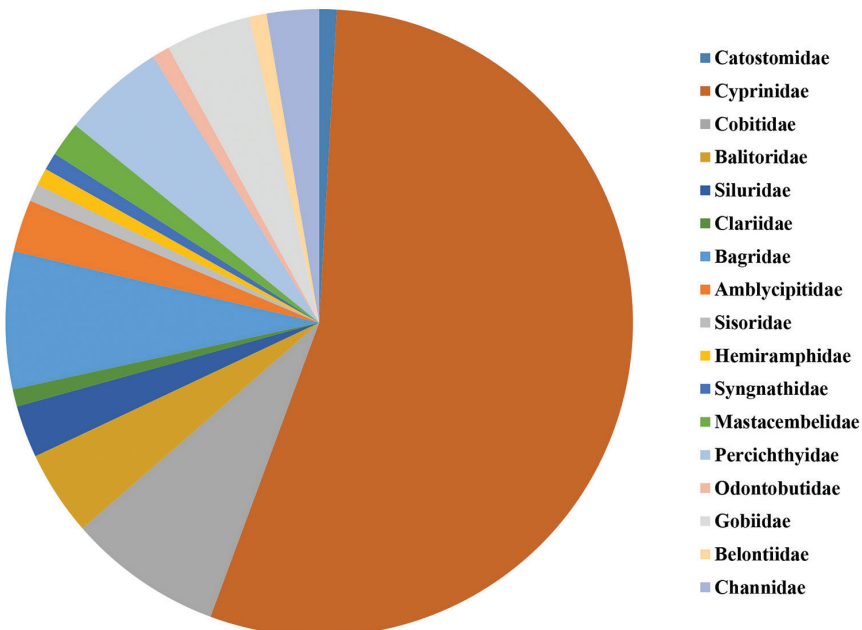
We performed Mantel tests and partial Mantel tests (Legendre and Legendre 2012) with 9999 permutations to assess the correlations (Spearman’s method) between eight pairwise similarity matrices and the matrices of geographical drivers (difference in area, length, average precipitation, annual average runoff, average altitude, average gradient and average temperature among streams; Table 1) to explore the po-

tential mechanisms that explained beta diversity patterns. The partial Mantel tests were used to remove the effect of covariation because an inter-correlation between matrices of difference in area, length, average precipitation, annual average runoff, average altitude, average gradient and average temperature was detected ( $P < 0.05$ ). All the analyses were performed in R 3.2.0 (R Development Core Team 2014) using the packages BAT (Cardoso et al. 2015), BETAPART package (Baselga and Orme 2012) and VEGAN (Oksanen et al. 2015).

## Results

### Fish species composition

The fish specimens sampled and identified in the Luoxiao Mountains were categorized into 113 species and 17 families (Figure 2; Appendix 2). The number of Cypriniformes was the greatest, accounting for 68.1% of the total number of fish species, followed by Siluriformes and Perciformes, accounting for 14.2% each, and Beloniformes, accounting for 0.1% (Figure 2). In addition, according to the endangered categories of the Jiang et al. (2016), Least Concern fish species were the greatest, accounting for 77.9% of the catch (Appendix 3). Critically Endangered, Vulnerable, and Near Threatened fish species accounted for 7.1% (Appendix 3).



**Figure 2.** Fish composition from the streams of the Luoxiao Mountains.

## Beta diversity patterns

The fish composition similarity in the Luoxiao Mountains had a mean value of 0.50 and 0.67, based on BAS and POD frameworks respectively (SD  $\pm$  0.06 and SD  $\pm$  0.05, respectively; Table 2). The spatial turnover and replacement components ( $\beta_{sim}$  and  $\beta_{-3}$ , 0.36  $\pm$  0.08, and 0.39  $\pm$  0.13) were greater than its nestedness and richness difference components ( $\beta_{snc}$  and  $\beta_{Rich}$ , 0.14  $\pm$  0.09 and 0.28  $\pm$  0.16). FS and SC had a high  $\beta_{sor}$  and  $\beta_{jac}$  (0.55  $\pm$  0.07 and 0.54  $\pm$  0.11; 0.71  $\pm$  0.05 and 0.69  $\pm$  0.09), a high spatial turnover and replacement components (0.39  $\pm$  0.07 and 0.45  $\pm$  0.12) in LY and nestedness and richness difference components (0.22  $\pm$  0.10 and 0.41  $\pm$  0.17) in SC (Table 2).

At the same time, fish composition similarity ( $\beta_{sor}$  and  $\beta_{jac}$ ) for the entire fish fauna had a mean value of 0.66 and 0.76 (SD  $\pm$  0.24 and 0.21, Table 3). The spatial turnover and replacement components ( $\beta_{sim}$  and  $\beta_{-3}$ , 0.41  $\pm$  0.03 and 0.32  $\pm$  0.03) were higher than the nestedness and richness difference components ( $\beta_{snc}$  and  $\beta_{rich}$ , 0.25  $\pm$  0.02 and 0.44  $\pm$  0.02). The greatest  $\beta_{sor}$  and  $\beta_{jac}$  (0.93 $\pm$ 0.16 and 0.96 $\pm$ 0.13) and spatial turnover and replacement components (0.80  $\pm$  0.04 and 0.49  $\pm$  0.03) was in Hemiramphidae, followed by Amblycipitidae, and the lowest was in Syngnathidae. The greatest nestedness and richness difference components (0.53  $\pm$  0.25 and 0.65  $\pm$  0.26) was in Syngnathidae, followed by Siluridae, and the lowest was in Hemiramphidae (Table 3).

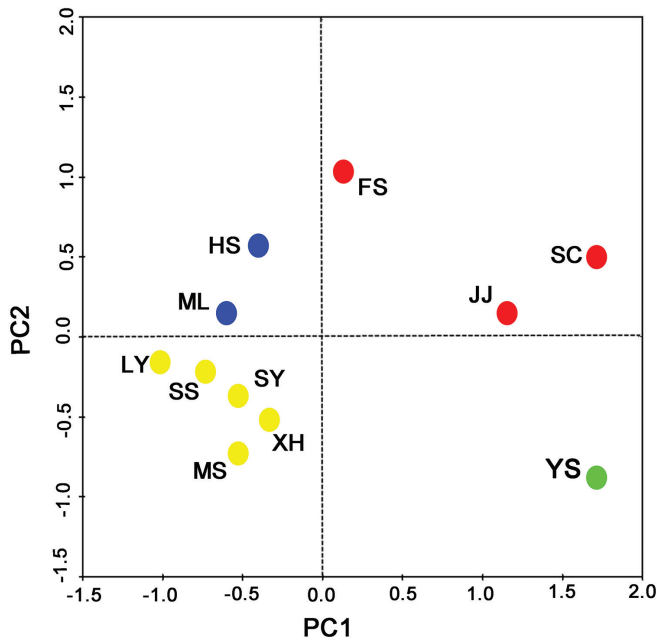
The PCA showed that fish composition similarity of LY, SS, SY, XH, and MS were similar based on BAS and POD frameworks; FS, JJ and SC were similar; HS and ML were similar; and YS was uniquely divided into other areas, respectively (Figure 3).

**Table 2.** Fish compositional similarity by BAS and POD frameworks in the streams of Luoxiao Mountains. JJ: Jinjiang River; YS: Yuanshui River; HS: Heshui River; SS: Shushui River; SC: Suichuan River; SY: Shangyou River; MS: Mishui River; ML: Miluo River; FS: Fushui River; XH: Xiuhe River; LY: Liuyang River; ES: Eastern stream of Luoxiao Mountain; WS: Western stream of Luoxiao Mountains.

Stream	$\beta$					
	BAS		POD			
	$\beta_{sor}$	$\beta_{sim}$	$\beta_{snc}$	$\beta_{jac}$	$\beta_{-3}$	$\beta_{rich}$
JJ	0.52 $\pm$ 0.08	0.32 $\pm$ 0.11	0.19 $\pm$ 0.13	0.68 $\pm$ 0.07	0.33 $\pm$ 0.16	0.35 $\pm$ 0.19
YS	0.48 $\pm$ 0.08	0.27 $\pm$ 0.06	0.21 $\pm$ 0.11	0.65 $\pm$ 0.07	0.27 $\pm$ 0.11	0.37 $\pm$ 0.17
HS	0.52 $\pm$ 0.08	0.39 $\pm$ 0.09	0.13 $\pm$ 0.09	0.68 $\pm$ 0.07	0.42 $\pm$ 0.14	0.26 $\pm$ 0.16
SS	0.49 $\pm$ 0.07	0.38 $\pm$ 0.07	0.11 $\pm$ 0.07	0.66 $\pm$ 0.06	0.42 $\pm$ 0.12	0.24 $\pm$ 0.14
SC	0.54 $\pm$ 0.11	0.31 $\pm$ 0.09	0.22 $\pm$ 0.10	0.69 $\pm$ 0.09	0.28 $\pm$ 0.11	0.41 $\pm$ 0.17
SY	0.48 $\pm$ 0.03	0.37 $\pm$ 0.10	0.11 $\pm$ 0.09	0.65 $\pm$ 0.03	0.42 $\pm$ 0.15	0.22 $\pm$ 0.14
MS	0.47 $\pm$ 0.05	0.36 $\pm$ 0.06	0.11 $\pm$ 0.08	0.64 $\pm$ 0.05	0.41 $\pm$ 0.12	0.22 $\pm$ 0.14
ML	0.50 $\pm$ 0.04	0.38 $\pm$ 0.10	0.12 $\pm$ 0.09	0.66 $\pm$ 0.04	0.42 $\pm$ 0.14	0.24 $\pm$ 0.15
FS	0.55 $\pm$ 0.07	0.39 $\pm$ 0.06	0.16 $\pm$ 0.10	0.71 $\pm$ 0.05	0.39 $\pm$ 0.15	0.32 $\pm$ 0.19
XH	0.48 $\pm$ 0.04	0.36 $\pm$ 0.08	0.11 $\pm$ 0.08	0.65 $\pm$ 0.03	0.42 $\pm$ 0.14	0.23 $\pm$ 0.15
LY	0.49 $\pm$ 0.03	0.39 $\pm$ 0.07	0.10 $\pm$ 0.07	0.66 $\pm$ 0.03	0.45 $\pm$ 0.12	0.21 $\pm$ 0.12
ES	0.50 $\pm$ 0.07	0.34 $\pm$ 0.09	0.16 $\pm$ 0.11	0.66 $\pm$ 0.06	0.36 $\pm$ 0.14	0.30 $\pm$ 0.17
WS	0.50 $\pm$ 0.06	0.38 $\pm$ 0.07	0.12 $\pm$ 0.09	0.67 $\pm$ 0.05	0.42 $\pm$ 0.13	0.25 $\pm$ 0.15
Total	0.50 $\pm$ 0.06	0.36 $\pm$ 0.08	0.14 $\pm$ 0.09	0.67 $\pm$ 0.05	0.39 $\pm$ 0.13	0.28 $\pm$ 0.16

**Table 3.** BAS and POD frameworks based on all species and 17 families in the streams of Luoxiao Mountain. Values are mean ± standard deviation.

Family	BAS			POD		
	$\beta_{sor}$	$\beta_{sim}$	$\beta_{snc}$	$\beta_{jac}$	$\beta_{.3}$	$\beta_{rich}$
Catostomidae	0.70±0.32	0.39±0.05	0.31±0.03	0.77±0.30	0.25±0.03	0.51±0.03
Cyprinidae	0.65±0.24	0.41±0.03	0.25±0.02	0.76±0.20	0.32±0.02	0.43±0.02
Cobitidae	0.66±0.26	0.37±0.04	0.28±0.03	0.75±0.23	0.28±0.03	0.47±0.03
Balitoridae	0.69±0.25	0.49±0.04	0.20±0.02	0.78±0.21	0.39±0.03	0.39±0.02
Siluridae	0.64±0.30	0.26±0.03	0.38±0.03	0.73±0.29	0.17±0.02	0.56±0.03
Clariidae	0.70±0.32	0.39±0.05	0.31±0.03	0.77±0.30	0.25±0.03	0.51±0.03
Bagridae	0.67±0.24	0.41±0.03	0.27±0.02	0.77±0.21	0.32±0.03	0.45±0.03
Amblycipitidae	0.78±0.23	0.58±0.04	0.21±0.03	0.85±0.19	0.40±0.03	0.45±0.03
Sisoridae	0.56±0.22	0.34±0.03	0.22±0.02	0.69±0.18	0.31±0.03	0.38±0.02
Hemiramphidae	0.93±0.16	0.80±0.04	0.14±0.03	0.96±0.13	0.49±0.03	0.47±0.03
Syngnathidae	0.53±0.25	0	0.53±0.25	0.65±0.26	0	0.65±0.26
Mastacembelidae	0.64±0.22	0.43±0.03	0.21±0.02	0.75±0.18	0.38±0.03	0.37±0.02
Percichthyidae	0.69±0.24	0.49±0.04	0.20±0.02	0.78±0.20	0.40±0.03	0.39±0.02
Odontobutidae	0.65±0.25	0.49±0.04	0.16±0.02	0.76±0.20	0.33±0.16	0.35±0.19
Gobiidae	0.66±0.25	0.42±0.04	0.24±0.02	0.76±0.22	0.32±0.03	0.44±0.02
Belontiidae	0.53±0.23	0.31±0.03	0.23±0.02	0.67±0.20	0.28±0.03	0.39±0.02
Channidae	0.58±0.22	0.35±0.03	0.23±0.02	0.71±0.19	0.30±0.02	0.41±0.02
All species	0.66±0.24	0.41±0.03	0.25±0.02	0.76±0.21	0.32±0.03	0.44±0.02



**Figure 3.** Results of the principal component analysis (PCA) on the compositional similarity of fish species in the streams of the Luoxiao Mountains. JJ: Jinjiang River; YS: Yuanshui River; HS: Heshui River; SS: Shushui River; SC: Suichuan River; SY: Shangyou River; MS: Mishui River; ML: Miluo River; FS: Fushui River; XH: Xiuhe River; LY: Liuyang River.



**Table 4.** Effects of geographical drivers on pairwise compositional similarity and its partitioned components obtained from BAS and POD frameworks in the streams of Luoxiao Mountain, Jiangxi Province. Significant results ( $P < 0.05$ ) are in bold.

		Area	Length	Average precipitation	Annual average runoff	Average altitude	Average gradient	Average temperature	
BAS	$\beta_{sor}$	<i>r</i>	0.162	0.134	-0.008	0.273	0.055	-0.162	-0.070
		<i>P</i>	0.190	0.203	0.454	0.069	0.333	0.850	0.647
	$\beta_{sim}$	<i>r</i>	-0.318	-0.245	0.187	-0.289	0.248	0.128	0.058
		<i>P</i>	0.931	0.871	0.127	0.888	<b>0.032</b>	0.272	0.415
	$\beta_{sne}$	<i>r</i>	0.386	0.304	-0.168	0.436	-0.179	-0.221	-0.098
		<i>P</i>	<b>0.022</b>	0.062	0.805	<b>0.011</b>	0.861	0.943	0.658
POD	$\beta_{jac}$	<i>r</i>	0.155	0.122	-0.017	0.254	0.067	-0.147	-0.076
		<i>P</i>	0.150	0.244	0.521	0.072	0.328	0.808	0.680
	$\beta_{-3}$	<i>r</i>	-0.366	-0.285	0.164	-0.401	0.236	0.219	0.081
		<i>P</i>	0.961	0.913	0.160	0.970	<b>0.028</b>	0.051	0.370
	$\beta_{rich}$	<i>r</i>	0.365	0.284	-0.145	0.430	-0.176	-0.237	-0.096
		<i>P</i>	<b>0.019</b>	0.076	0.758	<b>0.015</b>	0.851	0.981	0.630

We found almost no significant effects of geographical drivers on overall beta diversity for the Luoxiao Mountains (Table 4). The correlation between BAS and POD frameworks and difference in length, average precipitation, mean temperature, and average gradient were not significant in the Luoxiao Mountains. The correlation between  $\beta_{sne}$  ( $\beta_{rich}$ ) and differences in area and annual average runoff was significant. The correlation between  $\beta_{sim}$  ( $\beta_{-3}$ ) and difference in average altitude was also significant (Table 4).

## Discussion

### Fish species composition

Studies on fish composition and diversity in streams is the basis for the conservation and management of stream fishes (Liu et al. 2017; Zhang et al. 2018). In this study, the fish specimens sampled and classified in the stream of the Luoxiao Mountains were categorized into 113 species. Compared with species numbers of the Shiwanda Mountains (102 species; Zhao and Zhang 2001), Wuyi Mountains (117 species; Song et al. 2017), and the Tibetan Plateau (114 species; Wu and Tan 1991), the fish abundance in the Luoxiao Mountains was also higher.

### Beta diversity patterns

Abiotic and biotic factors and their ecological processes in different stream sizes varies substantially (Zhang et al. 2018). At least in streams, local species richness of fishes, habitat diversity and complexity often increase in large streams (Roberts and Hitt 2010; Zhang et al. 2018). Comparing alpha diversity and beta diversity at local and landscape

scales is an important, yet little-understood, area of basic and applied ecological research (Kessler et al. 2009). However, most studies on fish diversity of streams have focused on alpha diversity, whereas fewer studies have investigated beta diversity (Tisseuil et al. 2013; Johnson and Angeler 2014). Knowledge of beta diversity patterns can go beyond the systematic conservation planning method that only considers the location of protected area in relation to natural physical and biological patterns (Margules and Pressey 2000; Wiersma and Urban 2005). The efficiency of protected areas not only relies on species richness, but also on how well the complementarity among sites increases biodiversity conservation (Howard et al. 1998; Bush et al. 2016; Socolar et al. 2016). In this study, as turnover brought the larger contribution to beta diversity, additional conservation efforts must target an increase in the number of protected areas, which should be spread across each one of the regions, to maximize the protection of species diversity.

### **Biogeographical processes**

The modern freshwater fish fauna of Eurasia originated in the early Tertiary (Chen et al. 1986; Liu and Quan 1996; Zhang 2012). At the same time, the primitive species of the Danioninae and Barbinae became the main component of the fish fauna with the flattened land and the warming climate (Chen et al. 1986; Tang et al. 2001). During the dramatic changes of landscape and climate of the Eurasian continent in the late Oligocene and the end of the Pliocene, the primitive species component had been reduced rapidly (Chen et al. 1986; Tang et al. 2001). After the Quaternary ice age, only some offspring fishes of the old Tertiary Period remained (Chen et al. 1986; Tang et al. 2001). Moreover, Labeoninae, Gastromyzontidae, Balitoridae and Sisoridae were dominant during the uplift of the Tibetan Plateau (Chen et al. 1986; Yang et al. 1982; Tang et al. 2001). At the same time, a large area of alluvial plains appeared in eastern China, and special habitats were created under the influence of the East Asian monsoon (Chen et al. 1986; Zhang and Chen 1997). The cold-water fishes, such as Leuciscinae and Gobioninae became the endemic fishes of the river plain in East Asia (Hypophthalmichthyinae, Culterinae, Xenocyprininae, Acheilognathinae, Gobiobotinae) and the warm-water fishes the endemic fishes of Southeast Asia (Botiinae, Clariidae, Amblycipitidae, Belontiidae, Channidae, Mastacembelidae). Since then, these taxa have become the major faunal component in southern China (Chen et al. 1986). In this study, Culterinae, Gobioninae, and Acheilognathinae had a high species composition (Appendix 2). At the same time, the spatial turnover component is the main contributor of beta diversity in Hemiramphidae, Amblycipitidae, Catostomidae, Clariidae, Balitoridae, and Percichthyidae, indicating that it would be necessary to conserve habitats in the Luoxiao Mountains.

### **Threats to fish diversity**

The headwater stream is a tributary of a larger river, which is often located in a mountainous area with high altitude. Compared with large rivers, it had relatively

simple habitat structure, poor nutrition, obvious hydrological change, and low species diversity (Vannote et al. 1980; Grossman et al. 1990; Zhang et al. 2018). Therefore, the ecosystem of the stream is more fragile, its resistance to external disturbance and resilience is lower, and it would be more difficult to recover once it is damaged by humans. Fish, as the apex consumers of the stream, are very important to the stability and functioning of the stream ecosystems (Nogueira et al. 2010; Yan et al., 2011; Arthington et al. 2016; Liu et al. 2017). During the long evolution process, fishes have gradually adjusted their corresponding morphological characteristics, phenological rhythms, and life history countermeasures so that they could adapt to the unique natural environment of the stream (Lytle and Poff 2004; Osorio et al. 2011; Ren et al. 2016). However, due to habitat loss, water pollution, alien-species invasions, forest overcutting, climate change, overfishing etc., the fish biodiversity of most streams in China have been seriously threatened (Dudgeon et al. 2006; Allan and Castillo 2007). For example, numerous small dams in mountain streams were established (Huang et al. 2008; Hu et al. 2009). Dam constructions modified these small fast-flowing streams, which led to the decline of fish species adapted to rapid streams (Hu et al. 2009). In addition, a large number of fishing methods such as traps, gill nets, and electro-fishing has led to overfishing which has also caused a dramatic decline in fish biodiversity (Huang and Gong 2007; Zhang et al. 2010). Heavy metal pollution has affected the aquatic ecosystem in the Luoxiao Mountains (He et al. 1998). The contents of heavy metals have greatly exceeded the recommended standards (Xu et al. 2016). In this study, critically endangered (*Myxocyprinus asiaticus*), vulnerable (*Leptobotia elongata*, *Pseudobagrus pratti*, *Liobagrus marginatus*, *Siniperca roulei*), and near threatened (*Onychostoma barbatulum*, *Siniperca obscura*, *Siniperca undulata*) fish species accounted for 7.1% of the species recovered. At the same time, the PCA results showed that the fish composition among the streams sampled in the Luoxiao Mountains were similar. As turnover brought the larger contribution to beta diversity, additional conservation efforts must target an increase in the number of protected areas, which should be spread across each of the regions, to maximize the protection of species diversity.

### Conservation implications

Freshwater fishes were thought to be the world's most threatened group of vertebrates after amphibians (Bruton 1995; Hiddink et al. 2008; Liu et al. 2017) and, without protection, 20% of the world's freshwater fishes may become extinct in the next 50 years (Moyle and Leidy 1992; Fu et al. 2003). Although endangered fish have raised public awareness, conservation strategies of fish biodiversity in China are concentrated on endangered species and economic fish (Fu et al. 2003; Liu et al. 2017). In addition, protected areas mainly occur in terrestrial conservation strategies, but freshwater habitats are commonly protected only incidentally as part of their inclusion within terrestrial reserves (Huang et al. 2013). For example, conservation areas of plants, animals, and wetlands in Jiangxi Province have been established, but there are very few freshwater protected areas nor are there any fish passage facilities in the rivers (Huang et al. 2013). In this study,

species turnover component is the main pattern of beta diversity, implying that a larger number of protected areas would be necessary to conserve the regional biodiversity in the Luoxiao Mountains. Therefore, in order to protect fish biodiversity, the establishment of freshwater protected areas in the streams of the Luoxiao Mountains should be considered.

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Species	JJ	YS	HS	SS	SC	SY	MS	ML	FS	XH	LY
<i>Pseudolaubuca sinensis</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Sinibrama macrops</i>	1	1	0	0	1	1	1	1	0	1	0
<i>Chanodichthys erythropterus</i>	0	0	1	0	0	0	0	0	1	1	1
<i>Culter alburnus</i>	0	1	0	1	0	1	1	0	1	0	0
<i>Chanodichthys mongolicus</i>	0	0	1	0	0	0	0	0	0	0	1
<i>Chanodichthys dabryi</i>	0	1	0	1	0	0	0	0	1	0	1
<i>Culter oxycephaloides</i>	0	0	0	0	0	0	0	0	0	1	1
<i>Parabramis pekinensis</i>	0	0	0	0	1	1	1	1	0	0	0
<i>Megalobrama terminalis</i>	0	0	0	0	0	0	0	0	0	1	1
<i>Megalobrama amblycephala</i>	0	0	0	1	1	0	0	0	1	0	0
<i>Xenocypris macrolepis</i>	0	1	0	1	1	1	0	0	0	1	0
<i>Xenocypris davidi</i>	1	1	1	1	0	0	0	0	0	1	1
<i>Plagiognathops microlepis</i>	0	1	0	0	0	0	0	0	0	0	1
<i>Distochodon tumirostris</i>	1	0	0	0	0	0	0	0	1	1	0
<i>Hypophthalmichthys molitrix</i>	0	1	0	1	1	1	0	0	0	0	1
<i>Hypophthalmichthys nobilis</i>	0	1	0	0	1	1	0	0	0	0	1
<i>Abbottina rivularis</i>	0	1	1	1	1	0	1	1	0	1	1
<i>Pseudorasbora parva</i>	1	1	0	1	0	0	1	0	1	0	1
<i>Pseudogobio vaillanti</i>	0	1	0	0	0	0	0	0	0	0	0
<i>Pseudogobio guilinensis</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Hemibarbus labeo</i>	0	1	0	0	1	0	0	0	0	0	0
<i>Hemibarbus maculatus</i>	0	1	0	0	1	1	0	1	0	0	1
<i>Huigobio chenhsienensis</i>	0	1	0	0	1	0	0	0	0	0	0
<i>Sarcocheilichthys sinensis</i>	1	1	0	0	1	0	0	0	0	1	1
<i>Sarcocheilichthys kiangsiensis</i>	0	1	0	0	0	0	0	0	0	1	0
<i>Sarcocheilichthys nigripinnis</i>	0	0	0	0	0	0	1	0	0	1	1
<i>Squalidus argentatus</i>	0	1	1	1	1	1	1	0	1	0	1
<i>Rhinogobio typus</i>	1	0	1	0	0	0	1	0	0	1	1
<i>Platysmacheilus exiguus</i>	0	0	0	1	1	0	0	0	0	0	0
<i>Saurogobio dabryi</i>	1	1	1	0	1	1	1	1	0	1	1
<i>Saurogobio xiangjiangensis</i>	0	0	0	0	1	0	0	0	0	0	1
<i>Microphysogobio kiatingensis</i>	0	0	0	1	1	0	0	0	0	0	0
<i>Microphysogobio fukiensis</i>	0	0	0	1	0	0	1	0	0	1	0
<i>Gobiobotia filifer</i>	0	0	0	0	1	1	0	0	0	0	0
<i>Gobiobotia longibarba</i>	0	0	0	0	0	0	0	1	0	0	0
<i>Acheilognathus macropterus</i>	0	0	0	1	1	0	0	0	0	0	1
<i>Acheilognathus gracilis</i>	0	1	0	1	1	0	0	0	0	1	1
<i>Acheilognathus chankaensis</i>	0	0	0	1	0	1	0	1	0	0	0
<i>Acheilognathus tonkinensis</i>	0	0	0	0	0	0	1	1	1	0	1
<i>Acheilognathus barbatulus</i>	0	1	0	0	0	0	0	0	0	0	0
<i>Rhodeus ocellatus</i>	0	1	0	1	1	1	1	1	1	1	1
<i>Rhodeus lighti</i>	0	0	0	0	0	0	0	1	1	0	0
<i>Acrossocheilus fasciatus</i>	0	1	1	1	0	0	1	0	0	1	0
<i>Acrossocheilus paradoxus</i>	0	0	0	1	1	0	1	0	0	0	0
<i>Acrossocheilus hemispinus</i>	0	0	1	0	0	0	1	1	0	0	0
<i>Acrossocheilus parallens</i>	0	1	1	1	1	1	1	1	0	1	1
<i>Spinibarbus hollandi</i>	0	1	1	0	1	1	0	0	0	0	0
<i>Onychostoma barbatulum</i>	0	1	0	0	0	0	0	0	0	0	0
<i>Carassius auratus</i>	1	1	1	1	1	1	1	1	1	1	1
<i>Cyprinus carpio</i>	1	1	1	1	1	1	1	1	1	1	1
<i>Garra orientalis</i>	0	0	0	0	1	0	0	0	0	1	0
<i>Cobitis sinensis</i>	0	1	0	0	1	0	0	1	1	1	0

Species	JJ	YS	HS	SS	SC	SY	MS	ML	FS	XH	LY
<i>Misgurnus anguillicaudatus</i>	1	1	1	1	1	1	1	1	1	1	1
<i>Paramisgurnus dabryanus</i>	0	1	0	1	1	0	0	0	0	0	0
<i>Schistura fasciolata</i>	0	1	0	0	0	0	0	0	0	0	0
<i>Schistura incerta</i>	0	0	1	0	0	0	0	0	0	0	0
<i>Leptobotia elongata</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Parabotia banarensis</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Parabotia fasciata</i>	0	1	0	0	1	0	0	0	0	0	0
<i>Parabotia maculosa</i>	0	1	0	0	1	0	0	0	0	0	0
<i>Erromyzon sinensis</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Lepturichthys fimbriata</i>	0	0	0	1	1	0	1	0	0	0	0
<i>Vanmanenia stenosoma</i>	0	0	0	1	1	0	0	0	0	1	0
<i>Vanmanenia pingchowensis</i>	0	1	0	1	0	0	0	0	0	0	0
<i>Pseudogastromyzon changtingensis</i>	0	0	1	1	0	0	0	0	0	0	0
<i>Silurus asotus</i>	1	1	1	1	1	1	1	1	1	1	1
<i>Silurus meridionalis</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Pterocryptis cochinchinensis</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Clarias fuscus</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Hemibagrus macropterus</i>	0	0	0	0	1	0	1	1	0	0	1
<i>Pseudobagrus crassilabris</i>	0	0	0	0	1	0	1	0	0	0	0
<i>Pseudobagrus tenuis</i>	0	1	0	1	1	0	1	1	0	1	0
<i>Pseudobagrus ondon</i>	0	1	0	0	0	0	1	0	0	0	0
<i>Pseudobagrus pratti</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Pseudobagrus albomarginatus</i>	0	0	0	0	0	0	0	0	0	1	0
<i>Tachysurus fulvidraco</i>	1	1	1	1	1	1	1	1	1	1	1
<i>Tachysurus nitidus</i>	0	0	0	0	0	0	1	0	0	1	0
<i>Liobagrus anguillicauda</i>	0	0	1	0	0	0	0	0	0	0	0
<i>Liobagrus marginatus</i>	0	1	0	0	0	0	0	0	0	0	0
<i>Liobagrus nigricauda</i>	0	1	1	1	0	0	0	1	0	0	0
<i>Glyptothorax sinense</i>	0	1	0	1	1	0	0	1	0	0	0
<i>Hyporhamphus intermedius</i>	0	0	0	0	0	0	0	0	1	0	0
<i>Monopterus albus</i>	1	1	1	1	1	1	1	1	1	1	1
<i>Macragnathus aculeatus</i>	0	1	0	0	0	0	1	0	0	0	0
<i>Sinobdella sinensis</i>	0	1	0	0	1	0	0	1	0	1	0
<i>Siniperca chuatsi</i>	0	1	0	0	1	0	1	0	0	1	0
<i>Siniperca kneri</i>	0	1	0	0	1	0	0	0	0	0	0
<i>Siniperca obscura</i>	0	0	0	0	1	0	0	0	0	0	0
<i>Siniperca roulei</i>	1	0	0	0	1	0	0	0	0	0	0
<i>Siniperca scherzeri</i>	0	0	0	0	0	1	0	0	1	1	0
<i>Siniperca undulata</i>	0	0	0	0	0	1	0	0	0	1	0
<i>Odontobutis sinensis</i>	0	1	0	1	0	0	1	0	0	0	0
<i>Rhinogobius cliffordpopei</i>	1	1	1	1	0	0	1	0	0	1	0
<i>Rhinogobius duospilus</i>	0	1	0	0	0	0	0	0	0	0	0
<i>Rhinogobius giurinus</i>	0	1	0	1	1	1	1	1	0	1	0
<i>Rhinogobius lindbergi</i>	0	0	0	1	1	0	0	0	0	0	0
<i>Rhinogobius leavelli</i>	0	0	0	1	0	0	1	0	0	0	0
<i>Macropodus opercularis</i>	0	1	0	1	1	0	1	0	0	0	0
<i>Channa argus</i>	1	1	0	0	1	0	1	1	0	1	0
<i>Channa asiatica</i>	0	1	0	1	1	1	1	0	0	0	0
<i>Channa maculata</i>	0	0	0	0	1	1	1	0	0	0	0

## Appendix 2

The proportion of order, family, and subfamily of fish species.

Order	Species (proportion)	Family	Species (proportion)	Subfamily	Species (proportion)
Cypriniformes	77(68.1%)	Catostomidae	1(0.9%)	Danioninae	2(3.2%)
		Cyprinidae	62(54.9%)	Leuciscinae	4(6.5%)
		Cobitidae	9(8.0%)	Culterinae	15(24.2%)
		Balitoridae	5(4.4%)	Xenocyprininae	4(6.5%)
		Siluriformes	16(14.2%)	Siluridae	3(2.7%)
		Clariidae	1(0.9%)	Gobioninae	17(27.4%)
		Bagridae	8(7.1%)	Gobiobotinae	2(3.2%)
		Amblycipitidae	3(2.7%)	Acheilognathinae	7(11.3%)
		Sisoridae	1(0.9%)	Barbinae	6(9.7%)
Beloniformes	1(0.9%)	Hemiramphidae	1(0.9%)	Cyprininae	2(3.2%)
Syngnathiformes	3(2.7%)	Syngnathidae	1(0.9%)	Labeoninae	1(1.6%)
		Mastacembelidae	2(1.8%)		
Perciformes	16(14.2%)	Percichthyidae	6(5.3%)		
		Odontobutidae	1(0.9%)		
		Gobiidae	5(4.4%)		
		Belontiidae	1(0.9%)		
		Channidae	3(2.7%)		
		Total	113(100%)		113(100%)

## Appendix 3

Endangered categories of fish species in the Luoxiao Mountains. Key: DD: Data Deficient; LC: Least Concern; NT: Near Threatened; VU: Vulnerable; EN: Endangered; CR: Critically Endangered.

Species	Endangered categories	
	Jiang et al. (2016)	IUCN (2017)
<i>Myxocyprinus asiaticus</i>	CR	DD
<i>Zacco platypus</i>	LC	DD
<i>Opsariichthys bidens</i>	LC	LC
<i>Mylopharyngodon piceus</i>	LC	DD
<i>Ctenopharyngodon idella</i>	LC	DD
<i>Elopichthys bambusa</i>	LC	DD
<i>Squaliobarbus curriculus</i>	LC	DD
<i>Hemiculter leucisculus</i>	LC	LC
<i>Hemiculter bleekeri</i>	LC	DD
<i>Hemiculterella sawagei</i>	LC	LC
<i>Hemiculterella wui</i>	LC	DD
<i>Pseudohemiculter dispar</i>	LC	VU
<i>Pseudolaubuca sinensis</i>	LC	LC
<i>Sinibrama macrops</i>	LC	LC
<i>Chanodichthys erythropterus</i>	LC	LC
<i>Culter alburnus</i>	LC	DD
<i>Chanodichthys mongolicus</i>	LC	LC
<i>Chanodichthys dabryi</i>	LC	LC
<i>Culter oxycephaloides</i>	LC	DD
<i>Parabramis pekinensis</i>	LC	DD

Species	Endangered categories	
	Jiang et al. (2016)	IUCN (2017)
<i>Megalobrama terminalis</i>	LC	DD
<i>Megalobrama amblycephala</i>	LC	LC
<i>Xenocypris macrolepis</i>	LC	LC
<i>Xenocypris davidi</i>	LC	DD
<i>Plagiognathops microlepis</i>	LC	LC
<i>Distoichodon tumirostris</i>	LC	LC
<i>Hypophthalmichthys molitrix</i>	LC	NT
<i>Hypophthalmichthys nobilis</i>	LC	DD
<i>Abbottina rivularis</i>	LC	DD
<i>Pseudorasbora parva</i>	LC	LC
<i>Pseudogobio vaillanti</i>	LC	LC
<i>Pseudogobio guilinensis</i>	LC	DD
<i>Hemibarbus labeo</i>	LC	DD
<i>Hemibarbus maculatus</i>	LC	DD
<i>Huigobio chenhsienensis</i>	LC	LC
<i>Sarcocheilichthys sinensis</i>	LC	LC
<i>Sarcocheilichthys kiangsiensis</i>	LC	DD
<i>Sarcocheilichthys nigripinnis</i>	LC	DD
<i>Squalidus argentatus</i>	LC	DD
<i>Rhinogobio typus</i>	LC	DD
<i>Platysmacheilus exiguus</i>	LC	LC
<i>Saurogobio dabryi</i>	LC	DD
<i>Saurogobio xiangjiangensis</i>	LC	DD
<i>Microphysogobio kiatingensis</i>	DD	LC
<i>Microphysogobio fukiensis</i>	DD	LC
<i>Gobiobotia filifer</i>	LC	DD
<i>Gobiobotia longibarba</i>	DD	DD
<i>Acheilognathus macropterus</i>	LC	DD
<i>Acheilognathus gracilis</i>	LC	DD
<i>Acheilognathus chankaensis</i>	LC	DD
<i>Acheilognathus tonkinensis</i>	LC	DD
<i>Acheilognathus barbatulus</i>	LC	LC
<i>Rhodeus ocellatus</i>	LC	DD
<i>Rhodeus lighti</i>	LC	LC
<i>Acrossocheilus fasciatus</i>	LC	DD
<i>Acrossocheilus paradoxus</i>	LC	DD
<i>Acrossocheilus hemispinus</i>	LC	LC
<i>Acrossocheilus parallens</i>	LC	LC
<i>Spinibarbus hollandi</i>	LC	DD
<i>Onychostoma barbatulum</i>	NT	DD
<i>Carassius auratus</i>	LC	LC
<i>Cyprinus carpio</i>	LC	VU
<i>Garra orientalis</i>	LC	LC
<i>Cobitis sinensis</i>	LC	LC
<i>Misgurnus anguillicaudatus</i>	LC	LC
<i>Paramisgurnus dabryanus</i>	LC	DD
<i>Schistura fasciolata</i>	DD	DD
<i>Schistura incerta</i>	DD	DD
<i>Leptobotia elongata</i>	VU	VU
<i>Parabotia banarescui</i>	LC	DD
<i>Parabotia fasciata</i>	LC	LC
<i>Parabotia maculosa</i>	LC	LC

Species	Endangered categories	
	Jiang et al. (2016)	IUCN (2017)
<i>Erromyzon sinensis</i>	DD	DD
<i>Lepturichthys fimbriata</i>	DD	LC
<i>Vanmanenia stenosoma</i>	DD	DD
<i>Vanmanenia pingchowensis</i>	DD	DD
<i>Pseudogastromyzon changtingensis</i>	DD	DD
<i>Silurus asotus</i>	LC	LC
<i>Silurus meridionalis</i>	LC	LC
<i>Pterocryptis cochinchinensis</i>	LC	LC
<i>Clarias fuscus</i>	LC	LC
<i>Hemibagrus macropterus</i>	LC	LC
<i>Pseudobagrus crassilabris</i>	LC	DD
<i>Pseudobagrus tenuis</i>	DD	DD
<i>Pseudobagrus ondon</i>	DD	LC
<i>Pseudobagrus pratti</i>	VU	DD
<i>Pseudobagrus albomarginatus</i>	LC	DD
<i>Tachysurus fulvidraco</i>	LC	LC
<i>Tachysurus nitidus</i>	LC	DD
<i>Liobagrus anguillicauda</i>	DD	DD
<i>Liobagrus marginatus</i>	VU	DD
<i>Liobagrus nigricauda</i>	DD	EN
<i>Glyptothorax sinense</i>	LC	DD
<i>Hyporhamphus intermedius</i>	LC	DD
<i>Monopterus albus</i>	LC	LC
<i>Macragnathus aculeatus</i>	LC	DD
<i>Sinobdella sinensis</i>	DD	LC
<i>Siniperca chuatsi</i>	LC	DD
<i>Siniperca kneri</i>	LC	DD
<i>Siniperca obscura</i>	NT	LC
<i>Siniperca roulei</i>	VU	DD
<i>Siniperca scherzeri</i>	LC	DD
<i>Siniperca undulata</i>	NT	NT
<i>Odontobutis sinensis</i>	LC	DD
<i>Rhinogobius cliffordpopei</i>	LC	DD
<i>Rhinogobius duospilus</i>	DD	DD
<i>Rhinogobius giurinus</i>	LC	LC
<i>Rhinogobius lindbergi</i>	DD	DD
<i>Rhinogobius leavelli</i>	LC	LC
<i>Macropodus opercularis</i>	LC	LC
<i>Channa argus</i>	LC	DD
<i>Channa asiatica</i>	LC	LC
<i>Channa maculata</i>	LC	LC



# ***Gnathopalystes aureolus* (He & Hu, 2000): new combination for *Pseudopoda aureola* (Araneae, Sparassidae), with the first description of the female from Hainan Island, China**

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## **Abstract**

The taxonomic status of *Pseudopoda aureola* (He & Hu, 2000) is revised based on the re-examination of the type specimen and fresh material collected from the type locality. The cheliceral dentition, with a large denticle field between two anterior and three posterior teeth, the male palps with simple RTA arising distally, narrow, not filiform embolus and sheet-like membranous conductor, and the female epigyne with a visible median septum indicate that the species does not belong to *Pseudopoda* or to the originally assigned genus, *Heteropoda*. Based on these characters, the species is here transferred to *Gnathopalystes*. The male is redescribed and the female is described and illustrated for the first time.

## **Keywords**

Biodiversity, Hainan, huntsman spiders, taxonomy

## **Introduction**

*Heteropoda aureola* was first described in the genus *Heteropoda* Latreille, 1804, based on male specimens from Jianfengling Mountain, Hainan Province, China by He and

Hu (2000). Jäger (2014) transferred it to *Pseudopoda* Jäger, 2000 based on the original description and illustrations, which showed an RTA with a bifid apex arising medially from the tibia. However, the author pointed out that a definite characterization would only be possible with the examination of type material or material from the type locality.

Recently, the authors examined material from Jianfengling Mountain (type locality of *P. aureola*) and found specimens which, when compared to the holotype, were confirmed as *P. aureola*. However, the presence of male palps with simple RTA, arising distally (medially or basally in *Pseudopoda*), narrow, not filiform embolus (broadened and flattened in *Pseudopoda*, filiform in *Herteropoda*), sheet-like membranous conductor (sheath-like in *Herteropoda*) and female epigyne with visible median septum (inconspicuous in *Pseudopoda*) indicated that this species did not belong to either *Heteropoda* or *Pseudopoda*. When comparing this material to that of other species recorded from China, the authors noted a strong similarity between *P. aureola* and *Gnathopalystes taiwanensis* Zhu & Tso, 2006 (described from Taiwan and recorded in China) which suggested that the species might actually belong to *Gnathopalystes*.

Rainbow (1899) established *Gnathopalystes* to include *G. ferox* Rainbow, 1899. Simon (1903) synonymized it with *Palystes* L. Koch, 1875, and this decision was generally accepted until Croeser (1996) revised *Palystes* and revalidated *Gnathopalystes* to accommodate the non-African species of the genus. Nevertheless, he diagnosed the genus using only habitus characters, such as eye arrangement and cheliceral dentition, because the type species, *G. ferox*, was known solely from an immature female. From that time on, most authors considered *Gnathopalystes* a valid genus and several new species were described (Jäger 1998; Zhu and Tso 2006; Saha and Raychaudhuri 2007; Jäger 2014). Currently, the genus includes nine species, most of which are distributed in Vanuatu, Solomon Is., Papua New Guinea, New Guinea, Taiwan, Indonesia, and Malaysia.

Although *Gnathopalystes* has not been revised, and most species are poorly described and illustrated, we decided to remove *P. aureola* from *Pseudopoda* and transfer it to *Gnathopalystes* based on characters provided by Croeser (1996) and Jäger (1998). *Gnathopalystes aureolus* comb. n. has a straight posterior eye row, wider than the recurved anterior eye row, with AME smaller than ALE, PLE equal or slightly larger than PME (PLE significantly larger than PME in *Heteropoda* and *Pseudopoda*). It also shows a large denticle field between the anterior and posterior teeth, which extends from the proximal teeth to the margin of the non-sclerotized arthrodistal membrane (often close to the three anterior teeth in *Heteropoda* and *Pseudopoda*). In addition to the transfer, we provide a redescription of the male of *G. aureolus* comb. n. and describe the female for the first time.

## Materials and methods

Specimens were examined with an Olympus SZX16 stereomicroscope; details were further investigated with an Olympus BX51 compound microscope. All illustrations were made using an Olympus drawing tube. Epigynes were examined and illustrated after dissection from the spider bodies. Photos were made with a Canon G10 digital



camera (14.7 megapixels) mounted on an Olympus SZX16 stereomicroscope. The digital images depicting the habitus and genital morphology are a composite of multiple images taken at different focal planes along the Z-axis and assembled using the software package Helicon Focus 3.10. Left palps are depicted unless otherwise stated. The illustration of schematic course of internal duct system follows Jäger (2000). Most hairs and macrosetae are usually not depicted in the palp and epigyne drawings. Positions of regular appendages are given according to clock positions, based on the left male palp in ventral view. Measurements are given in millimeters.

Leg measurements are shown as: total length (femur, patella, tibia, metatarsus, tarsus). Number of spines is listed for each segment in the following order: prolateral, dorsal, retrolateral, ventral (in femora and patellae ventral spines are absent and fourth digit is omitted in the spination formula). Abbreviations follow Zhong et al. (2017, 2018):

<b>ALE</b>	anterior lateral eyes;	<b>PH</b>	prosoma height;
<b>AME</b>	anterior median eyes;	<b>PL</b>	prosoma length;
<b>AW</b>	anterior width of prosoma;	<b>PLE</b>	posterior lateral eyes;
<b>CH</b>	clypeus height;	<b>PME</b>	posterior median eyes;
<b>FE</b>	femur;	<b>Pp</b>	palpus;
<b>Mt</b>	metatarsus;	<b>PW</b>	prosoma width;
<b>OL</b>	opisthosoma length;	<b>Ta</b>	tarsus;
<b>OW</b>	opisthosoma width;	<b>Ti</b>	tibia. I, II, III, IV—legs I to IV.
<b>Pa</b>	patella;		

Abbreviations for the collection depositories:

<b>HBU</b>	Hubei University, Wuhan, China;
<b>MTJ</b>	Museum of Tianjing, Tianjing, China

## Taxonomy

**Family Sparassidae Bertkau, 1872**

**Genus *Gnathopalystes* Rainbow, 1899**

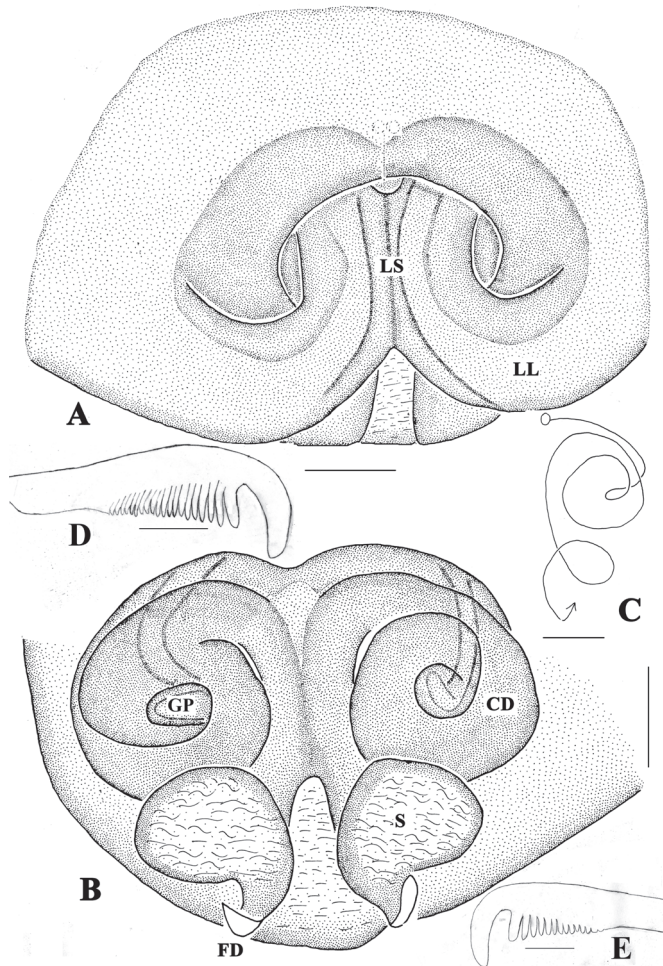
***Gnathopalystes aureolus* (He & Hu, 2000), comb. n.**

Figures 1–4

*Heteropoda aureola* He & Hu, 2000: 17, figs 1–2 (holotype male from Jianfengling Mountain, Hainan, China, deposited in MTJ, examined).

*Pseudopoda aureola* Jäger, 2014: 184 (transferred from *Heteropoda*).

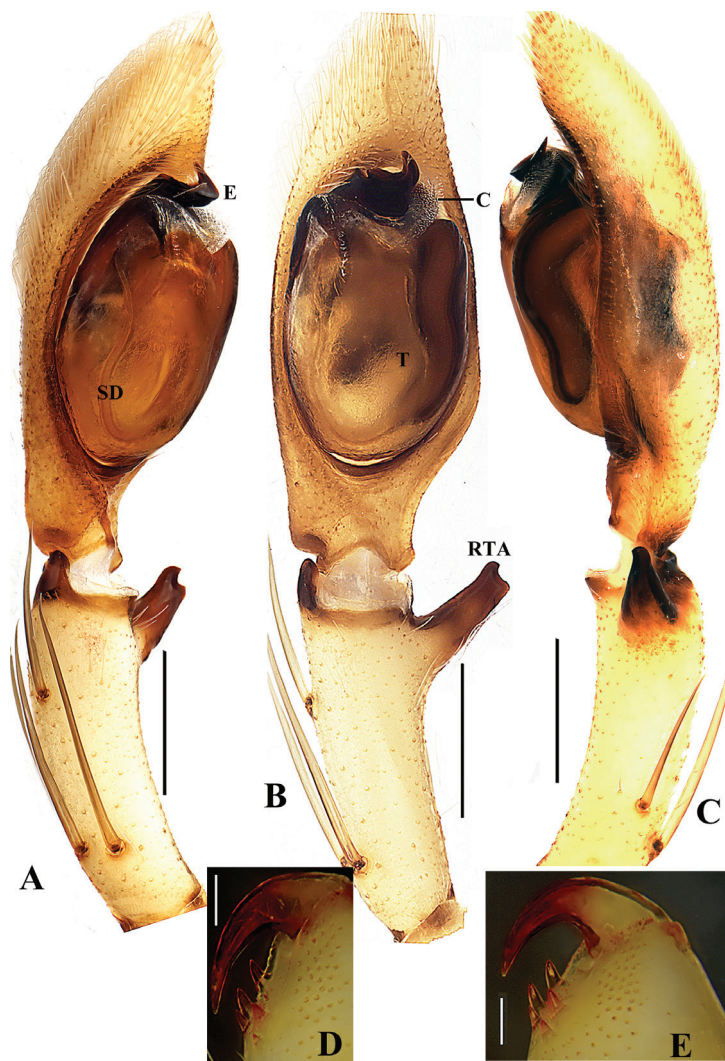
**Material examined.** 1 male (holotype, MTJ), Jianfengling Mountain, Hainan Island, China, 8 April 1980, Shengli Liu leg.; 1 male and 1 female (HBU), Jianfengling



**Figure 1.** *Gnathopalystes aureolus* (He & Hu, 2000) comb. n. **A** Epigyne, ventral view **B** Vulva, dorsal view **C** Schematic course of internal duct system, dorsal view **D–E** Female tarsal claws of leg I (**D** prolateral **E** retrolateral). Abbreviations: C – conductor; CD – copulatory duct; FD – fertilization duct; GP – glandular projection; LL – lateral lobes; LS – lobal septum. Scale bars: 0.2 mm (**A–C**); 0.1 mm (**D, E**).

Mountain [22°37.93'N, 120°63.29'E, 560 m], Hainan Island, China, 9 June 2013, Fengxiang Liu leg.

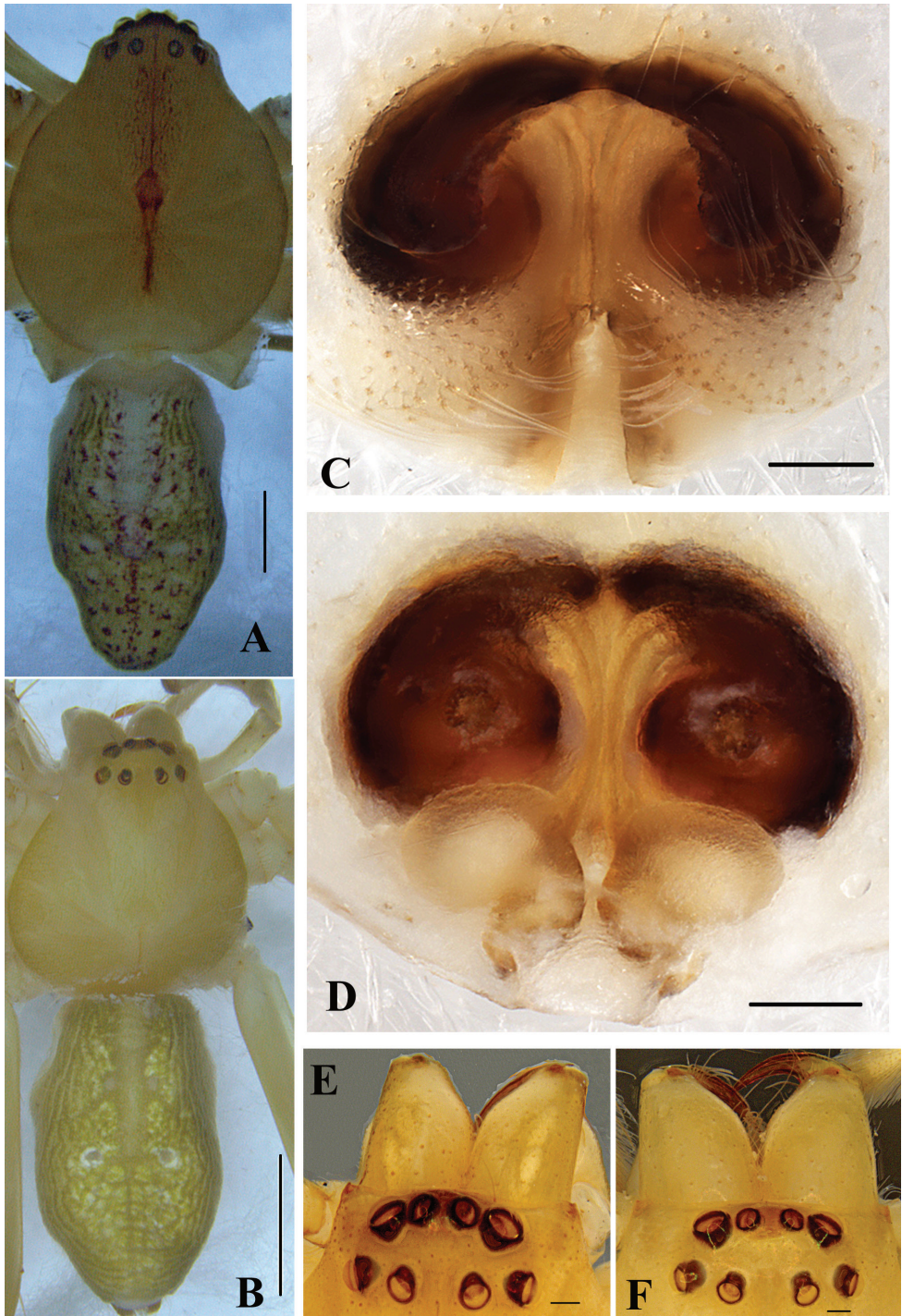
**Diagnosis.** Females of *G. aureolus* comb. n. resemble those of *G. taiwanensis* in eye arrangement (straight posterior row and recurved anterior row, with posterior row wider than anterior, AME smaller than ALE), cheliceral dentition (large denticle field between two anterior and three posterior teeth), and epigyne (with coiled copulatory ducts). They differ by the epigyne with lateral lobes contiguous (separated in *G. taiwanensis*), anterior margin of lobal pockets distinct (absent in *G. taiwanensis*) and left part of vulva connected to the right part (separated in *G. taiwanensis*). The females of *G. taiwanensis* and *G. aureolus* comb. n. can be distinguished from other *Gnathopalystes*



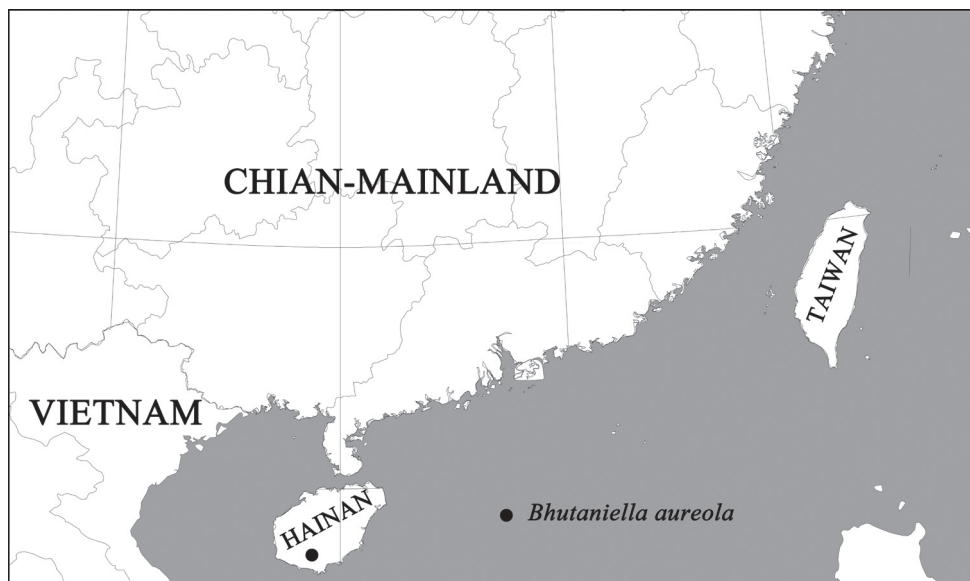
**Figure 2.** *Gnathopalystes aureolus* (He & Hu, 2000) comb. n. **A–C** Left male palp (**A** prolateral **B** ventral **C** retrolateral) **D** Male left cheliceral teeth, ventral view **E** Female left cheliceral teeth, ventral view. Abbreviations: C – conductor; E – embolus; RTA – retrolateral tibial apophysis; SD – sperm duct; T – tegulum. Scale bars: 0.2 mm.

species by the three posterior teeth on the retromargin of cheliceral fang furrow (four to five posterior teeth in other *Gnathopalystes* species). Males can be distinguished by the palp with clavate and straight RTA (RTA broad at base, tapering to apex, and bent in *G. kochi* (Simon, 1880), the only other known male to date) (Figs 1, 2, 3C–F).

**Description. Male.** Measurements: PL 4.26, PW 3.50, AW 1.37, PH 0.88, OL 4.28, OW 2.20. Eyes: AME 0.15, ALE 0.26, PME 0.18, PLE 0.21, AME–AME 0.20, AME–ALE 0.11, PME–PME 0.38, PME–PLE 0.25, AME–PME 0.45, ALE–PLE



**Figure 3.** *Gnathopalystes aureolus* (He & Hu, 2000) comb. n. **A** Male habitus, dorsal view **B** Female habitus, dorsal view **C** Epigyne, ventral view **D** Vulva, dorsal view **E** Male eyes, dorsal view **F** Female eyes, dorsal view. Scale bars: 1mm (**A**, **B**); 0.2 mm (**C**-**F**).



**Figure 4.** Collection locality of *Gnathopalystes aureolus* comb. n. in Hainan Island, China.

0.30, CH AME 0.24, CH ALE 0.12. Leg and palp measurements: Pp 5.15 (1.62, 0.79, 1.31, -, 1.43), I 22.77 (5.86, 1.11, 6.79, 7.08, 1.93), II 24.66 (7.00, 1.41, 6.86, 7.66, 1.73), III 17.49 (5.54, 1.20, 4.94, 4.45, 1.36), IV 21.35 (6.76, 1.22, 5.75, 6.04, 1.58). Leg formula: II-I-IV-III. Spination: Pp 120, 101, 3011; Fe I 223, II 323, III 222, IV 322; Pa I-IV 101; Ti I 2026, II 2025, III-IV 2024; Mt I-IV 2024. Cheliceral furrow with large denticle field extending from proximal teeth to the margin of the non-sclerotized arthrodial membrane, with approximately 18 denticles. Promargin of cheliceral furrow with two teeth, the distal one significantly larger than proximal one, retromargin with three teeth, the two distal ones similar sized, the proximal one significantly larger (Figure 2D). Dorsal shield of prosoma generally yellowish brown without distinct pattern, with a reddish longitudinal line medially, with a heavy reddish fovea and faint brown marks. Eye region slightly darker, eye borders dark. Sternum bright brown. Labium, gnathocoxae and chelicerae bright brown with orange margins. Legs and pedipalps bright brown. Opisthosoma yellowish-brown colored with dorsal pattern of scattered reddish marks (Figure 3A). Palp as in diagnosis. Tibia slightly shorter than cymbium with three prolateral, one dorsal, and one retrolateral spine. Cymbium with dense long hairs. Cymbial scopula distinct, elliptical and located on distal half of cymbium. Tegulum significantly large, almost occupying four-fifths of the alveolus. Subtegulum not visible in ventral view. Sperm duct U-shaped, tapering. Conductor irregular, with sharp tip in prolateral view, arising from tegulum at 11-o'clock-position. RTA simple, unbranched, arising distally from tibia (Figs 2A–C).

**Female.** Measurements: PL 3.95, PW 3.93, AW 1.90, PH 0.68, OL 5.24, OW 3.00. Eyes: AME 0.15, ALE 0.23, PME 0.17, PLE 0.2, AME–AME 0.25, AME–ALE 0.11, PME–PME 0.48, PME–PLE 0.27, AME–PME 0.49, ALE–PLE 0.36, CH

AME 0.25, CH ALE 0.10. Leg and palp measurements: Pp 3.93 (1.05, 0.77, 0.99, -, 1.12), I 20.28 (5.60, 1.72, 6.05, 5.51, 1.40), II 20.10 (5.95, 1.70, 5.81, 5.25, 1.39), III 13.28 (4.32, 0.97, 3.81, 3.17, 1.01), IV 16.37 (4.85, 1.03, 4.18, 5.03, 1.28). Leg formula: I-II-IV-III. Spination: Pp 202, 101, 2121, 2021; Fe I 023, II 010, III-IV 121; Pa I 000, II-IV 000; Ti I III- IV 2014, II 1013; Mt I-IV 2024. Cheliceral furrow as in male, with approximately 23 denticles (Figure 2E). Dorsal shield of prosoma generally pale brown without distinct pattern. Eye region slightly darker, eye borders dark. Sternum, labium, gnathocoxae and legs as in male. Opisthosoma yellowish-brown colored with two small white round marks medially and laterally (Figure 3B). Epigyne as in diagnosis. Anterior bands absent. Lateral lobes fused. Lobal septum wide, significantly short. Copulatory ducts coiled, with two small glandular projections, without integument. Fertilization ducts arising posterolaterally, widely separated (Figs 1, 3C–D).

**Distribution.** China (Hainan) (Fig. 4).

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# A new species of *Hyperimerus* Girault from China, with a key to species of the genus (Hymenoptera, Chalcidoidea, Pteromalidae)

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## Abstract

A new species of *Hyperimerus* Girault (Hymenoptera: Pteromalidae), *H. sichuanicus* Yang & Xiao, **sp. n.**, is reported and described for the first time from mainland China. A key to the worldwide species of *Hyperimerus* and illustrations of external features of the species are also provided.

## Keywords

China mainland, key, *Hyperimerus*, new species, Pteromalidae, taxonomy

## Introduction

The genus *Hyperimerus* was erected by Girault (1917) to include *Hyperimerus corvus* Girault from California, USA, and the genus was placed in the subfamily Erimerinae of Callimomidae. Subsequently, *Hyperimerus* was put in the tribe Asaphini under Pteromalidae by Peck (1951). Graham (1969) upgraded the tribe Asaphini to subfamily Asaphinae. Since then, several researchers reported *Hyperimerus* from Europe (Graham 1969; Bouček 1977; Dzhankmen 1978; Kalina 1989; Ulrich 1999). Schender et al. (2014) reviewed the genus and redescribed the two species, *H. corvus* Girault and *H. pusillus* (Walker). Until now, two valid species of *Hyperimerus* are known in the world.

Only one species, *H. pusillus* (Walker), has previously been reported in China (Huang and Xiao 2005). In this study, one new species, *H. sichuanicus* Yang & Xiao, sp. n., is described.

## Materials and methods

All specimens for the present study were swept using an insect net and preserved in 95% ethanol. They were subsequently air dried, point-mounted and examined with a LEICA MZ APO stereomicroscope. Photographs were taken under the Nikon Multi-zoom AZ100 system, and the plates were compiled using Adobe Photoshop software. In addition, the author also examined the specimens of *Hyperimerus* deposited in the National History Museum, London in 2002. All type specimens of the new species are deposited in the Institute of Zoology, Chinese Academy of Sciences, China (IZCAS).

Morphological terminology mostly follows that of Bouček (1988) and Gibson et al. (1997). All specimens were examined and identified based on the studies of Graham (1969), Gibson and Vikberg (1998), Bouček and Rasplus (1991) and Schender et al. (2014). The new species is described based on the holotype specimen. Body length excluding the ovipositor sheaths is measured in millimetres (mm); other measurements are given as ratios. Abbreviations of morphological terms used are:

- Fu<sub>n</sub>** funicular segment number;  
**POL** posterior ocellar distance;  
**OOL** ocellocular distance;  
**Gt<sub>n</sub>** gastral tergum number.

## Taxonomy

### Key to species

- 1 Fu<sub>1</sub> shorter than pedicel; ovipositor sheaths shortly protruded, shorter than 1/2 length of gaster ..... **2**
- Fu<sub>1</sub> longer than pedicel; ovipositor sheaths distinctly protruded, ca. 2/3 length of gaster ..... ***H. corvus* Girault**
- 2 Clava as long as Fu<sub>5</sub>-Fu<sub>7</sub> combined; marginal fringe of outer margin of fore wing longer than length of uncus; propodeum with irregularly and densely areolate sculptures (Fig. 5), most cells of sculpture as big as propodeal spiracles ..... ***H. sichuanicus* sp. n.**
- Clava shorter than length of Fu<sub>5</sub>-Fu<sub>7</sub> combined; marginal fringe of outer margin of fore wing shorter than length of uncus; propodeum with irregularly and sparsely sculptures (Fig. 10), most cells of sculpture more than 2× as big as propodeal spiracles ..... ***H. pusillus* (Walker)**

### ***Hyperimerus* Girault, 1917**

*Hyperimerus* Girault, 1917: 5. Type species: *Hyperimerus corvus* Girault, by original designation.

*Hyperimerus* Girault: Graham 1969: 83–84; Huang and Xiao 2005: 281–282; Schender et al. 2014: 408–420.

*Mespilon* Graham, 1957: 406. Type species: *Mespilon exiguum* Graham, by original designation. Synonymized by Bouček 1965: 549.

**Diagnosis.** Body with dense hairy, head and mesosoma with engraved reticulate sculpture. Head subtriangular in frontal view; antennal scrobes deep, not reaching anterior ocellus; malar sulci distinct; antennal insertion obviously below centre of face; formula 11173. Head in dorsal view, occiput with horseshoe-like carina. Pronotum quadrangular; notauli complete, frenal line on scutellum indistinct; propodeal sculptures irregular; fore wing with pilosity, speculum absent; postmarginal vein longer than marginal vein. Gaster convex, petiole transverse,  $Gt_1$  and  $Gt_2$  large and smooth, ovipositor sheath exerted.

*Hyperimerus* is similar to *Asaphes* in the subfamily Asaphinae, but it can be recognized by the antenna with one anellus and seven funicular segments (two anelli and six funicular segments in *Asaphes*), frenal line on scutellum indistinct (distinct in *Asaphes*), petiole transverse (petiole longer than broad and with longitudinal ridges in *Asaphes*).

**Biology.** *Hyperimerus* is recorded as parasites of *Pseudococcus* (Hemiptera: Pseudococcidae), *Psylla* (Hemiptera: Psyllidae), *Lymantria* (Lepidoptera: Erebididae), *Choristoneura*, *Zeiraphera* (Lepidoptera: Tortricidae), *Chrysopa* (Neurop: Chrysopidae), *Hemerobius*, and *Symphorobius* (Neuroptera: Hemerobiidae) (Graham 1969; Burks 1979; Schender et al. 2014; Noyes 2018). The genus is associated with the following plants: *Fagus sylvatica*, *Pyrus communis* (Dzhanokmen 1978; Ghahari et al, 2010).

**Distribution.** China (Sichuan, Tibet); Holarctic, Oriental, and Neotropical regions.

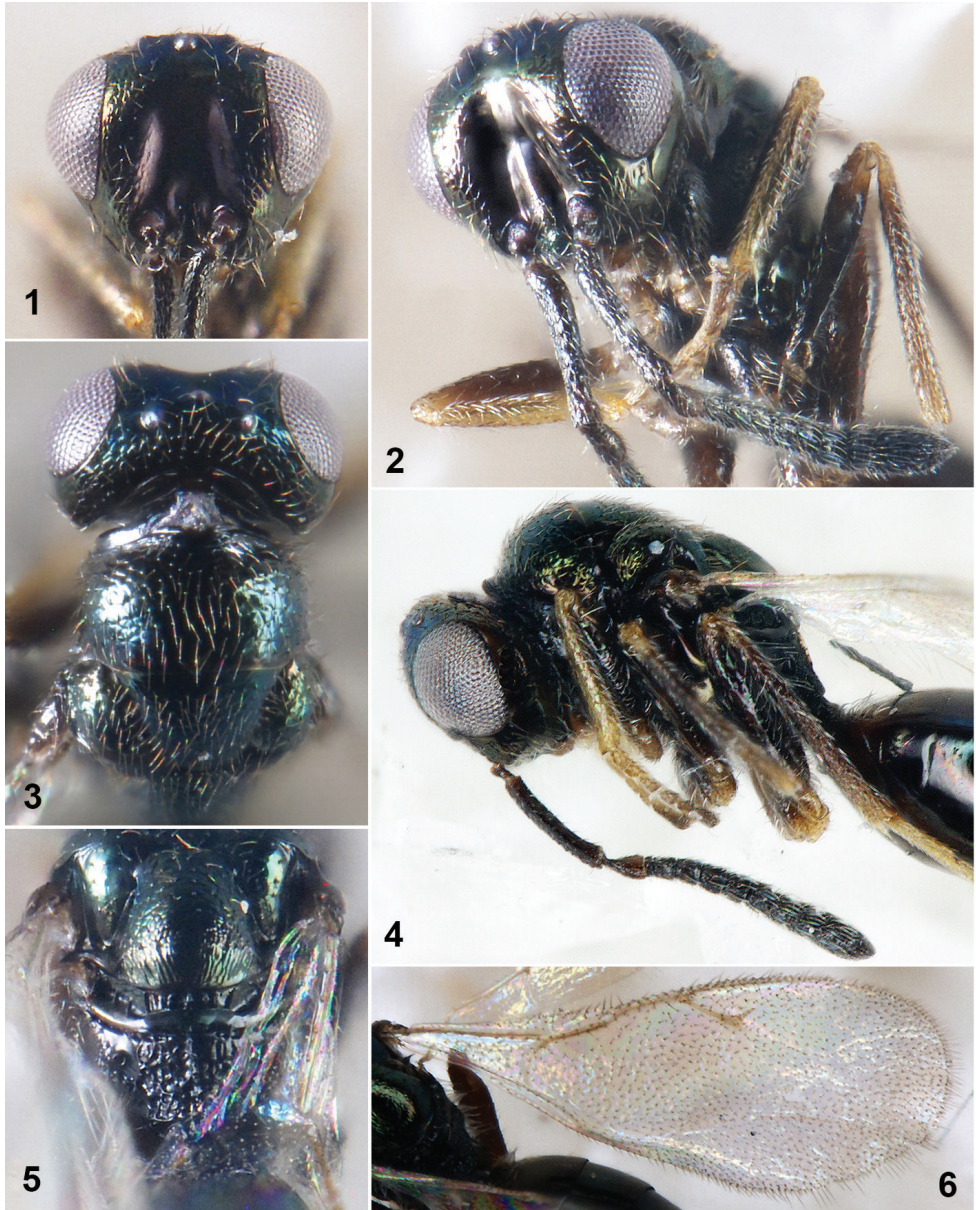
### ***Hyperimerus sichuanicus* Yang & Xiao, sp. n.**

<http://zoobank.org/36329DB9-9E34-44E8-8CEB-D7773E07A6AA>

Figs 1–6

**Diagnosis.** Antenna with  $Fu_1$  shorter than pedicel;  $Fu_7$  quadrate, clava as long as  $Fu_5$ - $Fu_7$  combined; marginal fringe of outer margin of fore wing longer than length unicus; postmarginal vein 1.65× as long as stigmal vein; propodeum with irregularly and densely sculptured, most of them as big as propodeal spiracles; ovipositor sheaths protruded, not longer than 1/2 length of gaster.

**Description.** *Female* (holotype). Body length 1.4 mm. Head and body dark green with bluish reflection. Mandibles brown, antennae dark brown. Coxae concolorous with body, remainder of legs brown except tibiae yellowish brown, colour gradation from yellowish brown to brown at tarsi.



**Figures 1–6.** *Hyperimerus sichuanicus* sp. n., female holotype **1** Head in frontal view **2** Head in lateral view **3** Head and pronotum in dorsal view **4** Antenna and thorax in lateral view **5** Propodeum in dorsal view **6** Fore wing in dorsal view.

Head in frontal view  $1.26\times$  as wide as high (Fig. 1); eyes height  $0.62\times$  head height; eyes separated by  $1.24\times$  their height. Antennal scrobes deep and glabrous, not reaching anterior ocellus (Fig. 2), interantennal crest distinct. Clypeal suture obvious, clypeus and supraclypeal area slightly prominent, clypeal margin truncate. Antennal insertion on lower ocular line, close to clypeal margin, distance from upper margin of torulus

to lower margin of anterior ocellus  $7\times$  distance from lower margin of torulus to lower margin of clypeus. Gena smooth and convex; malar sulci distinct, malar space  $0.67\times$  eyes height. Antenna (Fig. 4) clavate; scape  $1.14\times$  eyes height, reaching anterior ocellus; length of pedicel and flagellum combined longer than head width ( $1.16\times$ ); pedicel conical,  $2\times$  as long as broad in lateral view; anellus transverse; each funicular segment and clava segment with single row of sensilla; ratio of length and width from  $Fu_1$  to  $Fu_7$  as 3:3, 4:3.5, 4:4, 5:4, 5:4, 5:5, 5:5; clava equal to length of  $Fu_5$ - $Fu_7$  combined. Head in dorsal view,  $1.95\times$  as wide as long (Fig. 3); vertex convex, POL  $2.75\times$  OOL.

Head  $1.27\times$  as broad as mesosoma. Mesosoma convex,  $1.76\times$  as long as broad. Pronotum, mesoscutum and anterior area of scutellum with engraved reticulation. Pronotum  $0.91\times$  as broad as mesoscutum. Mesoscutum  $2\times$  as broad as long; notauli deep and complete. Scutellum weakly convex, frenal line obscured medially, frenum with longitudinal rugae (Fig. 5), frenum ca.  $1/5$  length of scutellum. Dorsellum glabrous,  $4\times$  as broad as long,  $0.17\times$  as long as propodeum. Propodeum (Fig. 5)  $0.71\times$  as long as scutellum, median carina distinct; areolate sculptures on median area irregular and dense, most cells as big as propodeal spiracles; area otherwise glabrous; propodeal spiracles ovate, separated from metanotum by  $1.5\times$  spiracular width; callus densely hairy laterally and posteriorly. Prepectus smooth and with sparse hairy. Upper mesepisternum setose and finely reticulate, area otherwise smooth. Fore wing (Fig. 6)  $2.63\times$  as long as broad, speculum absent; marginal fringe of outer margin of fore wing longer than length of uncus; costal cell with sparse setae; ratio of submarginal vein: marginal vein: postmarginal vein: stigmal vein as 49:18:26:16; stigmal vein oblique, stigma slightly expand, uncus ca.  $1/4$  length of stigmal vein.

Gaster ovate,  $1.88\times$  as long as broad, shorter than head and mesosoma combined. Petiole short,  $0.5\times$  as long as broad, dorsum with longitudinal ridges. Each gastral tergite smooth;  $Gt_1$  with long hairy around petiole;  $Gt_1$  and  $Gt_2$  large and smooth,  $0.47\times$  length of gaster. Ovipositor sheaths distinctly produced in dorsal view; gaster in lateral view, ovipositor sheaths  $0.47\times$  length of gaster,  $0.76\times$  length of hind tibia.

Male. Body slender, scape yellowish-brown, antenna segmented clearly, others similar to the female.

**Remarks.** The new species is similar to *H. corvus*, but different by the antenna with  $Fu_1$  shorter than pedicel, ovipositor sheaths shortly protruded. It is also very close to *H. pusillus* in having the  $Fu_1$  shorter than pedicel, ovipositor sheaths shorter than  $1/2$  length of gaster, but can be recognized by the characters listed in the key.

**Material examined.** Holotype. China, ♀, Sichuan: Kangding,  $30.04^\circ\text{N}$ ,  $101.57^\circ\text{E}$ , 15.VI.2017, leg. Yanzhou Zhang (Hyp-2018-06, original number ZYZ-2017-28). Paratypes.  $1\text{♀}1\text{♂}$ , Sichuan: Kangding, 29.VI.2017, leg. Yanzhou Zhang (Hyp-2018-01, Hyp-2018-02, original number ZYZ-2017-08);  $1\text{♀}$ , Sichuan: Kangding, 2.VIII.2017, leg. Yanzhou Zhang (Hyp-2018-03, original number ZYZ-2017-20);  $1\text{♂}$ , Sichuan: Kangding, 29.VI.2017, leg. Yanzhou Zhang (Hyp-2018-08, original number ZYZ-2017-029).

**Etymology.** Named after the location of the type material.

**Hosts.** Unknown.

**Distribution.** China (Sichuan).

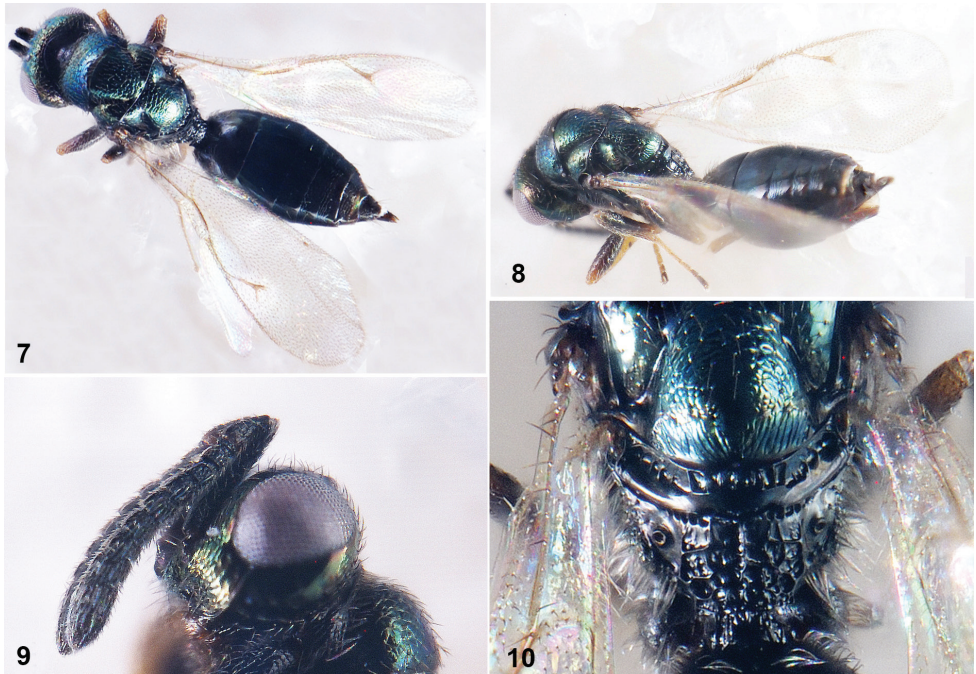
***Hyperimerus pusillus* (Walker, 1833)**

Figs 7–10

*Cyrtogaster pusilla* Walker, 1833: 383. Holotype female (BMNH No. 3300), examined.  
*Hyperimerus pusillus* (Walker): Graham 1969: 83; Bouček 1993: 1306; Huang and Xiao 2005: 281–282; Schender et al. 2014: 414–417.

*Mespilon exiguum* Graham, 1957: 406. Synonymized by Graham 1969: 83.

**Diagnosis.** Female. Body (Fig 7, 8) length approximately 1.5 mm, dark green. Head width  $1.32\times$  height in frontal view. Eyes height  $0.58\times$  head height; eyes separated by  $1.07\times$  their height. Gena with malar sulci distinct, malar space  $0.55\times$  eyes height. Antenna (Fig. 9) with scape reaching anterior ocellus; pedicel and flagellum combined  $1.2\times$  head width; pedicel broader than  $Fu_1$ , clava shorter than length of  $Fu_5 - Fu_7$  combined. Scutellum with frenum ca.  $1/3$  length of scutellum. Propodeum with areolate sculptures irregular and sparse, most of sculptures  $2\times$  as big as propodeal spiracles (Fig. 10). Marginal fringe of outer margin of fore wing shorter than length of uncus; marginal vein shorter than postmarginal vein ( $0.59\times$ ), postmarginal vein approximately  $1.85\times$  as long as stigmal vein. Petiole  $0.4\times$  as long as broad. Gaster  $1.91\times$  as long as broad; narrower than thorax, shorter than the length of head and mesosoma



**Figures 7–10.** *Hyperimerus pusillus* (Walker, 1833) **7** Body in dorsal view **8** Body in lateral view **9** Head and antenna in lateral view **10** Propodeum in dorsal view.

combined. Ovipositor sheaths slightly produced in dorsal view; gaster in lateral view, ovipositor sheaths 0.26× length of gaster, 0.44× length of hind tibia.

**Material examined.** China, 1♀2♂, Sichuan: Kangding, 15.VI.2017, leg. Yanzhou Zhang (Hyp-2018-07, original number ZYZ-2017-28; Hyp-2018-09, original number ZYZ-2017-29); 1♀, Sichuan: Kangding, 2.VIII. 2017, leg. Yanzhou Zhang (Hyp-2018-04, original number ZYZ-2017-21); 1♀, Sichuan: Kangding, 29.VI.2017, leg. Yanzhou Zhang (Hyp-2018-11, original number ZYZ-2017-42); 1♂, Tibet: Zhamo, 2700m, VII. 19.1978, leg. Fasheng Li.

**Hosts.** Parasitoids of *Psylla pyricola* (Ghahari et al., 2010), *Phenacoccus aceris*, *Psylla ulmi* (Dzhanokmen, 1978).

**Distribution.** China (Sichuan, Tibet), Holarctic, Oriental, and Neotropical regions.

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# Morphological and cytogenetic characteristics of *Neobisium (Blothrus) slovacum* Gulička, 1977 (Pseudoscorpiones, Neobisiidae), the northernmost troglobitic species of the subgenus *Blothrus* in Europe

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## Abstract

A redescription is provided of the adult, tritonymph and deutonymph life stages of the troglobitic *Neobisium (Blothrus) slovacum* Gulička, 1977, which is known from Slovakia and Hungary. Material examined included 35 previously deposited museum specimens and 15 newly collected specimens. In addition, the karyotype and distribution of 18S rDNA clusters are described, using fluorescence in situ hybridization (FISH). The male karyotype of *N. slovacum* comprises 69 chromosomes, with a predominance of biarmed chromosomes, and an XO sex chromosome system. Two pairs of signals for 18S rDNA on biarmed chromosomes (submetacentric and metacentric) of different sizes were identified. The present study provides the first information about the distribution of these clusters in the arachnid order Pseudoscorpiones. The geographic distribution of the species is summarized and mapped. *Neobisium slovacum* is endemic to the Slovak and Aggtelek Karst area in southern Slovakia and north-western

Hungary, where it has been recorded from 16 caves. One of these, Hačavská cave (in Slovakia), is the northernmost locality known for any species of the subgenus *Blothrus*.

### Keywords

Distribution, endemic, FISH, sex chromosome, Slovak Karst, troglobitic, 18S rDNA

### Introduction

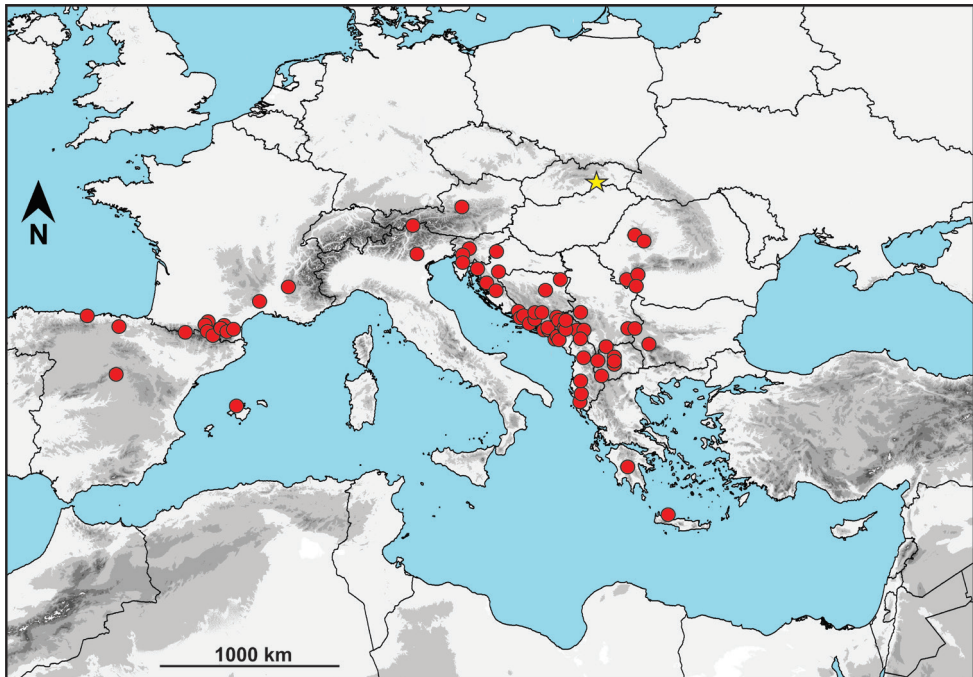
In Europe, troglobitic pseudoscorpions of the genus *Neobisium* Chamberlin, 1930 occur in four subgenera: *Blothrus* Schiödte, 1847, *Ommatoblothrus* Beier, 1956, *Heoblothrus* Beier, 1963 and *Pennobisium* Čurčić, 1988. Of these, *Blothrus* is the most frequent genus in European caves (Ducháč and Mlejnek 2000a), and includes about 90 species (Harvey 2013). The majority of these species are distributed in southern Europe (Harvey 2013; Fig. 1), with only six species occurring in the Carpathian Mountains (Novák 2014).

In Slovakia, the sole species of the subgenus *Blothrus* is *Neobisium slovacum* Gulička, 1977, which has clear troglomorphic characters (Gulička 1977a, b). This species is endemic to the Western Carpathians (Gulička 1977a, Ducháč and Mlejnek 2000a), with a distribution limited to the Slovak and Aggtelek Karst, a well-known karst area on the border between Slovakia and Hungary. This species was first collected by Lysenko in 1966 in the Diviacia chasm (Lysenko 1972), but it was not identified at that time. The specimens were rediscovered in Dr P.H. Verner's collection thirty years later and correctly assigned to this species by Ducháč (Ducháč and Mlejnek 2000a, b).

*Neobisium slovacum* is a strictly troglobitic species, with specific morphological adaptations to the cave environment. Compared to epigean relatives, the body of *N. slovacum* is pale, the legs and pedipalps are elongated, and eyes are absent. The remarkably elongated appendages provide an advantage in locating and capturing prey in the cave environment. The specimens are usually found on walls, in stony debris with clay sediments and near organic material (bat guano) in caves (Kováč et al. 2010).

The species was originally described from Stará brzotínska cave by Gulička (1977a), based on an adult holotype (sex not indicated) and two paratype nymphs. The description of the type material was incomplete because it omitted many important features necessary for proper species delimitation and identification. The depository of the type material is not known. Ducháč (1996) examined an additional specimen from the type locality and added some characteristics that were not reported in the original description. However, some morphological features useful for identification remained unknown. Ducháč (2002) summarized all previous collections of *N. slovacum* in his Ph.D. thesis, which contained descriptions of 53 adults, two tritonymphs and one deutonymph. However, these were still incomplete, with characters such as the chaetotaxy of sternites, morphometric data of legs I and IV, and the number of teeth on the cheliceral and palpal fingers not being specified.

Later, Ducháč (2004a, b) described in detail the male genitalia and the chaetotaxy of the genital opercula of *N. slovacum* and provided partial morphological descriptions of deutonymphs and tritonymphs, but the drawings and measurements were inadvert-

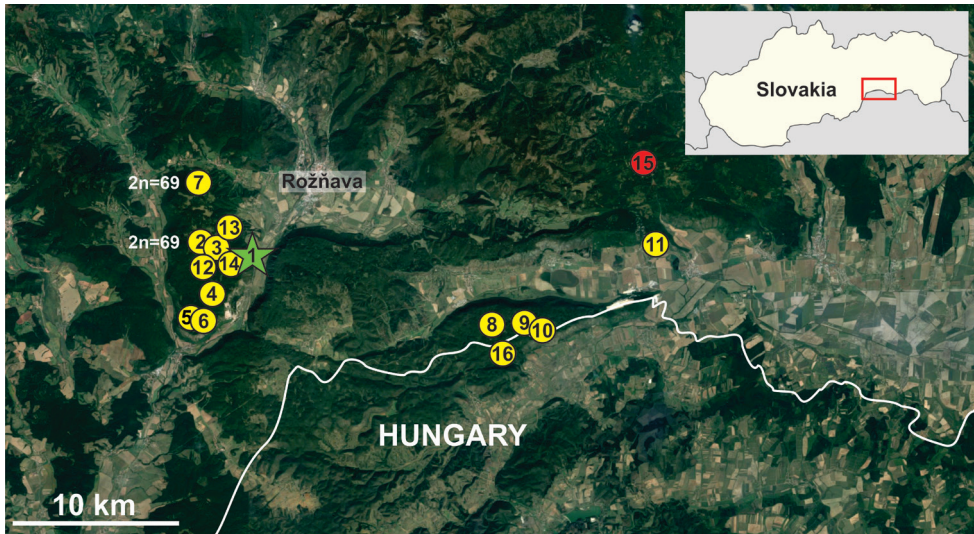


**Figure 1.** Distribution of the subgenus *Blothrus* in Europe. Red circles represent type localities of species. Yellow star indicates the northernmost occurrence of the subgenus in Slovakia (*Neobisium slovacum*).

ently omitted by the journal. Intraspecific variability of *N. slovacum* from the Slovak Karst was discussed by Ducháč (1999), who compared it with all Carpathian species of the subgenus *Blothrus*.

After Stará brzotínska cave, the species was found at 14 other localities in the Slovak Karst (Fig. 2, Table 1). Of these, Šingliarova chasm was the northernmost locality for the species, as well as for subgenus. Outside Slovakia, *N. slovacum* has been recorded from Meteor cave in the Aggtelek Karst in Hungary (Ducháč and Mlejnek 2000b). The distribution of *N. slovacum* in caves of the Slovak and Aggtelek Karst was discussed by Ducháč and Mlejnek (2000a, b). More recent findings of *N. slovacum* have been published by Vlk (2001) from Natrhnutá chasm, by Christophoryová (2009) from Šingliarova chasm, and by Kováč et al. (2010) from Slničná, Vlčia and Velká Peňažnica chasms.

Although *N. slovacum* seems to be restricted to the cave systems of the Slovak and Aggtelek Karst area, the possibility exists that the isolation of populations in separate cave systems could have led to diversification and cryptic speciation in this region. The detection of cryptic species diversity has recently been based mainly on the analysis of mitochondrial and nuclear gene sequences in terrestrial (e.g. Opatova and Arnedo 2014) and subterranean (e.g. Harrison et al. 2014) arachnid groups. However, cytogenetic techniques can also be useful for the identification of cryptic species within various orders of arachnids, such as harvestmen (Štáhlavský et al. 2018a), spiders (Řezáč



**Figure 2.** Distribution of *Neobisium slovacum* in karst in Slovakia and Hungary. Green star indicates the type locality and red spot the northernmost locality of the subgenus *Blothrus*. The karyotype is recorded for two localities. Localities numbered as in Table 1.

et al. 2018), scorpions (Štáhlavský et al. 2018b) and pseudoscorpions (Zaragoza and Štáhlavský 2008). This is especially notable in some species of edaphic pseudoscorpions characterized by limited dispersal ability, for which distinct karyotypes may be found even in very close localities (Kotrbová et al. 2016). It is therefore useful to combine descriptions the external morphology with karyotype analysis (Plíšková et al. 2016), as is done here.

Summarizing the previously published data, 61 adults and six nymphs have been collected from Slovakia. Despite this, knowledge of the morphology of *N. slovacum* remained incomplete. All previous descriptions were deficient in important details. Consequently, the aims of the present study are to: (1) complete and examine previously studied material of *N. slovacum*, (2) describe newly collected specimens and provide additional information on the variability of morphometric and morphological characters, (3) analyse the *N. slovacum* karyotype and (4) analyse the distribution of *N. slovacum* in caves of the Western Carpathians.

## Methods

To locate previously studied material of *N. slovacum*, the natural history museums in Vienna, Bratislava and Berlin, and the zoology departments of Charles University in Prague and Comenius University in Bratislava were contacted. In total, 35 specimens from eight localities in the Slovak Karst were obtained, including one specimen from the type locality. These specimens have been deposited in the Department of Zoology of Charles University in Prague and the Natural History Museum in Vienna

**Table 1.** List of collection localities of *Neobisium slovacum* in the Slovak Karst area (1–15 Slovakia; 16 Hungary). Abbreviations: a.s.l., above sea level; N, latitude; W, longitude (see map, Fig. 2).

Code	Locality	N	W	m a.s.l.
1	Stará brzotínska cave (type locality)	48°36'32"	20°28'15"	258
2	Zvonivá chasm	48°37'04"	20°25'33"	675
3	Zombor chasm	48°36'48"	20°26'38"	645
4	Diviacia chasm	48°35'02"	20°26'30"	597
5	Čikova diera cave	48°34'14"	20°24'50"	526
6	Fialová cave	48°34'12"	20°24'55"	544
7	Šingliarova chasm	48°39'21"	20°25'04"	677
8	Obrovská chasm	48°34'04"	20°41'05"	535
9	Pri salaši 2 chasm	48°34'21"	20°43'20"	543
10	Natrhnutá chasm	48°34'00"	20°43'56"	508
11	Erňa cave	48°36'56"	20°50'36"	410
12	Slnečná chasm	48°35'60"	20°24'83"	564
13	Vlčia chasm	48°36'89"	20°27'05"	660
14	Veľká Peňažnica chasm	48°35'83"	20°27'12"	667
15	Hačavská cave	48°39'50"	20°49'47"	795
16	Meteor cave	48°33'11"	20°42'26"	443

(NHMW). The recently collected specimens of *N. slovacum* (15 specimens) were obtained at three localities of the Slovak Karst: Šingliarova chasm, Hačavská cave and Zvonivá chasm. The new material from Hačavská cave and Šingliarova chasm was deposited in the Natural History Museum in Vienna (NHMW) and material from the Zvonivá chasm in the Department of Zoology, Charles University in Prague.

Recently collected specimens were identified using the identification keys in Beier (1963), Christophoryová et al. (2011) and Novák (2014). Morphological terminology follows Chamberlin (1931) and Judson (2007); nomenclature follows Harvey (2013). All specimens were studied as temporary slide mounts using lactic acid as the medium, then rinsed in water and returned to 70% ethanol. Morphological and morphometric analyses were performed using a Leica DM1000 compound microscope with ICC50 Camera Module (LAS EZ application, 1.8.0). Measurements (in mm) were taken from digital images using the AxioVision 40LE application. Reference points for measurements follow Chamberlin (1931).

### Cytogenetic analysis

One male from Zvonivá chasm (locality 2: Table 1 and Fig. 2) and two males from Šingliarova chasm (locality 7: Table 1 and Fig. 2) were used for the cytogenetic analysis.

The chromosomes were prepared by the “spreading” method described in Štáhlavský and Král (2004), slightly modified as noted below. The dissected gonads were hypotonized in 0.075 M KCl for 30 min and then fixed in a methanol:glacial acetic acid (3:1) solution for at least 20 min. The tissue was then dissociated in a drop of 60% acetic acid

on a clean microscope slide and the suspension was moved with tungsten needles on the surface until the fluid evaporated. The chromosomes were stained with 5% Giemsa solution in Sörensen phosphate buffer for 30 min. Chromosomes were documented using an Olympus IX81 inverted microscope equipped with a Hamamatsu ORCA-AG monochromatic camera. In total, ten metaphases II were measured using the LEVAN plugin (Sakamoto and Zacaro 2009) for IMAGEJ 1.47 software (<http://imagej.nih.gov/ij/>). For the identification of rDNA clusters, FISH with an 18S rDNA probe was used for all three males. This probe was prepared from the scorpion *Euscorpium sicanus* (Koch, 1837), as described by Štáhlavský et al. (2018a). The probe was labelled by PCR with biotin-14-dUTP (Roche) using a Nick Translation Kit (Abbott Molecular) following the manufacturer's guidelines. The FISH protocol was performed following Forman et al. (2013), on the same chromosome preparation used for the standard karyotype analysis after washing Giemsa in methanol:glacial acetic acid (3:1) solution. During the FISH procedure, the slides were treated with RNase A (200 µg/ml in 2× saline-sodium citrate) (60 min, 37 °C). The chromosomes were then denatured in 70% formamide in 2× saline-sodium citrate (3 min 30 s, 68 °C). Finally, the probe mixture (20 ng of probe, 25 ng of salmon sperm DNA (Sigma Aldrich), 10 µl of 50% formamide, 10% dextran sulphate in 2× saline-sodium citrate) was applied to each slide and hybridization was performed overnight in a black box at 37 °C. The following day, the probe was detected with Cy3-conjugated streptavidin. The signal was intensified by application of biotinylated anti-streptavidin and Cy3-conjugated streptavidin. Chromosomes were counterstained with DAPI (Fluoroshield, Sigma-Aldrich) and documented using an Olympus IX81 microscope equipped with a Hamamatsu ORCA-AG monochromatic CCD camera. The images were pseudocoloured (red for Cy3 and blue for DAPI) and superimposed with Cell^R software (Olympus Soft Imaging Solutions GmbH).

## Results

### *Neobisium (Blothrus) slovacum* Gulička, 1977

Figures 3–5

*Neobisium (Blothrus) slovacum* Gulička, 1977a: 6–8, figs 1–4; Gulička 1977b: 24, fig. 1; Harvey 1991: 383; Ducháč 1996: 154–157, figs 1–9; Ducháč 1999: 176–179, figs 1–2, 6–8; Ducháč and Mlejnek 2000a: 48–49, figs 1–2; Ducháč and Mlejnek 2000b: 19–20, figs 1–2; Ducháč 2004a: 53–54, figs 1–2; Ducháč 2004b: figs 1–10, table 1; Kárpáthe gyi 2007: 86; Christophoryová et al. 2011: 36, fig. 3D; Novák 2014: 394–400.

**Diagnosis (adults).** *Neobisium slovacum* is an eyeless, troglobitic species that differs from other Carpathian species of the subgenus *Blothrus* in following combination of characters: posterior margin of carapace usually with 4 setae; a subocular seta usually present (sometimes missing on one side); epistome absent; cheliceral hand with 5 setae; anterolateral process of coxa I long, broad and apically pointed, medial process



**Figure 3.** Male of *Neobisium slovacum* from Hačavská cave. Scale line: 1 mm.

prominent, rounded, with strong denticles; palpal trochanter without tubercles; palpal femur ratio in the range 5.69–8.81 mm; fixed palpal finger with unequally long teeth; telotarsus IV with 2 long tactile setae. Chromosomes of male  $2n = 69$ .

**Type locality.** Slovakia, Slovak Karst, Stará brzotínska cave (48°36'32"N; 20°28'15"E, 258 m a.s.l., length 120 m).

**Holotype.** Adult of undetermined sex collected from stony debris on clay sediment on 26 September 1974. Apparently lost.

**Paratypes.** 2 nymphs, from type locality, collected on 10 August 1975. Apparently lost.

**Note.** The depository of the type material is unknown. Searches for the holotype and paratypes in various institutions (listed in Methods section) were unsuccessful.

**Material examined** (see Table 1 for coordinates and altitudes of localities):

**Previously studied material.** **Čikova diera cave** (length 60 m, depth 26 m): 1 ♀ (det. V. Ducháč), 21 February–13 March 1988, leg. R. Mlejnek; 1 ♂ (det. V. Ducháč), 12 May 1988, leg. R. Mlejnek; 1 ♀ (det. V. Ducháč), 9 June 1989, leg. R. Mlejnek; **Diviačia chasm** (length 468 m, depth 127 m): 1 ♂, 2 ♀ (det. V. Ducháč), 1966, leg. V. Lysenko; **Erňa cave** (length 60 m, depth 10 m): 2 ♀, 1 specimen represented by appendages only, sex unknown (det. V. Ducháč), 20 July 1999, leg. R. Mlejnek; **Fialová cave** (length 21 m, depth 5.5 m): 1 ♀ (det. V. Ducháč), 15 April 1989, leg. R. Mlejnek; 1 ♂ (det. V. Ducháč), 23 September 1989, leg. R. Mlejnek; 1 ♂, 1 ♀, 1 tritonymph (det. V. Ducháč), 17 January 1998, leg. R. Mlejnek; 1 ♂ (det. V. Ducháč), 11 May 1998, leg. R. Mlejnek; **Obrovská chasm** (depth 100 m): 1 ♂ (det. V. Ducháč), 8 May 1988, leg. R. Mlejnek; **Pri salaši 2 chasm** (length 50 m, depth 36 m): 1 specimen represented by appendages only (det. V. Ducháč), 8 May 1988, leg. R. Mlejnek; **Stará brzotínska cave** (length 120 m): 1 ♀ (NHMW 28661) (det. V. Ducháč), 6 June 1982, leg. P. Moravec; **Šingliarova chasm** (length 140 m, depth 72 m): 3 ♂, 1 ♀ and 1 ♀ damaged badly (det. V. Ducháč), 12 May 1988, leg. R. Mlejnek; 1 ♂ (det. V. Ducháč), 7 June–19 October 1998, leg. R. Mlejnek; 3 ♂, 3 ♀, 2 specimens (sex unknown) represented by appendages only (det. V. Ducháč), 16 May 1998, leg. R. Mlejnek; **Unknown locality**: 1 ♂ (NHMW 28664), 2 ♀ (NHMW 28664) (det. V. Ducháč), 4 March 2003, leg. R. Mlejnek, locality data missing.

**Remarks.** Faunistic data and descriptions of the 35 specimens listed above were provided by Ducháč (1996, 1999, 2002, 2004a, b) and Ducháč and Mlejnek (2000a, b). It was generally not possible to correlate specimens with an individual literature source, because only the numbers of individuals and variability of some characters were mentioned. Of these specimens, 27 were used in the present study to add information on previously ignored characters. The remaining eight specimens were not examined in detail, either because the locality was unknown or because they were not in a suitable condition.

**Newly obtained material.** **Hačavská cave** (length 200 m): 2 ♂, 1 ♀ (NHMW 28662); hand sampling, in stony debris with clay sediment, back part of the cave, 150 m in the cave from entrance, 3 April 2017, leg. V. Papáč; **Šingliarova chasm** (length 140 m, depth 72 m): 1 ♂, 2 ♀, 1 deutonymph (NHMW 28659), pitfall trapping, 11 October 2003, leg. A. Mock; 2 ♂, 1 ♀, hand sampling, 2 May 2007, leg. Ľ. Kováč; 2 ♂, 1 ♀ (NHMW 28660), hand sampling, on cave walls and rocks, Second Hall, 25 August 2012, leg. P. Luptáčík; **Zvonivá chasm** (length 494 m): 2 ♀, hand sampling, 5 May 2006, leg. Ľ. Kováč.

**Cytogenetic analysis.** **Šingliarova chasm**: 2 May 2007: 2 ♂, hand sampling, leg. Ľ. Kováč; **Zvonivá chasm**: 5 May 2006: 1 ♂, hand sampling, leg. Ľ. Kováč.

**Remarks.** The newly collected specimens are described here, except for those used in the cytogenetic analyse. The record of the four specimens (NHMW 28659) from Šingliarova chasm was previously published by Christophoryová (2009). Hačavská





**Figure 4.** Back part of Hačavská cave, a new locality for the occurrence of *N. slovacum* in the Slovak Karst (photograph: V. Papáč); northernmost known locality of a member of the subgenus. Indicated as a yellow star on map (Fig. 1).

cave represents a new locality for the species in Slovakia and is the northernmost locality of any member of the subgenus *Blotbrus* (Figs 2, 4). In total, 14 adults and one

**Table 2.** Morphometric data for males and females of *Neobisium slovacum* (measurements in mm). Abbreviations: M, median; Min, minimum; Max, maximum; N, number of individuals measured; SD, standard deviation;  $\bar{x}$ , arithmetic mean.

Characteristics	Males			Females		
	Min–Max	M/ $\bar{x}$ ±SD	N	Min–Max	M/ $\bar{x}$ ±SD	N
Body, length	2.80–3.97	3.54/3.18±1.05	11	3.00–3.90	3.38/3.42±0.30	14
Carapace, length	0.91–1.19	1.04/1.00±0.24	19	0.95–1.18	1.05/1.06±0.07	18
Carapace, posterior width	0.86–1.08	0.97/0.92±0.25	15	0.85–1.20	0.97/1.00±0.11	15
Carapace, length/posterior width ratio	0.98–1.13	1.07/1.01±0.28	15	0.92–1.24	1.08/1.08±0.09	14
Chelicera, length	0.61–0.78	0.69/0.66±0.16	19	0.67–0.80	0.69/0.71±0.04	18
Chelicera, width	0.29–0.40	0.36/0.34±0.08	20	0.35–0.43	0.37/0.38±0.02	19
Chelicera, length/width ratio	1.79–2.06	1.92/1.82±0.43	19	1.74–1.95	1.89/1.87±0.06	18
Chelicerall movable finger, length	0.37–0.49	0.44/0.42±0.10	20	0.42–0.50	0.46/0.46±0.02	19
Palpal trochanter, length	0.65–0.82	0.72/0.70±0.17	19	0.66–0.84	0.74/0.74±0.05	19
Palpal trochanter, width	0.20–0.35	0.28/0.26±0.07	20	0.25–0.35	0.29/0.29±0.03	20
Palpal trochanter, length/width ratio	2.03–3.09	2.58/2.50±0.65	19	2.09–2.89	2.50/2.54±0.18	19
Palpal femur, length	1.66–1.93	1.76/1.67±0.41	18	1.52–1.85	1.74/1.73±0.08	20
Palpal femur, width	0.20–0.27	0.22/0.21±0.05	20	0.19–0.32	0.23/0.23±0.03	20
Palpal femur, length/width ratio	6.52–8.81	7.96/7.38±1.87	18	5.69–8.74	7.65/7.60±0.74	20
Palpal patella, length	1.46–1.67	1.56/1.49±0.37	18	1.41–1.61	1.49/1.51±0.06	19
Palpal patella, width	0.20–0.30	0.24/0.24±0.06	20	0.22–0.32	0.26/0.26±0.03	20
Palpal patella, length/width ratio	5.20–6.75	6.17/5.79±1.48	18	5.00–6.83	5.88/5.82±0.53	19
Palpal hand, length with pedicel	1.01–1.32	1.17/1.12±0.27	19	1.05–1.25	1.15/1.16±0.05	19
Palpal hand, length without pedicel	1.01–1.14	1.06/1.01±0.26	17	0.90–1.14	1.04/1.04±0.06	19
Palpal hand, width	0.33–0.47	0.40/0.38±0.09	20	0.36–0.52	0.41/0.42±0.05	19
Palpal hand, length with pedicel/width ratio	2.43–3.40	2.95/2.82±0.70	19	2.21–3.19	2.80/2.76±0.26	18
Palpal finger, length	1.81–2.09	1.92/1.82±0.45	18	1.81–2.06	1.91/1.92±0.08	18
Palpal finger, length/palpal hand length with pedicel	1.52–1.69	1.61/1.53±0.37	18	1.59–1.77	1.66/1.66±0.05	16
Palpal chela, length	2.57–3.19	2.91/2.77±0.67	19	2.73–3.21	2.91/2.93±0.14	17
Palpal chela, width	0.33–0.47	0.40/0.38±0.09	20	0.36–0.52	0.41/0.42±0.05	17
Palpal chela, length/width ratio	6.43–7.94	7.33/6.97±1.70	19	5.67–8.08	7.04/6.99±0.63	15
Leg I trochanter, length	0.30–0.38	0.34/0.32±0.08	18	0.28–0.37	0.33/0.33±0.03	18
Leg I trochanter, depth	0.18–0.23	0.20/0.19±0.05	18	0.17–0.24	0.21/0.21±0.02	18
Leg I trochanter, length/depth ratio	1.48–1.79	1.60/1.53±0.39	17	1.33–1.94	1.55/1.62±0.17	17
Leg I femur, length	0.88–1.02	0.94/0.89±0.23	17	0.75–1.03	0.90/0.91±0.08	17
Leg I femur, depth	0.09–0.14	0.11/0.11±0.03	18	0.10–0.15	0.11/0.12±0.02	17
Leg I femur, length/depth ratio	6.79–9.78	8.18/7.83±2.10	17	6.67–9.36	7.70/7.84±0.79	17
Leg I patella, length	0.50–0.71	0.63/0.60±0.15	19	0.56–0.69	0.62/0.62±0.04	18
Leg I patella, depth	0.10–0.15	0.12/0.11±0.03	19	0.11–0.16	0.12/0.12±0.01	16
Leg I patella, length/depth ratio	4.20–6.80	5.32/5.11±1.38	19	4.00–6.00	5.12/5.07±0.53	16
Leg I tibia, length	0.64–0.82	0.73/0.69±0.17	18	0.65–0.77	0.72/0.71±0.04	19
Leg I tibia, depth	0.08–0.10	0.09/0.09±0.02	18	0.08–0.11	0.09/0.09±0.01	19
Leg I tibia, length/depth ratio	7.33–9.63	7.95/7.67±1.99	17	6.50–9.38	7.67/7.90±0.84	19
Leg I basitarsus, length	0.40–0.49	0.45/0.42±0.11	17	0.37–0.47	0.43/0.43±0.03	20
Leg I basitarsus, depth	0.06–0.09	0.08/0.08±0.02	19	0.06–0.10	0.09/0.08±0.01	20
Leg I basitarsus, length/depth ratio	4.89–7.50	5.13/5.11±1.42	17	4.44–7.83	4.89/5.15±0.78	20

Leg I telotarsus, length	0.48–0.63	0.55/0.53±0.13	18	0.40–0.62	0.56/0.55±0.05	18
Leg I telotarsus, depth	0.07–0.10	0.08/0.08±0.02	18	0.07–0.10	0.08/0.08±0.01	16
Leg I telotarsus, length/depth ratio	6.11–8.43	6.87/6.49±1.73	17	5.00–8.86	6.75/6.66±1.04	16
Leg IV trochanter, length	0.50–0.63	0.55/0.52±0.13	17	0.49–0.65	0.55/0.56±0.04	19
Leg IV trochanter, depth	0.17–0.28	0.22/0.21±0.06	17	0.19–0.28	0.22/0.23±0.03	19
Leg IV trochanter, length/depth ratio	1.79–3.11	2.59/2.41±0.71	16	1.96–2.95	2.50/2.48±0.31	19
Leg IV femoropatella, length	1.44–1.75	1.62/1.51±0.40	16	1.40–1.75	1.58/1.56±0.08	17
Leg IV femoropatella, depth	0.18–0.30	0.23/0.22±0.06	17	0.21–0.31	0.24/0.24±0.03	16
Leg IV femoropatella, length/depth ratio	5.47–8.72	6.91/6.45±1.83	16	5.10–7.33	6.55/6.47±0.67	15
Leg IV tibia, length	1.40–1.64	1.49/1.41±0.36	17	1.32–1.56	1.43/1.44±0.08	17
Leg IV tibia, depth	0.12–0.17	0.13/0.13±0.03	19	0.11–0.16	0.13/0.13±0.01	18
Leg IV tibia, length/depth ratio	9.33–12.67	10.97/10.45±2.76	17	9.64–13.00	11.50/11.35±0.85	17
Leg IV basitarsus, length	0.51–0.64	0.56/0.54±0.14	17	0.49–0.60	0.56/0.55±0.03	18
Leg IV basitarsus, depth	0.10–0.13	0.11/0.10±0.03	19	0.09–0.13	0.11/0.11±0.01	18
Leg IV basitarsus, length/depth ratio	4.25–5.82	5.27/4.95±1.33	17	4.46–6.44	5.05/5.19±0.51	18
Leg IV telotarsus, length	0.81–0.93	0.87/0.82±0.21	16	0.74–0.96	0.85/0.85±0.06	17
Leg IV telotarsus, depth	0.09–0.11	0.10/0.10±0.03	17	0.09–0.13	0.11/0.10±0.01	18
Leg IV telotarsus, length/depth ratio	7.73–10.33	8.24/7.94±2.24	15	6.25–10.67	8.27/8.28±1.24	17

deutonymph were identified in the new material from Šingliarova chasm, Hačavská cave and Zvonivá chasm.

**Redescription.** In total, 40 adults, 1 tritonymph and 1 deutonymph were examined in the present study. Measurements of adults are given in Table 2.

**Adults.** Body yellowish; carapace, chelicerae and pedipalps light brown. Vestitural setae of body and pedipalps long and pointed. Carapace rectangular, without granulation, longer than broad, epistome and eyes absent, a subocular seta usually present (sometimes missing on one side). Tergites and sternites undivided. Chelicera with 5 setae on hand, 1 seta on movable finger, spinneret well developed in female, weak in male, rallum of 8 blades, 2 distal blades dentate. Anterolateral process of coxa I long, broad and apically pointed, medial process prominent, rounded, with strong denticles. Pedipalps slender, chelal fingers with normal number of trichobothria (8 on fixed and 4 on movable finger), sensillum  $p_1$  slightly distal of  $st$ ,  $p_2$  nearer to  $st$  than to  $sb$ , situated close to dental margin. Palpal trochanter without tubercles. Legs elongated, I–IV with 2 tarsal segments (basitarsus and telotarsus separated). Subterminal setae of telotarsi I and IV with short, smooth, ventral rami and small dorsal denticles in distal part. Telotarsus IV with 2 long tactile setae, telotarsus I without tactile seta. Claws with a small dorsal denticle about one-third from base.

**Males (20 specimens studied).** Chaetotaxy of carapace: total 16–21 setae, posterior margin mostly with 4 setae, exceptionally with 3 setae in 2 males and 5 setae in 1 male, anterior margin mostly with 4 setae, exceptionally with 3 setae in 2 males and 5 setae in 2 males. Chaetotaxy of tergites I–X: 4, 4, 4–6, 4–6, 4–7, 4–6, 5–7, 5–7, 6–7, 5–7. Chaetotaxy of sternites IV–X: 7–15, 8–12, 8–11, 8–11, 9–11, 8–10, 7–9. Anterior genital operculum with 9–19 setae, posterior genital operculum with 29–38 setae in total, comprised of 19–26 medial and 8–15 marginal setae. Chelicera: fixed finger with 12–22 and movable finger with 10–20 unequally long teeth. Pedipalp: fixed fin-

ger with 133–172 unequally long teeth and movable finger with 106–152 equally long teeth. See Table 2 for measurements.

**Females (20 specimens studied).** Chaetotaxy of carapace: total 16–20 setae, posterior margin mostly with 4 setae, exceptionally with 2 setae in 1 female, anterior margin mostly with 4 setae exceptionally with 5 setae in 2 females. Chaetotaxy of tergites I–X: 4–5, 4–5, 4–6, 4–6, 4–6, 5–6, 5–7, 5–8, 5–7, 5–7. Chaetotaxy of sternites IV–X: 6–13, 6–10, 8–11, 8–11, 8–12, 8–11, 7–9. Anterior genital operculum with 7–13 setae, posterior operculum with 12–18 setae. Chelicera: fixed finger with 14–20 and movable finger with 11–19 unequally long teeth. Pedipalp: fixed finger with 131–171 unequally long teeth and movable finger with 118–149 equally long teeth. See Table 2 for measurements.

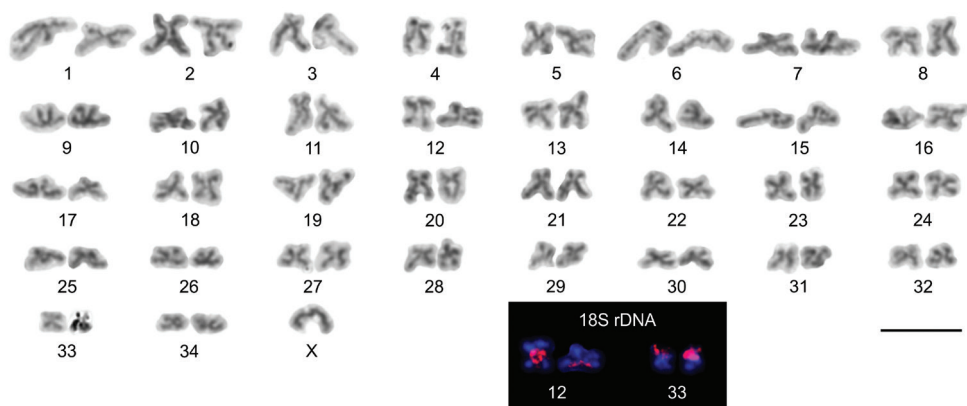
**Tritonymph (1 specimen studied).** With same general characteristics as adults. Chaetotaxy of carapace: total 17 setae, posterior and anterior margin with 4 setae each. Chaetotaxy of tergites I–X: 4, 5, 5, 4, 6, 6, 6, 6, 6, 6. Chaetotaxy of sternites IV–X: 8, 11, 10, 11, 10, 8, 8. Chelicera: rallum of 8 blades, 2 distal blades dentate, fixed finger with 14 and movable finger with 13 unequally long teeth. Pedipalps: chela with 7 trichobothria on fixed finger and 3 on movable finger; fixed finger with 112 equally long marginal teeth, movable finger with 97 equally long marginal teeth.

**Measurements of tritonymph.** Body length 3.43. Chelicera: 0.54/0.27 ( $\times 2.00$ ); movable finger length 0.35. Pedipalps: trochanter 0.53/0.23 ( $\times 2.30$ ), femur 1.10/0.19 ( $\times 5.79$ ), patella 0.96/0.20 ( $\times 4.80$ ), palpal hand 0.81/0.24 ( $\times 3.38$ ), hand length without pedicel 0.73, movable finger length 1.26, movable finger and hand length ratio 1.56, palpal chela 1.95/0.24 ( $\times 8.13$ ). Leg I: femur 0.59/0.10 ( $\times 5.90$ ), patella 0.39/0.10 ( $\times 3.90$ ), tibia 0.47/0.07 ( $\times 6.71$ ), basitarsus 0.27/0.08 ( $\times 3.38$ ), telotarsus 0.41/0.10 ( $\times 4.10$ ). Leg IV: trochanter 0.38/0.18 ( $\times 2.11$ ), femoropatella 0.96/0.18 ( $\times 5.33$ ), tibia 0.88/0.11 ( $\times 8.00$ ), basitarsus 0.35/0.10 ( $\times 3.50$ ), telotarsus 0.57/0.12 ( $\times 4.75$ ).

**Note.** A description of this tritonymph was previously published by Ducháč (2004b). Because of damage to the specimen, it was not possible to measure the carapace and the trochanter of leg I.

**Deutonymph (1 specimen studied).** With same general characteristics as adults. Chaetotaxy of carapace: total 19 setae, posterior margin with 5, anterior margin with 4 setae. Chaetotaxy of tergites I–X: 3, 4, 5, 6, 6, 6, 6, 6, 5. Chaetotaxy of sternites IV–X: 8, 8, 8, 8, 8, 8, 7. Chelicera: rallum of 6 blades, 2 distal blades dentate; fixed finger with 14 and movable finger with 11 unequal teeth. Pedipalps: chelal with 6 trichobothria on fixed finger and 2 on movable finger; fixed finger with 73 equally long teeth, movable finger with 66 equally long teeth.

**Measurements of deutonymph.** Body length 2.57. Carapace: 0.68/0.66 ( $\times 1.03$ ). Chelicera: 0.41/0.24 ( $\times 1.71$ ); movable finger length 0.26. Pedipalps: trochanter 0.38/0.19 ( $\times 2.00$ ), femur 0.76/0.15 ( $\times 5.07$ ), patella 0.62/0.20 ( $\times 3.10$ ), palpal hand 0.57/0.27 ( $\times 2.11$ ), hand length without pedicel 0.53, movable finger length 0.91, movable finger and hand length ratio 1.60, palpal chela 1.33/0.27 ( $\times 4.93$ ). Leg I: trochanter 0.20/0.13 ( $\times 1.54$ ), femur I 0.40/0.09 ( $\times 4.44$ ), patella 0.26/0.08 ( $\times 3.25$ ), tibia 0.29/0.07 ( $\times 4.14$ ), basitarsus 0.16/0.07 ( $\times 2.29$ ), telotarsus 0.27/0.08 ( $\times 3.38$ ). Leg



**Figure 5.** *Neobisium slovacum*, male karyotype from Šingliarova chasm after Giemsa staining. Inset (black box) shows chromosomes detected by FISH with 18S rDNA (red signal). Based on two sister metaphase II plates. Scale bar: 10  $\mu$ m.

IV: trochanter 0.24/0.16 ( $\times 1.50$ ), femoropatella 0.69/0.15 ( $\times 4.60$ ), tibia 0.54/0.09 ( $\times 6.00$ ), basitarsus 0.24/0.09 ( $\times 2.67$ ), telotarsus 0.39/0.09 ( $\times 4.33$ ).

**Karyotype.** The diploid complement of all three males analysed was 69 chromosomes (Fig. 5). The karyotype contains 19 pairs of metacentric (No. 2, 5, 8, 9, 13, 14, 16, 18, 19, 22, 23, 24, 26, 27, 29, 30, 32, 33, 34), ten pairs of submetacentric (No. 1, 4, 7, 10, 12, 17, 21, 25, 28, 31), three pairs of subtelocentric (No. 3, 11, 20) and two pairs of acrocentric (Nos. 6, 15) autosomes. The autosomes gradually decreased in length from 2.31% to 0.91% of the diploid set. An XO sex chromosome system was identified in this species. The X chromosome shows an acrocentric morphology and constitutes 1.13% of the diploid set. Two pairs of 18S rDNA clusters were detected by FISH in this species. The 18S rDNA probe signals were localized close to the centromeres on the long arm of submetacentric chromosome pair No. 12 and on the short arm of metacentric chromosome pair No. 33 (Fig. 5).

## Discussion

The original description of *N. slovacum* by Gulička (1977a) included only a few morphological and morphometric features, but it was sufficient for recognition of the species. The recently examined specimens fit well with this description, except for minor variability in the chaetotaxy of the tergites and measurements of chelicera.

Ducháč (2004b) provided descriptions of the deutonymph and tritonymph, but the figures and table of measurements are missing on the web site of the journal and no printed version of the paper is available. According to the editor of the journal (Dr Eduard Stloukal, pers. com.), the figures and table were never published.

The redescription of previously studied and newly obtained material in the present paper gives a better assessment of intraspecific variability and adds some previously

unstudied characters, such as the chaetotaxy of sternites, measurements of legs and the number of teeth on cheliceral and palpal fingers, presence of subocular setae, anterolateral and medial processes of coxa I, positions of chelal sensilla  $p_1$  and  $p_2$ , form of subterminal seta, tactile seta of legs I and IV and denticulation of leg claws. The individuals show great variability in some characters, such as palpal femur and trochanter length, palpal chela length/width ratio, femur I and telotarsus I length/depth ratio, tibia IV and telotarsus IV length/depth ratio, femoropatella IV length/depth ratio in males, number of teeth on chelal and palpal fingers, chaetotaxy of sternites, and chaetotaxy of the male genital operculum. However, no significant differences were observed in chaetotaxy or measurements between populations of different caves. The greatest variability was observed in the palpal femur length/width ratio, with a range of 5.69–8.81 mm. Novák (2014) separated *Blothrus* species of the Carpathian Mountains in couplet 1 of his identification key between those with a palpal femur ratio of up to 6 and those, including *N. slovacum*, with a ratio of 6.5 or more. According to our measurements, this couplet will not always be reliable for identifying *N. slovacum*.

Concerning the karyotype, no differences were observed between the three males analysed. It should be noted that the material used for cytogenetic analysis comes only from two localities (Fig. 2) near each other in the northern part of the Plešivecká Plateau (Slovak Karst). In this case, dispersion of individuals between caves and hence gene flow between populations cannot yet be excluded. The number of chromosomes in *N. slovacum* ( $2n = 69$ ) is similar to that of *Neobisium* (*Neobisium*) *carcinoides* (Hermann, 1804) ( $2n = 67$ ; Sokolow 1926) and falls within the known range ( $2n = 30–71$ ) reported in a preliminary analysis of species of this genus (Šťáhlavský et al. 2012b). Other cytogenetic features of *N. slovacum* are consistent with previous findings for pseudoscorpions. The chromosomes gradually decrease in length and their morphology is variable, which is typical for karyotypes of pseudoscorpions with higher number of chromosomes (Šťáhlavský et al. 2009, 2012a). The sex chromosome system is X0, which is assumed to be the ancestral state for pseudoscorpions (Troiano 1990). However, the morphology of the X chromosome is usually metacentric in this order (Šťáhlavský et al. 2012a, 2013, Kotrbová et al. 2016), whereas an acrocentric X was identified in *N. slovacum*. This morphology of the X chromosome was previously documented in only one population of *Olpium pallipes* (Lucas, 1846) (Olpiidae) within pseudoscorpions (Šťáhlavský et al. 2006). It was supposed to be an effect of pericentric inversion from Greece in the population concerned. This type of chromosomal rearrangement can also be presumed for *N. slovacum*, because all other neobisiids thus far karyotyped possess biarmed X chromosomes (Šťáhlavský et al. 2013). Interestingly, two pairs of 18S rDNA clusters were identified in *N. slovacum*, even though one pair is considered to be the ancestral state for arachnids (Forman et al. 2013). However, a multiplication of the 18S rDNA clusters seems to be frequent, at least in arachnids with limited dispersal ability (Svojanovská et al. 2016, Šťáhlavský et al. 2018a). In such groups the variability in the number and position of rDNA clusters suggests intensive chromosomal changes that may also be expected in *N. slovacum*, in view of its subterranean habitat. It should be noted that no information is available about the number

and position of rDNA clusters in any other pseudoscorpions, and the presence of two pairs of rDNA clusters might be typical for this order.

Summarizing all known distributional records, *N. slovacum* is currently known from 15 localities in the Slovakia and one locality in Hungary (Table 1 and Fig. 2). Based on this distribution, it can be expected to occur in the karst areas between the localities and near the border of the two countries. There are no valid records of species of the subgenus *Blothrus* in countries lying north of Slovakia in Europe or elsewhere. Beier (1936) described *Neobisium (Blothrus) vulpinum* Beier, 1936 from Krkonoše, western Sudetenland (a mountain range now within parts of the Czech Republic and Poland). However, Beier (1963) later stated that the locality details of the types were erroneous and synonymized this name with *N. (B.) minutum* (Tömösváry, 1883). The size of the caves and chasms occupied by *N. slovacum* range from small (Fialová cave, length 21 m; Vlčia chasm, length 29 m) to large (Zvonivá chasm, length 494 m; Diviačia chasm, length 468 m), situated at elevations between 258 and 795 m a.s.l. Air temperature in the caves, measured at the time of collection (using a digital thermometer), ranged from 5.4 °C (Zvonivá chasm) to 9.7 °C (Stará brzotínska cave). Air relative humidity during the collection of specimens was 94–100%.

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# A new species of *Erythrolamprus* from the oceanic island of Tobago (Squamata, Dipsadidae)

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## Abstract

Tobago is a small island on the southeast edge of the Caribbean Plate with a continental flora and fauna. Using DNA sequences from Genbank, new sequences, and morphological data from the snakes *Erythrolamprus epinephalus*, *E. melanotus*, *E. reginae*, and *E. zweifeli*, the species status of specimens of a Tobago snake previously considered to be *Erythrolamprus reginae* was assessed. *Erythrolamprus zweifeli*, long considered a subspecies of *E. reginae*, was found to be a northern Venezuela-Trinidad endemic and the sister to *E. reginae*. The trans-Andean species *E. epinephalus* is shown to be non-monophyletic while the Costa Rican lineage of *E. epinephalus* is weakly supported as the sister to the Tobago population. The Tobago *Erythrolamprus* is described as a distinct taxon based upon five specimens from four localities in lower montane rainforest. Much of the new species range includes the Main Ridge Forest Reserve of Tobago, the

oldest protected forest in the Western Hemisphere. All known locations fall within a 400-ha area, and its total geographic distribution is likely to be less than 4,566 ha. The restricted distribution of this new snake makes it a likely candidate for threatened status. The new species also becomes another biogeographic link between northern Venezuela and Tobago.

### Keywords

cryptic species, evolutionary species concept, lowland montane rainforest, sky islands, systematics

### Introduction

The Cordillera de Costa (CC) is a sky island archipelago that extends 925 km in an east-west orientation from western Venezuela, across the Northern Range of Trinidad to the island of Tobago. The CC is separated from the Andes by the Yaracuy River depression, and in the east, the CC is separated from the Guyana shield by the Llanos grasslands. The Gulf of Paria separates the Peninsula de Paria from Trinidad, and Trinidad is separated by 35 km of open water from Tobago. The CC formed between the late Cretaceous and the Miocene (Sisson et al. 2005). Rising and falling sea levels, marine incursions, changing climates, and tectonic events have continually remodeled the landscape isolating and reconnecting populations of organisms.

Tobago is at the eastern edge of the CC sky island complex and is slightly more than 300 km<sup>2</sup>, and its highest peak is about 576 m above sea level (ASL). The island has two physiographic regions: a flat coastal plain composed of a coral terrace in the southwest and the Main Ridge, a mass of metamorphic and igneous rocks, covered by dense tropical forest. The Main Ridge runs in a northeast-southwest direction.

Tobago's snake fauna contains 23 species, and eleven of these belong to the Dipsadidae clade. Molecular studies on the Western Hemisphere snake clade Dipsadidae (or Dipsadinae) (Zaher et al. 2009; Vidal et al. 2010; Grazziotin et al. 2012) suggest *Erythrolamprus* Boie 1826 is not monophyletic unless most of the snakes formerly placed in the genera *Liophis* Wagler, 1830, *Leimadophis* Fitzinger, 1843, and *Umbrivaga* Roze, 1964 are included. This action increased the number of *Erythrolamprus* species from six, mostly coral snake mimics, to 50 species (Uetz and Jacob 2018) with a variety of color patterns and habits. Thus, *Erythrolamprus* became one of the most species-rich genera of Neotropical snakes. This arrangement has not been accepted by everyone (Wallach et al. 2014). Here we consider the genus *Erythrolamprus* in the broadest sense, including the species traditionally allocated to *Leimadophis*, *Liophis*, and *Umbrivaga*, acknowledging that future taxonomic changes are likely.

There is no known synapomorphy for the genus *Erythrolamprus* (Myers 2011). That said, members of the genus are usually less than 1.6 m in total length; nine scales are normally present on the crown; the number of dorsal scale rows is 15–19 and in some species they are reduced once, in others, they may be reduced twice posteriorly; apical pits may be present or absent on some or all of the scales; ventral counts range from 129–212; subcaudal counts range from 38–106; the temporal formula is usually 1+2; the preocular is usually single; the postoculars are usually two; upper labials are usually eight; lower labials are usually ten, and two pair of enlarged chin shields are present.

*Erythrolamprus* ranges from Costa Rica southward to Argentina and occurs on both sides of the Andes as well as in the Lesser Antilles. Some taxa reach an elevation of 3,500 m ASL. Members of the genus have life styles that range from fossorial to terrestrial to semi-aquatic in habitats spanning rainforests, savannas, and páramo (Savage 2002).

### The genus *Erythrolamprus* in the Cordillera de la Costa

Eighteen species of *Erythrolamprus* occur in northern Venezuela, of these, two are Pantepui species: *E. trebbau* (Roze 1958a), *E. ingeri* (Roze 1958b). The remaining 16 species are associated with the CC either as montane species, lowland species, or species that are not restricted by elevation. Eight of the 16 species occur on the Guyana Shield and seven species of *Erythrolamprus* are recognized on Trinidad and Tobago: *E. aesculapii* (Linnaeus, 1758); *E. bizona* Jan 1863; *E. cobellus* (Linnaeus, 1758); *E. melanotus* (Shaw, 1802), *E. ocellatus* Peters, 1868; *E. zweifeli* (Roze, 1959); and *E. reginae* (Linnaeus, 1758) (Murphy et al. 2018). Both *E. aesculapii* and *E. bizona* are coral snake mimics, and each is known from a single specimen from Trinidad (but better known from elsewhere in their ranges).

*Erythrolamprus ocellatus* is a Tobago endemic, with a bright red dorsum and black ocelli, and is best considered an imperfect coral snake mimic, keeping in mind that there are no extant species of coral snakes on Tobago (Hodson and Lehtinen 2017). *Erythrolamprus cobellus* is a semi-aquatic, lowland species; while *E. melanotus* and *E. zweifeli* are forest species often associated with stream-edge habitats and mountains from sea level to at least 2,000 m. However, in Venezuela, *E. zweifeli* is usually associated with montane environments.

Noting significant differences in coloration, as well as distinct ventral and subcaudal counts from *E. reginae*, Rivas et al. (2012) returned *Erythrolamprus reginae zweifeli* to species status. They noted *E. zweifeli* differs from *E. reginae* in having a salt-and-pepper dorsal pattern or a more uniform olive-green or olive-brown pattern. Wallach et al. (2014) concurred and recognized the elevation of *zweifeli* to species level. *E. reginae* has a dorsum with dense pale and dark paravertebral flecking. The two species also differ in subcaudal counts (69–88 in *E. zweifeli* as opposed to 55–78 in *E. reginae*) with the ranges overlapping, but different means. Following this arrangement, *E. zweifeli* occurs throughout the Cordillera de Mérida and the CC in Venezuela, including Trinidad.

### Natural history of Trinidad and Tobago's *Erythrolamprus*

There are some ecological differences between the Trinidad and Tobago *Erythrolamprus*. The two poorly known coral snake mimics (*E. aesculapii*, *E. bizona*) are forest dwellers and snake predators (Campbell and Lamar 2004). Dietary differences between the two better known forest and forest-edge species are apparent. *Erythrolamprus melanotus* feeds on the microteiid lizards in the genus *Bachia*, the rain frog *Pristimantis urichi*, the puddle frog *Engystomops pustulosus* and the gecko *Gonatodes vittatus*, and unidenti-

fied fish have been reported. *Erythrolamprus zweifeli* feeds on stream frogs of the genus *Mannophryne*, hylid frogs, *Leptodactylus validus*, salamanders, lizards of the genus *Ameiva*, and small birds (Michaud and Dixon 1989; Murphy 1997; Esqueda et al. 2009). While the diets overlap the presence of *Bachia*, *Gonatodes*, and *Pristimantis urichi* in the diet of *E. melanotus* suggest it is hunting in more terrestrial situations in forests or at forest edges. *Mannophryne* in the diet of *E. zweifeli* suggests it is hunting along forested stream-edges. It supports the fact that *E. zweifeli* was the most common snake encountered during a study in a canal system used for water collection from a mountain stream located in Naiguatá, Venezuela (Silva et al. 1985; Silva and Valdez 1989).

*Erythrolamprus epinephalus* (Cope, 1862) is widespread and polytypic, ranging from Costa Rica to Ecuador, Colombia, and Venezuela and has not been previously associated with Trinidad or Tobago. The examination of a single specimen (USNM 22069) from Tobago led Dixon (1983b) to conclude that it was *Liophis* (= *Erythrolamprus*) *reginae* with an atypical color pattern that resembled a *Liophis* (= *Erythrolamprus*) *epinephalus* population from eastern Colombia. Dixon's remark was the only mention of *E. epinephalus* associated with Trinidad and Tobago. He noted the most striking difference in the Tobago animal was a dorsolateral tan stripe not present in the Trinidadian *E. zweifeli*.

Here, we examine the genetic divergence and morphology of a Tobago snake, previously considered part of the *E. reginae* group, in an attempt to understand its phylogenetic relationship to other *Erythrolamprus* and the biogeography in northeastern South America.

## Materials and methods

Museum material examined (Appendix 1) included 105 specimens of five *Erythrolamprus* species. Snakes were examined for external morphological data; scale nomenclature follows Dixon (1983a, b). Museum acronyms are as follows:

<b>AMNH</b>	American Museum of Natural History
<b>FMNH</b>	Field Museum of Natural History
<b>EBRG</b>	Museo de la Estación Biológica de Rancho Grande
<b>UMMZ</b>	University of Michigan Museum of Zoology
<b>USNM</b>	National Museum of Natural History
<b>UWIZM</b>	University of the West Indies Zoology Museum
<b>MBLUZ</b>	Museo de Biología, Universidad del Zulia
<b>MCNC</b>	Museo de Ciencias Naturales, Caracas

Locality data was converted into coordinates using Google Earth. Measurements of the body and tail lengths were taken to the nearest millimeter; ventral scale count methods follow Dowling (1951). The anal plate and terminal scutes were not included in the number of ventrals or subcaudals. The dorsal scale row counts were made about ten ventrals behind the head, at mid-body, and about ten ventrals anterior to the vent. Values for paired head scales are given in left/right order. Scales were measured to the

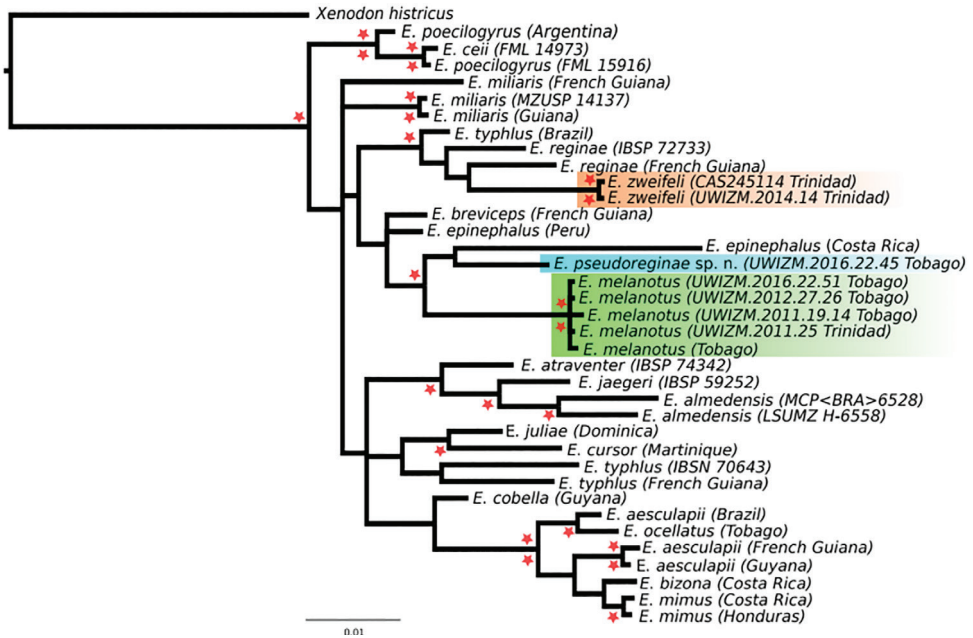
nearest 0.1 mm with the aid of a digital caliper and dissection microscope. Total length (TTL) and tail length (TL) measurements were taken to the nearest mm by carefully stretching the specimens along a ruler or placing a measuring tape along the length of the animal (Appendix 2). Statistical analyses were done with Excel-QI Macros ( $\alpha = 0.05$ ). Ventral and subcaudal counts were compared using ANOVA (Appendix 3).

DNA extraction, purification, and amplification protocols follow Jowers et al. (2013). Two mitochondrial gene fragments, 12S rDNA (primers 12Sa and 12Sb; Kocher et al. 1989), 16S rDNA (primers 16SL and 16SH; Palumbi 1996) and a nuclear gene fragment, *c-mos* (primers G73 and G74; Saint et al. 1998) were amplified. The lengths of the sequences were: 12S rDNA, 343 base pairs (bp); 16S rDNA, 425 bp; *c-mos*, 564 bp. We sequenced four *Erythrolamprus melanotus* (GenBank accession numbers are shown in Appendix 4) from Trinidad ( $n = 1$ ), Tobago ( $n = 3$ ), two *E. zweifeli* from Trinidad, and a new undescribed *Erythrolamprus* sp. from Tobago. We downloaded all *Erythrolamprus* sequences for the same loci from Genbank and used *Xenodon histricus* as the outgroup (Hodson and Lehtinen 2017).

Seaview v.4.2.11 (Gouy 2010) was used for preliminary alignments of sequences and were aligned thereafter in MAFFT (Kato et al. 2002), and phylogenetic analyses were conducted using the concatenated mitochondrial and nuclear (12S+16S rDNA+*c-mos*) alignment (with a length of 1332 bp) using a partitioned model of substitution by gene fragment. The most appropriate substitution model for each gene partition was determined by the Bayesian Information Criterion (BIC) in PartitionFinder v.2 (Lanfear 2012). The best-fitting models for the ribosomal and *c-mos* fragments were as follows: 12S rDNA + 16S rDNA (TRN+I+G), *c-mos* first and second codon positions (TrNef+I) and *c-mos* third codon position (HKY). Phylogenetic relationships between taxa were inferred using the Bayesian Inference (BI) optimality criterion under the best fitting substitution model for each gene partition. MrBayes Huelsenbeck et al. (2001) was used with default priors and Markov chain settings, and with random starting trees. Each run consisted of four chains of 30 million generations, sampled every 1,000 generations. Runs were evaluated for convergence and mixing by observing and comparing traces of each parameter in Tracer v.1.6 (<http://beast.bio.ed.ac.uk/tracer>) (Rambaut et al. 2014). We considered effective sampling size (ESS) values > 200 to be good indicators of parameter mixing. Phylogenetic relationships (Figure 1) were also estimated using a Maximum Likelihood (ML) approach, as implemented in the software RAxML v7.0.4 (Silvestro and Michalak 2010), under the best partition scheme under the GTR model. All analyses were performed using the CIPRES platform (Miller et al. 2010). *P*-uncorrected distances were calculated in MEGA V7 (Kumar et al. 2016) under complete deletion of gaps and missing data.

## Molecular results

Runs showed high Effective Sample Size convergence (> 2300), indicating adequate sampling of the posterior distribution. The *p*-uncorrected distances between *L. epinephalus* from Costa Rica and *E. sp.* from Tobago were the highest of all terminal monophyletic



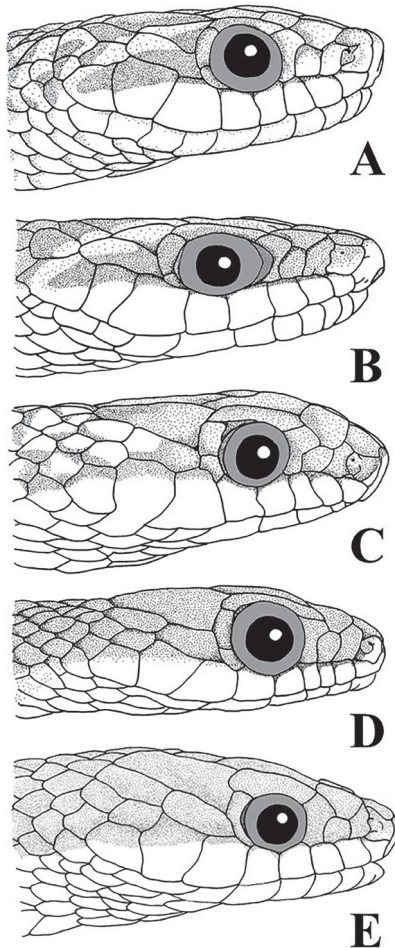
**Figure 1.** Bayesian inference tree of *Erythrolamprus* species from Genbank MtDNA 12S+16SrDNA+c-mos sequences (1332 bp). Red stars indicate Bayesian inference and ML posterior probabilities (> 95%) and bootstrap (> 70%) support values above and below nodes, respectively. Clade in orange shows *E. zweifeli*, in green *E. melanotus*, and in blue *E. pseudoreginae* sp. n. (AF158433) is from French Guiana, and *E. reginae* (JQ598983) is from Brazil.

clades (4.69%) indicating the high genetic divergence between both species (Appendix 5). The phylogenetic relationships of *Erythrolamprus* and the paraphyly of some species (*E. typhlus*, *E. poecilogyrus*, *E. epinephalus*, *E. aesculapii*) are similar to past published work (Hodson and Lehtinen 2017), suggesting the need for an in-depth systematic revision of the genus. Furthermore, the results show the paraphyly of *Erythrolamprus reginae*. *Erythrolamprus melanotus* from Trinidad and Tobago are monophyletic, and the Trinidad specimen shows no genetic differentiation from the most common Tobago haplotype. *Erythrolamprus* sp. from Tobago is the sister clade to *E. epinephalus* from Costa Rica. This clade, composed by the three species (*E. melanotus* + *E. epinephalus* + *E. sp.* Tobago), is strongly supported in the Bayesian analyses. The Trinidadian *E. zweifeli* form the sister clade to *E. reginae* from Guyana but are a distinct lineage.

## Morphological results

Figure 2 shows the similarities in the architecture of the scales when *Erythrolamprus* are viewed in profile. They all have a single preocular, two postoculars, and eight upper labials; the second and third upper labials are in contact with the loreal, the fourth and





**Figure 2.** A comparison of the five members of the *Erythrolamprus reginae* group. **A** *E. reginae* for Guyana (FMNH 30959) **B** *E. zweifeli* from Venezuela (FMNH 204477) **C** *E. melanotus* from Tobago (UWIZM.2012.42.19) **D** *E. pseudoreginae* sp. n. from Tobago (FLMNH 91621) **E** *E. epinephalus* from Venezuela (MBLUZ 1502).

fifth border the orbit, and the temporal formula is 1+2. Figure 3 compares the crowns and chins of four of these species (including *E. zweifeli* from three different populations). They all share nine plate-like scales on the crown in similar proportions and two pair of enlarged chin-shields. Figure 4 illustrates the distribution of the five species in northern South America, Trinidad, and Tobago.

Comparisons and summaries of the meristic characters for taxa under consideration are given in Table 1. Ventral counts for all *Erythrolamprus* taxa under consideration have ranges that overlap, although they have different means, some of which are significantly different. The ranges for the subcaudal counts are similar. The Tobago *E. pseudoreginae* sp. n. can be separated from *E. melanotus* but not the other taxa. The

**Table 1.** A comparison of the meristic and color pattern data for the five taxa in *Erythrolamprus* in the Trinidad and Tobago area. Key: \* based on our counts for Venezuelan specimens.

	<i>E. melanotus</i>	<i>E. reginae</i>	<i>E. zweifeli</i>	<i>E. pseudoreginae</i> sp. n.	<i>E. epinephalus</i>
Number of specimens	12	14	44	5	6
stripe on rows	4–5	3–4	3–4	3–4–5	variable
ventral range	139–154	129–147	134–157	143–154	144–157*
mean ventrals $\pm$ SD	146.66 $\pm$ 4.36	138.35 $\pm$ 4.71	142.54 $\pm$ 3.98	147.5 $\pm$ 3.35	151.33 $\pm$ 3.38
subcaudal range	53–58	68–79	72–85	76–79	65–75*
mean subcaudals $\pm$ SD	55.2 $\pm$ 1.4	72.0 $\pm$ 7.14	79.9 $\pm$ 4.20	77.5 $\pm$ 1.5	68.2 $\pm$ 3.38
postocular stripe	present	indistinct	present	indistinct	variable
ventral color	yellow	yellow to pale orange, usually with black checks	red with black checks, some ventrals solid black	uniform yellow to red with scattered fine speckling	variable
apical pit present	yes	yes	no	yes	yes

results of single factor ANOVAs are presented in Appendix 2. Some support the separation of *E. zweifeli* from *E. reginae*, *E. zweifeli* from the Tobago *E. pseudoreginae* sp. n., and *E. reginae* from the Tobago *E. pseudoreginae* sp. n.

Substantial genetic differences (0.047) (Appendix 5) and relatively minor morphological differences (different means for ventral counts, distinctive coloration, the absence of apical pits on dorsal scales) and its geographic isolation support the description of the Tobago population as a new species. Figure 5 compares the color morphs of *Erythrolamprus zweifeli* found in Trinidad and Venezuela with *Erythrolamprus reginae* from Guyana. Figure 6 illustrates *E. pseudoreginae* sp. n.

### *Erythrolamprus pseudoreginae* sp. n.

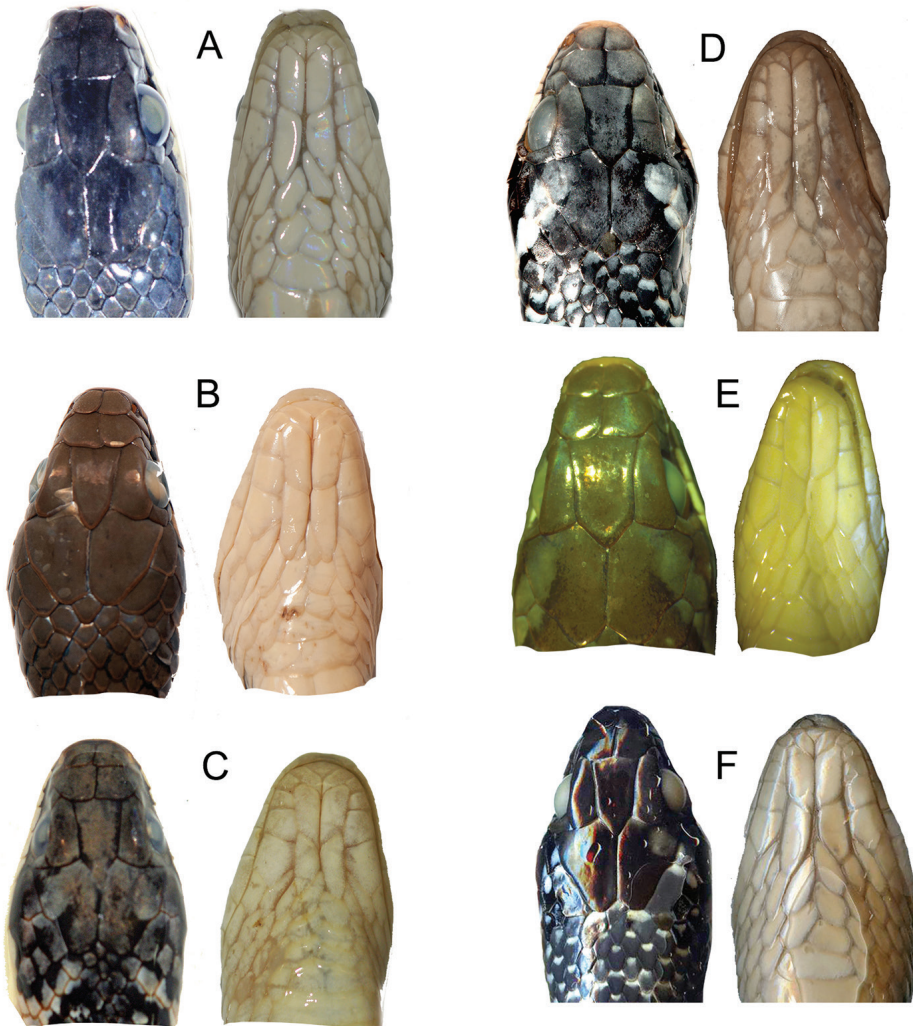
<http://zoobank.org/B5FAE467-C240-4EBB-9DA5-B3D44998757E>

Figures 2D, 3A, 6

*Liophis* sp. Hardy 1982: 86.

*Liophis reginae* [ssp.] Dixon 1983b: 12.

**Material. Holotype.** UWIZM.2016.22.45 collected 13 June 2016 by Alvin L. Braswell and Renoir J. Auguste on Gilpin Trace Trail, 8.5 km NNW Roxborough, St. John, Tobago (~11°16'55"N; 60°37'12"W, about 493 m ASL) at 0900 hrs. **Paratypes.** TOBAGO: St John: FLMNH 91621 Gilpin Trace Trail, 5.3 mi NNW Roxborough, 11°16'N, 60°37'W collected on 17 July by Kurt Auffenberg. USNM 228069 south of Charlotteville, at first creek crossing on Pigeon Peak Trace 11°17'N, 60°36'W collected 12 May 1979 at (14:00 hrs) by Dave Stephens; USNM 325089 NW of Roxborough on Gilpin Trace, ca. 0.5 mi from its junction with Roxborough–Bloody Bay Road, collected 11 November 1992; USNM 539191 approx. 6 km (airline) NNW of Roxborough, 0.5 mi from upper entrance of Gilpin Trace and Roxborough - Parlatuvier Road, 11°17'N, 60°35'W collected 11 July 2000.



**Figure 3.** A comparison of the scale arrangements on the crowns and ventral heads of the *Erythrolamprus* taxa under discussion. **A** *E. pseudoreginae* sp. n. from Tobago **B** *E. epinephalus* from Venezuela MBLUZ 1501 (dorsal view) and 1500 (ventral view) **C, D** Salt and pepper morph of *E. zweifeli* from Trinidad and Venezuela **E** An olive-brown morph of *E. zweifeli* Trinidad, FMNH 215827 **F** A melanistic morph of *E. zweifeli* from Venezuela EBRG 2745.

**Diagnosis.** Ventrals 143–154; subcaudals 76–79; second pair of chin shields longest; some anterior dorsal scales have an apical pit; lateral stripe on scale rows 3–4–5, dark stripe (row 3) and a pale stripe (rows 4–5) on posterior body and tail, the black stripe continues to the forebody as a series of black spots on scale row three; and the ventral surface has scattered flecks of pigment toward mid-body. Otherwise, the belly is uniform cream with fine speckling in preserved material, and red in life, tail uniform cream in preservative, red in life.

**Description of the holotype.** UWIZM.2016.22.45, an adult male, 525 mm total length, 148 mm tail; tail 28% of SVL. Rostral barely visible from above, broader than tall; internasals paired, shorter than prefrontal; frontal pentagonal; parietals longer than frontal; four post parietals; nasal divided, first lobe does contact the second labial; loreal subrectangular, higher than long, contacts upper labials 2–3; preocular single, T-shaped, contacts upper labials 3–4; postoculars 2/2, upper largest; temporals 1+2, primary temporal contacts upper labials 6–7/6–7; upper labials 8/8; 4–5 in orbit; lower labials 4/5 contact anterior chin shields, total of nine in contact with both pairs; lower labials 9/10; three gular scales; dorsal scales are smooth, some have a single apical pit, they are in 17 rows at mid body and reduced to 15 rows anterior to the cloaca; 146 ventrals; 77 subcaudals.

In life the crown is dark moss green with black spots, the upper labials are cream, with a dark stripe on the upper edge that runs from nasal to orbit, and widens posteriorly onto the temporals. Dorsal spots on scale rows 2–3 about two ventrals apart, start above the 12<sup>th</sup> ventral, and coalesce into a stripe at about the 96<sup>th</sup> ventral and extend posteriorly to the tip of the tail; lateral stripe mostly on scale row three on body, goes onto scale row one on tail. About one-third down the body, about ventral 40, scale rows 1–4 blue-gray, row five is brown, row six and above blue-gray; except for the mossy green on the anteriormost dorsal surface for about 40 ventrals. Ventral surface mostly uniform yellow to orange with light mottling starting about the 50<sup>th</sup> ventral; tail has a mid-line zigzag stripe.

**Variation:** The smallest specimen measured 347 mm SVL with a 129 mm tail; the largest specimen 420 mm SVL with a 119 mm damaged tail. Dorsal scale rows 17–17–15. Ventrals range from 143–154 ( $n = 5$ ,  $X = 147.5$ ,  $SD = 3.35$ ); subcaudals 76–79 ( $n = 2$ ,  $X = 77.5$ ,  $SD = 1.5$ ). Upper labials eight or nine, 2–3 contact loreal, 4–5 border the orbit (one specimen has 5–6 bordering the orbit on one side), the tallest can be seventh, (or eight if nine labials are present); the sixth labial is the largest in the area. Loreal is quadrangular to pentagonal. Lower labials 9–10; first four or five contact the anterior chin shields. Longest pair of chin shields is the second. Eye diameter is greater than eye-nostril distance. The dark posterior lateral stripe is usually on scale rows 2–3–4, but one specimen has it on scale rows 2–3 only.

Color in life. The following is based on the holotype (Figure 6) and a color photograph in Brown (2013). Crown and face olive brown, upper labials white, a short black subocular stripe extends from the nasal scale under the eye and posteriorly to the last labial. Immediately behind the head, the interstitial skin is yellow; dorsum brown with an indistinct vertebral stripe and scales partially edged with black pigment most obvious on posterior two-thirds of the body. First three scale rows are blue-gray and separated from brown dorsum by a row of black spots.

Color in alcohol. Head, body, and tail dark blue to brown with a black stripe on the posterior lateral body that becomes a series of dark spots extending anteriorly on the body. The belly is a uniform cream with fine speckling of pigment.

**Comparisons.** *Erythrolamprus pseudoreginae* sp. n. differs from *E. zweifeli* in the presence of apical pits on some dorsal scales, an almost uniform yellow to red venter,

and a dark stripe on the posterior body on scale rows 3–4 bordered above by a pale stripe on scale row five. The new species lacks the well-defined postocular stripe that runs from the postocular scales across the temporals to a point just above the rictus in most *E. zweifeli*. In *E. zweifeli* the postocular stripe may also have a pale dorsal border.

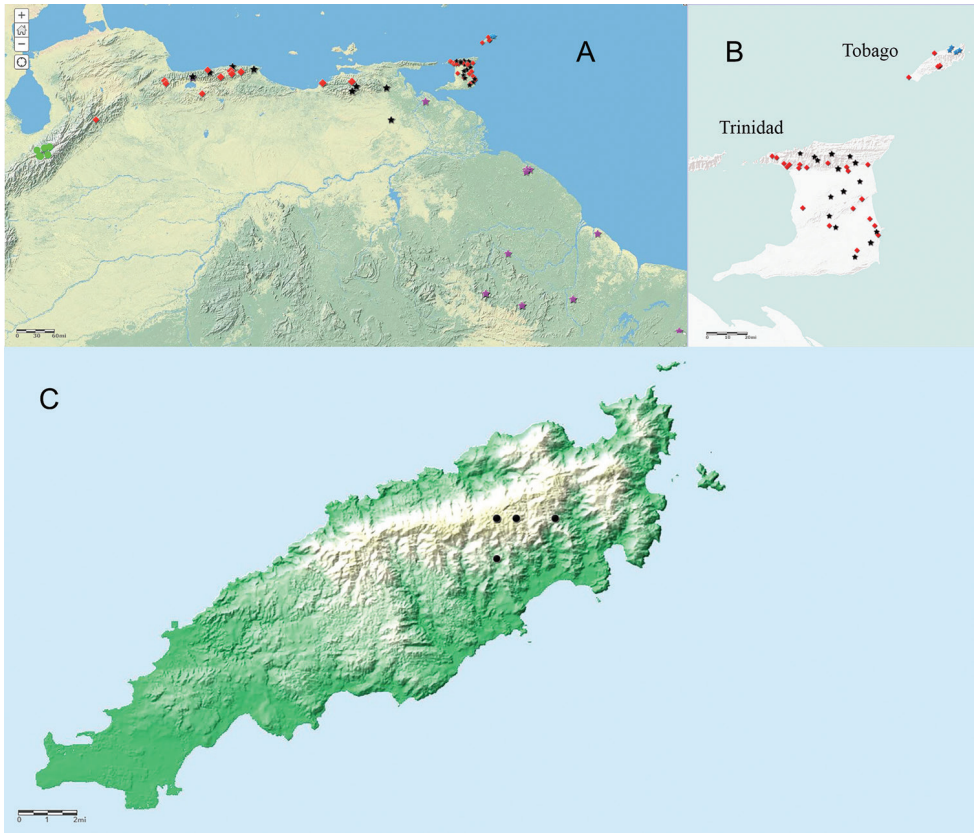
*Erythrolamprus pseudoreginae* sp. n. differs from all populations of *E. epinephalus* in having more than 75 subcaudal scales, except for some Venezuelan and Colombian populations. The *E. epinephalus* populations with more than 75 subcaudals have a dorsal or ventral pattern that includes transverse bars, black checks, or a pattern with irregular black spots on the outer edges of the ventral scales that may extend onto the first row of dorsal scales (Dixon 1983a, Escalona 2017).

The new species differs from *Erythrolamprus reginae* in having a uniform venter (*E. reginae*) has yellow to orange venter with black checks, and a dark stripe on the last fourth of the body on scale rows 3–4 which is not bordered by a pale stripe. *Erythrolamprus pseudoreginae* sp. n. has uniform yellow to red ventral surface and a very distinctive, pale posterior lateral stripe on row five above the black stripe on rows 3–4 that extends anteriorly as a row of dark spots. *Erythrolamprus reginae* has fewer ventrals and a lower mean ventral count than *E. pseudoreginae* sp. n.

The pattern will readily distinguish it from the two coral snake mimics (*Erythrolamprus aesculapii* and *E. bizona*) which are on Trinidad but not Tobago. The endemic Tobago Red Snake, *E. ocellatus*, has a bright red dorsum with black ocelli. The semi-aquatic *Erythrolamprus cobellus* has a uniform dark green or black dorsum and is known from Trinidad but not Tobago. The absence of a black stripe five scale rows wide on the vertebral line separates it from Shaw's Black Back Snake, *E. melanotus*, a species known from both islands.

**Distribution.** It occurs in northeastern Tobago and appears to be restricted to the forested ravines along the crest of the Main Ridge (Fig. 4). Tobago's Main Ridge is about 16 km long and covered with lower montane rain forest on schist soil above 224 m ASL. The ridge crest reaches elevations of 487–576 m ASL and forms steep terrain with deep gullies and fast-moving streams. The area receives about 318 cm of rainfall per year, and no month receives less than 10 cm (Beard, 1944). Tobago's Main Ridge Forest Reserve is the oldest protected forest in the Western Hemisphere (since April 1776) and encompasses 3958 hectares. At this writing, five specimens of *Erythrolamprus pseudoreginae* sp. n. are known, all of which came from the northeast end of the Main Ridge. The locality and elevation data available suggest it occurs within an area of about 400 ha at elevations between 430–500 m ASL. Three types of rainforest occur on Tobago: lowland rainforest covers 4,844 ha, lower montane rainforest covers 4,566 ha, and xerophytic rainforest covers 937 ha (Helmer et al. 2012). All of the localities for *E. pseudoreginae* sp. n. fall within the lower montane rainforest, suggesting its maximum area of occupancy may be 4,566 ha, if it is restricted to that forest type.

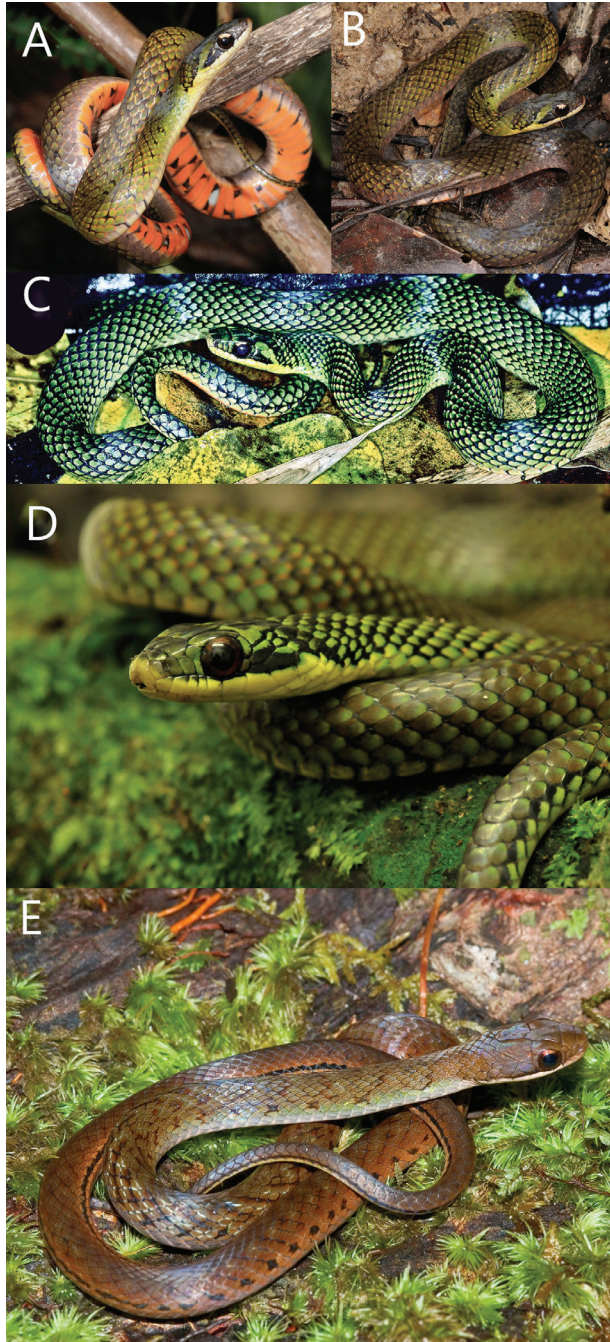
**Natural History.** *Erythrolamprus pseudoreginae* sp. n. is diurnal, all of the specimens with time of collection data were found in the morning or afternoon. Nothing is known about the diet and reproduction of this snake. Its close relatives have been reported to eat anurans, and it likely preys upon small ground-dwelling frogs.



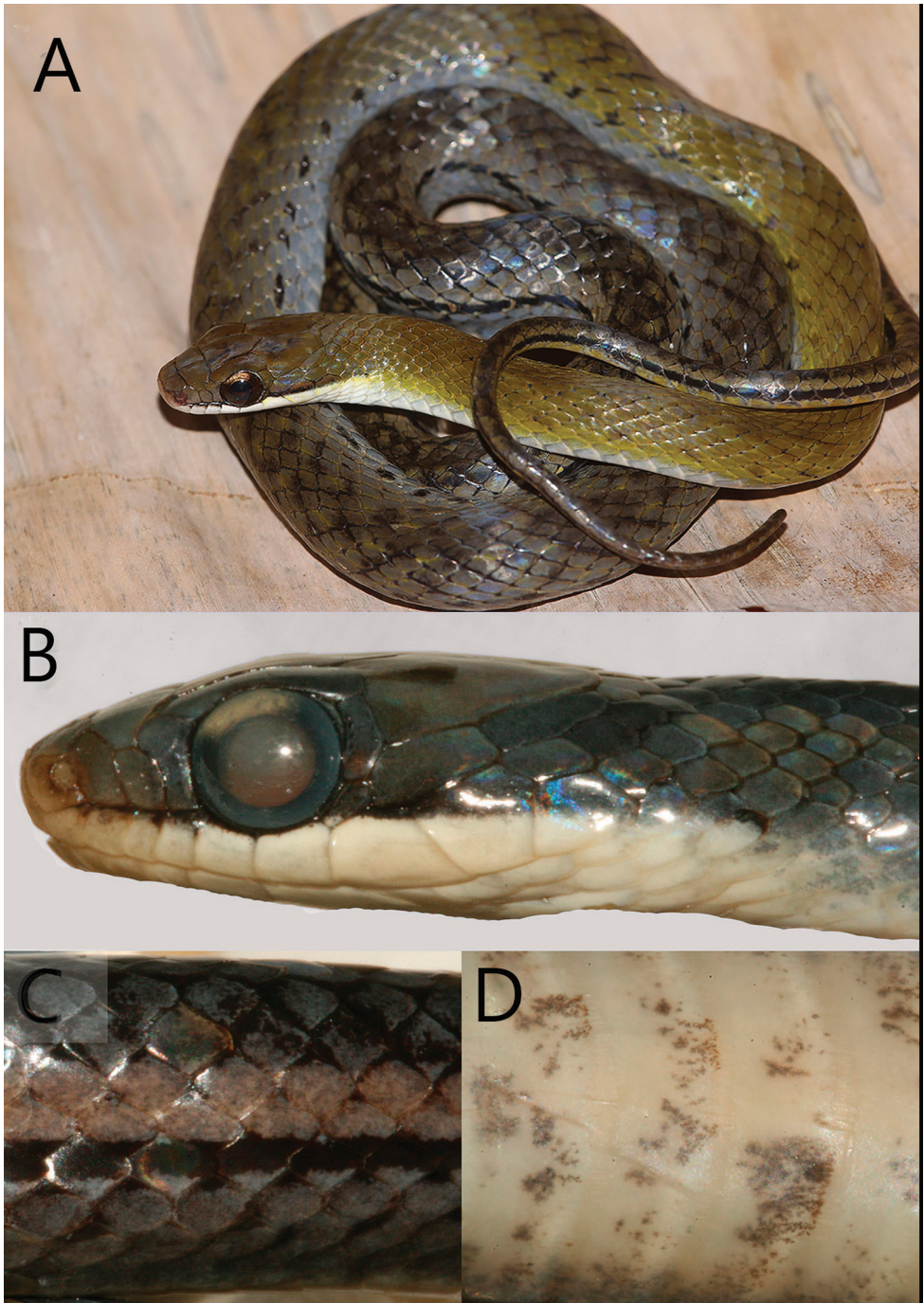
**Figure 4.** Geographic distribution of the five species of *Erythrolamprus* under discussion in this paper. **A** The distribution of the species of *Erythrolamprus* under discussion in northern Venezuela and Trinidad and Tobago **B** More detailed view of the distribution on Trinidad and Tobago **C** Tobago with the known localities for *E. pseudoreginae* sp. n. Note that two of the markers closely overlap. Key: black stars = *E. zweifeli* from Cordillera de Costa in Venezuela and the island of Trinidad; green circles = *E. epinephalus* from the Cordillera de Mérida, Venezuela. Note that these markers denote the closest population to Tobago based on Roze (1966). Specimens examined came from several different locations. Purple stars = *E. reginae* from the Guianas including Orinoco Delta in Venezuela; red stars = *E. melanotus* from Venezuela, Trinidad, and Tobago; blue star = *Erythrolamprus pseudoreginae* sp. n. on Tobago.

**Conservation.** Given the restricted distribution of this snake as well as the fact that most, if not all, of its distribution lies within the oldest protected forests in the Western Hemisphere it may be assumed that it is well protected. However, as the climate changes the microclimate found in the lowland montane rainforest may be expected to change and potentially make the local environment inhospitable for this species and the other endemic taxa found here.

**Etymology.** The epithet *pseudoreginae* was chosen because prior investigators considered this snake to be *Liophis reginae*. We suggest Tobago Stream Snake as the common English name for this snake.



**Figure 5.** **A, B** Variations in the olive color morph of *Erythrolamprus zweifeli* from Trinidad (photographs by Michael Patrikeev) **C** the middle photo shows the “salt and pepper” morph that occurs at higher elevation (photograph by JCM). Both color morphs are included in our molecular sample **D** *E. zweifeli* Rancho Grande, Parque Nacional Henri Pittier, Luis A. Rodriguez J. **E** the Royal Snake, *Erythrolamprus reginae* from Kaiteur, Guyana (photograph by P Kok).



**Figure 6.** *Erythrolamprus pseudoreginae*. **A** UWIZM 2016.22.45, holotype **B–D** FLMNH 91621 from Gilpin Trace, on Tobago’s Main Ridge. **B** Profile. Of the four specimens examined this was the only one that had nine upper labials (on one side only) **C** The posterior lateral stripe bordered by a dorsal light stripe **D** Venter mostly uniform with patches of scattered pigment.



## Discussion

*Erythrolamprus pseudoreginae* becomes the fifty-first species in the genus, and the eleventh member of the Tobago herpetofauna closely associated with the Main Ridge. The list of Main Ridge species includes the frogs *Mannophryne olmonae*, *Pristimantis charlottevillensis*, *P. turpinorum*, *Hyalinobatrachium orientale*; the lizards *Bachia* cf. *flavescens*, *Gonatodes ocellatus*, *Anolis* cf. *tigrinus*; and the snakes *Atractus fuliginosus*, *Erythrolamprus ocellatus*, and *Leptophis haileyi*.

Most of the Main Ridge endemic species seem to have their closest living relatives in the Coastal Ranges of Venezuela as opposed to the more proximal island of Trinidad or the Guiana Shield. The Coastal Range endemic *Mannophryne riveroi* is the sister to *M. olmonae* (Manzanilla et al. 2009, Lehtinen et al. 2011). Tobago's *Pristimantis charlottevillensis* appears to be most closely related to *P. terraebolivaris* and members of the *Pristimantis conspicillatus* group (Hedges et al. 2008). Jowers et al. (2015) proposed a Pliocene land bridge connection between Tobago and Venezuela to explain the presence of *Hyalinobatrachium orientale* on Tobago and northeast Venezuela. An alternative explanation is that Tobago was accreted to Venezuela on its movement to its current position.

With this study, only 21 of the 51 named *Erythrolamprus* species have been included in molecular studies; thus, the tree contains only 41% of the known species in the genus. Therefore, its topography is likely to change with additional taxa from more locations. *Erythrolamprus reginae* and *E. epinephalus* are polytypic and given their distributions and morphological variation they represent a considerable challenge to resolving the lineages found within these taxa. Some of the color patterns have evolved multiple times in the different lineages and when combined with the conserved morphology, separating these taxa by morphology becomes a conundrum. It seems likely that some of the currently recognized subspecies will be found more closely related to lineages other than the one they are currently assigned.

The phylogenetic analyses suggest part of *E. reginae* is the sister to *E. zweifeli*. The results show for the first time the Trinidadian *E. melanotus*, has no genetic divergence from the most common haplotype from Tobago. This lack of differentiation suggests some recent or ongoing gene flow between islands. The position of *E. ocellatus* from Tobago suggests that it forms a highly divergent lineage to the remaining Tobago's *Erythrolamprus* and may indicate a different time of colonization.

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## Appendix I

Locality data for specimens examined in this study. Coordinates represent georeferencing from Google Earth, variation from the exact collecting locality is expected.

Species	Voucher	Country	Coordinates
<i>epinephalus</i>	MBLUZ 1500	Venezuela	10°19'N; 72°35'W
	MBLUZ 1501	Venezuela	10°19'N; 72°35'W
	MBLUZ 1502	Venezuela	10°19'N; 72°35'W
	MBLUZ 1503	Venezuela	10°19'N; 72°35'W
	MCNC 5677	Venezuela	07°39'N; 72°21'W
	MCNC 7875	Venezuela	07°29'N; 72°27'W
<i>melanotus</i>	FMNH 61669	Colombia	07°09'N; 75°21'W
	FMNH 61670	Colombia	no specific locality
	FMNH 121224	Colombia	04°09'N; 73°38'W
	FMNH 165341	Colombia	10°26'N; 75°22'W
	FMNH 165399-408	Colombia	10°26'N; 75°22'W
	FMNH 165498	Colombia	10°26'N; 75°22'W
	FMNH 165644	Colombia	10°26'N; 75°22'W
	FMNH 165645	Colombia	10°26'N; 75°22'W
	FMNH 217232	Trinidad	10°43'N; 61°17'W
	FMNH 218779	Trinidad	10°43'N; 61°17'W
	FMNH 49947-50	Trinidad	10°29'N; 61°28'W
	FMNH 49945-46	Trinidad	10°16'N; 61°1'W
	FMNH 5674	Trinidad	10°39'N; 61°30'W
	FMNH 77902-03	Trinidad	10°39'N; 61°30'W
	FMNH 190749	Trinidad	10°09'N; 61°30'W
	FMNH 49938-44	Trinidad	10°34'N; 61°15'W
FMNH 69778	Venezuela	10°28'N; 67°07'W	
<i>reginae</i>	AMNH 3595	“Guiana”	no specific locality
	USNM 164210	Guyana	8°12'N; 59°46'W
	USNM 164208	Guyana	8°12'N; 59°46'W
	FMNH 30959	Guyana	10°29'N; 61°28'W
	FMNH 30962	Guyana	no specific locality
	UMMZ 53901	Guyana	no specific locality
	UMMZ53912	Guyana	no specific locality
	UMMZ 53968	Guyana	no specific locality
	UMMZ 53969	Guyana	no specific locality
	AMNH 17680	Guyana	6°47'N; 58°09'W
	FMNH 56149	Peru	4°36'S; 74°10'W
	FMNH 40234	Peru	11°48'S; 70°48'W
	AMNH 8132	Suriname	5°51'N; 55°12'W
	AMNH 4436	Venez. or Brazil	no specific locality
<i>pseudoreginae</i>	USNM 539191	Tobago	11°17'N; 60°35'W
	UWIZM.2016.22.45	Tobago	11°17'N; 60°36'W
	UWIZM 91621	Tobago	11°16'N; 60°37'W
	USNM 325089	Tobago	11°17'N; 60°36'W
	USNM 228069	Tobago	11°17'N; 60°36'W

Species	Voucher	Country	Coordinates
<i>Erythrolamprus</i> sp. <i>zweifeli</i>	USNM 549328	Guyana	05°17'N; 60°45'W
	FMNH 215827	Trinidad	10°43'N; 61°17'W
	FMNH 217226-27	Trinidad	10°43'N; 61°17'W
	FMNH 219615	Trinidad	10°43'N; 61°17'W
	USNM 17757-58	Trinidad	10°43'N; 61°17'W
	FMNH 49957-58	Trinidad	10°28'N; 61°28'W
	UWIMZ 2010.12.110	Trinidad	10°43'N; 61°25'W
	UWIMZ 2010.12.201	Trinidad	10°45'N; 61°26'W
	UWIMZ 2010.12.108a, b	Trinidad	10°16'N; 61°1'W
	UWIMZ 2010.12.107	Trinidad	no specific locality
	UWIMZ 2010.12.109	Trinidad	no specific locality
	USNM 252682-83	Trinidad	10°45'N; 61°17'W
	USNM 286922	Trinidad	10°30'N; 61°16'W
	AMNH 137503	Venezuela	10°01'N; 67°17'W
	AMNH 98260	Venezuela	10°06'N; 63°06'W
	USNM 217197	Venezuela	02°37'N; 66°19'W
	FMNH 120986	Venezuela	10°01'N; 67°17'W
	FMNH 204477	Venezuela	10°01'N; 67°17'W
	UMMZ 124225	Venezuela	10°01'N; 67°17'W
	UMMZ 124227-33	Venezuela	10°01'N; 67°17'W
	UMMZ 128390	Venezuela	10°01'N; 67°17'W
	USNM 217198	Venezuela	10°15'N; 68°21'W
	USNM 196332	Venezuela	10°13'N; 66°25'W
	AMNH 67877	Venezuela	10°06'N; 63°06'W
	AMNH 29317	Venezuela	10°09'N; 64°17'W
	AMNH 29332	Venezuela	10°29'N; 66°07'W
AMNH 29317	Venezuela	10°22'N; 63°17'W	
FMNH 17833-36	Venezuela	10°22'N; 63°17'W	
AMNH 29332	Venezuela	10°09'N; 64°17'W	

## Appendix 2

Morphometric data and sex for specimens of *Erythrolamprus* species examined. Key: m = male, f = female; j = juvenile; SVL = snout vent length mm; tail mm; D1–3 dorsal scale rows at anterior, midbody, and posterior body); V = ventral scales; S = subcaudal scales; nd = no data.

Museum	Voucher	Species	Sex	svl	tail	D1	D2	D3	V	S
MBLUZ	1500	epinephalus	?	335	88 d	17	17	15	153	51+
MBLUZ	1501	epinephalus	?	330	112	17	17	15	155	75
MBLUZ	1502	epinephalus	?	280	95	17	17	15	153	69
MBLUZ	1503	epinephalus	f	340	19	17	17	15	157	67
MCNC	5677	epinephalus	m	355	112	17	17	15	144	65
MCNC	7875	epinephalus	m	345	120	17	17	15	146	65
FMNH	165402	<i>melanotus</i>	f	332	90	17	17	15	142	54
FMNH	49946	<i>melanotus</i>	f	291	61	17	17	15	144	57
FMNH	49947	<i>melanotus</i>	f	230	52	17	17	15	139	53
FMNH	49950	<i>melanotus</i>	f	307	77	17	17	15	nd	nd
FMNH	190749	<i>melanotus</i>	f	305	72	17	17	15	142	54
FMNH	165644	<i>melanotus</i>	m	358	92	17	17	15	149	55
FMNH	165498	<i>melanotus</i>	m	325	97	17	17	15	144	54
FMNH	165407	<i>melanotus</i>	m	350	85	17	17	15	147	55
FMNH	49949	<i>melanotus</i>	m	271	76	17	17	15	147	56
FMNH	77903	<i>melanotus</i>	m	275	76	17	16	15	152	58
FMNH	69778	<i>melanotus</i>	m	370	72+	17	17	15	154	nd
FMNH	121224	<i>melanotus</i>	m	282	81	17	16	15	149	57
FMNH	61670	<i>melanotus</i>	nd	310	81	17	17	15	151	55
AMNH	4436	<i>reginae</i>	f	355	128	17	17	15	144	74
UMMZ	53912	<i>reginae</i>	f	420	117	17	17	15	133	79
UMMZ	53969	<i>reginae</i>	f	415	nd	17	17	15	136	nd
USNM	164210	<i>reginae</i>	f	428	nd	17	17	15	139	nd
AMNH	3595	<i>reginae</i>	f	443	nd	17	17	15	136	nd
FMNH	40234	<i>reginae</i>	j	128	44	17	17	15	147	74
AMNH	17680	<i>reginae</i>	m	313	120	17	17	15	137	73
AMNH	8132	<i>reginae</i>	m	445	nd	17	17	15	142	nd
FMNH	30959	<i>reginae</i>	m	443	d	17	17	15	139	nd
FMNH	56149	<i>reginae</i>	m	419	117	17	17	15	145	55
UMMZ	53901	<i>reginae</i>	m	428	186	17	17	15	139	78
UMMZ	53968	<i>reginae</i>	m	474	210	17	17	15	135	75
USNM	164208	<i>reginae</i>	m	308	nd	17	17	15	136	nd
FMNH	30962	<i>reginae</i>	nd	nd	nd	17	17	15	129	68
USNM	539191	<i>pseudoreginae</i>	f	408	nd	17	17	15	148	nd
USNM	228069	<i>pseudoreginae</i>	f	347	129	17	17	15	143	76
USNM	539191	<i>pseudoreginae</i>	f	408	nd	17	17	15	148	nd
FLMNH	91621	<i>pseudoreginae</i>	m	420	119	17	17	15	146	nd
FLMNH	91621	<i>pseudoreginae</i>	m	420	119	17	17	15	146	nd
USNM	325089	<i>pseudoreginae</i>	m	408	158	17	17	15	154	79
USNM	549328	<i>Erythrolamprus</i> sp.	m	361	117	17	17	15	148	64
AMNH	137503	<i>zweifeli</i>	f	456	167	17	17	15	146	83
FMNH	17836	<i>zweifeli</i>	f	380	165	17	17	15	138	82
FMNH	204477	<i>zweifeli</i>	f	454	180	17	17	15	142	85
UMMZ	128390	<i>zweifeli</i>	f	nd	nd	17	17	15	141	nd
UMMZ	124232	<i>zweifeli</i>	f	375	144	17	17	15	144	nd
UMMZ	1288390	<i>zweifeli</i>	f	402	162	17	17	15	141	84



Museum	Voucher	Species	Sex	svl	tail	D1	D2	D3	V	S
USNM	17757	<i>zweifeli</i>	f	471	187	17	17	15	143	76
USNM	252683	<i>zweifeli</i>	f	236	nd	17	17	15	140	nd
USNM	217197	<i>zweifeli</i>	f	434	167	17	17	15	134	72
USNM	252683	<i>zweifeli</i>	f	236	nd	17	17	15	140	nd
UWIZM	2010.12.109	<i>zweifeli</i>	f	245	nd	17	17	15	nd	nd
UWIZM	2010.12.107	<i>zweifeli</i>	f	355	152	17	17	15	139	79
UWIZM	2010.12.201	<i>zweifeli</i>	f	401	158	17	17	15	143	79
UWIZM	2010.12.109	<i>zweifeli</i>	f	245	nd	17	17	15	nd	nd
FMNH	17833	<i>zweifeli</i>	j	172	63	17	16	15	140	80
FMNH	17835	<i>zweifeli</i>	j	152	56	17	17	15	144	85
UMMZ	124229	<i>zweifeli</i>	j	305	117	17	17	15	139	83
UMMZ	124230	<i>zweifeli</i>	j	184	67	17	17	15	141	80
UMMZ	124227	<i>zweifeli</i>	j	nd	nd	17	17	15	142	84
UMMZ	124231	<i>zweifeli</i>	j	185	62	17	17	15	145	nd
UWIZM	2010.12.108b	<i>zweifeli</i>	j	136	45	17	17	15	134	75
AMNH	29317	<i>zweifeli</i>	m	365	152	17	17	15	145	79
AMNH	29332	<i>zweifeli</i>	m	297	114	17	17	15	151	76
AMNH	R-29317	<i>zweifeli</i>	m	369	148	17	17	15	143	82
AMNH	29332	<i>zweifeli</i>	m	322	115	17	17	15	142	74
AMNH	67877	<i>zweifeli</i>	m	361	148	17	17	15	149	82
FMNH	17834	<i>zweifeli</i>	m	384	101+	17	15	15	141	nd
FMNH	217226	<i>zweifeli</i>	m	340	139	17	17	15	138	77
FMNH	219615	<i>zweifeli</i>	m	d	nd	17	17	15	142	nd
FMNH	49957	<i>zweifeli</i>	m	398	174	17	15	15	145	79
FMNH	49958	<i>zweifeli</i>	m	456	nd	17	17	15	145	nd
FMNH	215827	<i>zweifeli</i>	m	354	155	17	16	15	140	78
FMNH	217227	<i>zweifeli</i>	m	367	157	17	15	15	141	nd
FMNH	120986	<i>zweifeli</i>	m	386	143	17	17	15	142	83
FMNH	215827	<i>zweifeli</i>	m	354	155	17	16	15	140	78
FMNH	217227	<i>zweifeli</i>	m	367	157	17	15	15	141	nd
UMMZ	124233	<i>zweifeli</i>	m	394	163	17	17	15	142	83
UMMZ	124225	<i>zweifeli</i>	m	363	149	17	17	15	144	80
UMMZ	124228	<i>zweifeli</i>	m	415	135+	17	17	15	143	nd
USNM	17758	<i>zweifeli</i>	m	349	nd	17	17	15	142	nd
USNM	252682	<i>zweifeli</i>	m	370	160	17	17	15	141	83
USNM	286922	<i>zweifeli</i>	m	165	55	17	17	15	149	80
USNM	196332	<i>zweifeli</i>	m	430	170	17	17	15	145	78
USNM	217198	<i>zweifeli</i>	m	509	203	17	17	15	140	75
USNM	252682	<i>zweifeli</i>	m	370	160	17	17	15	141	83
USNM	286922	<i>zweifeli</i>	m	165	55	17	17	15	149	80
AMNH	98260	<i>zweifeli</i>	nd	492	nd	17	16	15	144	nd
UWIZM	2010.12.108a	<i>zweifeli</i>	nd	360	nd	17	17	15	146	nd
UWIZM	2010.12.110	<i>zweifeli</i>	nd	373	157	17	15	15	138	80

### Appendix 3

(A) compares the single factor ANOVA results for ventral counts and (B) compares the single factor ANOVA results for subcaudal counts. Statistically significant results that resulted in the rejection of the null hypothesis are in bold.

A. ventrals		
	<i>zweifeli</i>	<i>pseudoreginae</i>
<i>zweifeli</i>		<b>p = 0.003</b> <b>df = 53</b>
<i>reginae</i>	<b>p = 0.00</b> <b>df = 59</b>	<b>p = 0.00</b> <b>df = 15</b>
B. subcaudals		
	<i>zweifeli</i>	<i>pseudoreginae</i>
<i>zweifeli</i>		p = 0.350 df = 7
<i>reginae</i>	<b>p = 0.004</b> <b>df = 38</b>	p = 0.230 df = 36

## Appendix 4

Material used for molecular analysis and GenBank numbers. Key: \* sequenced in this study.

Species	Museum voucher	Locality	12S	16S	c-mos
<i>Erythrolamprus aesculapii</i>	ROM 47474	Guyana	-	KY986512	KY986488
	IBSP 74046	Brazil	GQ457795	GQ457736	GQ457856
	MNHN 1996.7896	French Guiana	AF158462	AF158531	GQ9895814
<i>Erythrolamprus almadensis</i>	LSUMZ H-6558	Unknown	-	KY986517	KY986497
	MCP < BRA > 6528	?	JQ598808	JQ598871	JQ598979
<i>Erythrolamprus atraventer</i>	IBSP 74342	?	JQ598809	JQ598872	JQ598980
<i>Erythrolamprus bizona</i>	LSUMZ H-6360	Costa Rica	-	KY986513	KY986493
<i>Erythrolamprus breviceps</i>	MNHN 1996.7879	French Guiana	AF158464	AF158533	-
<i>Erythrolamprus ceii</i>	FML 14973	?	JQ598810	JQ598873	JQ598981
<i>Erythrolamprus cobella</i>	ROM 28372	Guyana	-	KY986514	KY986489
<i>Erythrolamprus cursor</i>	MNHN 1887.0120	Martinique	JX905307	JX905311	-
<i>Erythrolamprus epinephalus</i>	LSUMZ H-1547	Peru	-	KY986515	KY986487
	None	Costa Rica	GU018158	GU018176	-
<i>Erythrolamprus jaegeri</i>	IBSP 59252	?	GQ457809	GQ457749	GQ457869
<i>Erythrolamprus juliae</i>	SBH 194227	Dominica	AF158445	AF158514	-
<i>Erythrolamprus melanotus</i>	RML 0266	Tobago	-	KY986510	KY986492
<i>Erythrolamprus miliaris</i>	ROM 22837	Guyana	-	KY986511	KY986494
	MZUSP 14137	?	JQ598811	JQ598874	JQ598982
	None	French Guiana	AF158409	AF158480	-
<i>Erythrolamprus mimus</i>	LSUMZ H-6398	Honduras	-	KY986508	KY986496
	ICP 1105	Costa Rica	GU018157	GU018175	-
<i>Erythrolamprus ocellatus</i>	CAS 245326	Tobago	-	KY986518	KY986490
<i>Erythrolamprus poecilogyrus</i>	LSUMZ H-6972	Argentina	-	KY986516	KY986491
<i>Erythrolamprus reginae</i>	FML 15916	?	JQ598812	JQ598875	-
	IBSP 72733	?	JQ598813	JQ598876	JQ598983
	MNHN 1996.7846	French Guiana	AF158433	AF158501	-
<i>Erythrolamprus typhlus</i>	LSUMZ H-17725	Brazil	-	KY986509	KY986495
	IBSP 70643	?	GQ457811	GQ457751	GQ457871
	None	French Guiana	AF158410	AF158481	-
<i>Xenodon histricus</i>	MZUSP 13265	?	-	GQ457753	GQ457873
<i>Erythrolamprus pseudoreginae</i> *	UWIZM.2016.22.45	Tobago	MK287470	MK287477	MK287484
<i>Erythrolamprus melanotus</i> *	UWIZM.2011.19.14	Tobago	MK287471	MK287481	-
	UWIZM.2011.25	Trinidad	MK287472	MK287478	MK287485
	UWIZM.2016.22.51	Tobago	MK287473	MK287479	MK287486
	UWIZM.2012.27.26	Tobago	MK287474	MK287480	MK287487
<i>Erythrolamprus zweifeli</i> *	CAS245114	Trinidad	MK287475	MK287482	MK287488
	UWIZM.2014.14	Trinidad	MK287476	MK287483	MK287489

## Appendix 5

Table of p-uncorrected distances computed in MEGA7 (under a complete deletion option) of all species shown in Figure 2. The order of specimens from top to bottom follows Figure 2. *Erythrolamprus pseudoreginae* is marked in bold type and the genetic distance of its closest species (*E. epinephelus*) as recovered from the phylogenetic tree is shown in bold type and marker with a square.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 <i>Xenodon histricus</i>	-															
2 <i>E. poecilogyrus</i> (Argentina)	0.053	-														
3 <i>E. ceii</i> (FML 14973)	0.059	0.006	-													
4 <i>E. poecilogyrus</i> (FML 15916)	0.059	0.006	0.000	-												
5 <i>E. miliaris</i> (French Guiana)	0.059	0.021	0.026	0.026	-											
6 <i>E. miliaris</i> (MZUP 14137)	0.056	0.023	0.029	0.029	0.032	-										
7 <i>E. miliaris</i> (Guiana)	0.056	0.023	0.029	0.029	0.032	0.000	-									
8 <i>E. typhlus</i> (Brazil)	0.041	0.026	0.032	0.032	0.029	0.026	0.026	-								
9 <i>E. reginae</i> (IBSP 72733)	0.044	0.023	0.029	0.029	0.029	0.023	0.023	0.018	-							
10 <i>E. reginae</i> (French Guiana)	0.053	0.026	0.032	0.032	0.023	0.035	0.035	0.023	0.021	-						
11 <i>E. zueifeli</i> (CAS245114 Trinidad)	0.053	0.032	0.038	0.038	0.035	0.041	0.041	0.029	0.032	0.026	-					
12 <i>E. zueifeli</i> (2014.14 Trinidad)	0.053	0.032	0.038	0.038	0.035	0.041	0.041	0.029	0.032	0.026	0.000	-				
13 <i>E. breviceps</i> (French Guiana)	0.053	0.009	0.015	0.015	0.018	0.026	0.026	0.023	0.023	0.029	0.029	0.029	-			
14 <i>E. epinephelus</i> (Peru)	0.053	0.009	0.015	0.015	0.012	0.021	0.021	0.018	0.018	0.023	0.029	0.029	0.006	-		
15 <i>E. epinephalus</i> (Costa Rica)	0.085	0.053	0.059	0.059	0.059	0.062	0.062	0.053	0.059	0.070	0.070	0.070	0.050	0.047	-	
16 <b><i>E. pseudoreginae</i></b> (2016.22.45 Tobago)	0.067	0.023	0.029	0.029	0.026	0.035	0.035	0.032	0.032	0.038	0.041	0.041	0.021	0.015	<b>0.047</b>	-
17 <i>E. melanotus</i> (2016.22.51 Tobago)	0.056	0.023	0.023	0.023	0.032	0.029	0.029	0.032	0.029	0.041	0.038	0.038	0.021	0.021	0.047	0.029
18 <i>E. melanotus</i> (2012.27.26 Tobago)	0.056	0.023	0.023	0.023	0.032	0.029	0.029	0.032	0.029	0.041	0.038	0.038	0.021	0.021	0.047	0.029
19 <i>E. melanotus</i> (2011.19.14 Tobago)	0.056	0.023	0.023	0.023	0.032	0.029	0.029	0.032	0.029	0.041	0.038	0.038	0.021	0.021	0.047	0.029
20 <i>E. melanotus</i> (2011.25 Trinidad)	0.056	0.023	0.023	0.023	0.032	0.029	0.029	0.032	0.029	0.041	0.038	0.038	0.021	0.021	0.047	0.029
21 <i>E. melanotus</i> (Tobago)	0.056	0.023	0.023	0.023	0.032	0.029	0.029	0.032	0.029	0.041	0.038	0.038	0.021	0.021	0.047	0.029
22 <i>E. atraventer</i> (IBSP 74342)	0.065	0.021	0.026	0.026	0.032	0.035	0.035	0.044	0.038	0.041	0.038	0.038	0.021	0.026	0.070	0.041
23 <i>E. jaegeri</i> (IBSP 59252)	0.076	0.029	0.035	0.035	0.032	0.032	0.032	0.044	0.038	0.038	0.044	0.044	0.026	0.026	0.067	0.035
24 <i>E. almadensis</i> (MCP<BRA>6528)	0.067	0.023	0.029	0.029	0.026	0.029	0.029	0.032	0.038	0.038	0.044	0.044	0.026	0.021	0.050	0.026
25 <i>E. almadensis</i> (LSUMP H-6558)	0.056	0.023	0.029	0.029	0.026	0.029	0.029	0.026	0.032	0.038	0.044	0.044	0.026	0.021	0.050	0.029
26 <i>E. juliae</i> (Dominica)	0.065	0.021	0.026	0.026	0.023	0.026	0.026	0.029	0.029	0.035	0.041	0.041	0.018	0.012	0.047	0.021
27 <i>E. cursor</i> (Martinique)	0.059	0.015	0.021	0.021	0.023	0.032	0.032	0.029	0.029	0.035	0.035	0.035	0.012	0.012	0.050	0.026
28 <i>E. typhlus</i> (IBSN7070643)	0.079	0.044	0.044	0.044	0.047	0.056	0.056	0.053	0.059	0.059	0.053	0.053	0.041	0.041	0.056	0.044
29 <i>E. typhlus</i> (French Guiana)	0.076	0.035	0.035	0.035	0.038	0.047	0.047	0.044	0.050	0.050	0.050	0.050	0.032	0.032	0.053	0.041
30 <i>E. cobella</i> (Guyana)	0.059	0.015	0.021	0.021	0.023	0.032	0.032	0.029	0.029	0.035	0.029	0.029	0.006	0.012	0.047	0.023
31 <i>E. aesculapii</i> (Brazil)	0.062	0.032	0.038	0.038	0.041	0.044	0.044	0.041	0.047	0.041	0.041	0.041	0.029	0.035	0.070	0.038
32 <i>E. ocellatus</i> (Tobago)	0.056	0.026	0.032	0.032	0.041	0.032	0.032	0.035	0.041	0.041	0.041	0.041	0.029	0.029	0.065	0.032
33 <i>E. aesculapii</i> (French Guiana)	0.065	0.032	0.035	0.035	0.041	0.038	0.038	0.041	0.041	0.047	0.053	0.053	0.029	0.029	0.073	0.038
34 <i>E. aesculapii</i> (Guyana)	0.062	0.029	0.032	0.032	0.038	0.035	0.035	0.038	0.038	0.044	0.050	0.050	0.026	0.026	0.070	0.035
35 <i>E. bizona</i> (Costa Rica)	0.059	0.018	0.023	0.023	0.032	0.035	0.035	0.032	0.038	0.038	0.038	0.038	0.015	0.021	0.065	0.035
36 <i>E. mimus</i> (Costa Rica)	0.059	0.018	0.023	0.023	0.032	0.035	0.035	0.032	0.038	0.038	0.038	0.038	0.015	0.021	0.065	0.035
37 <i>E. mimus</i> (Honduras)	0.059	0.018	0.023	0.023	0.032	0.035	0.035	0.032	0.038	0.038	0.038	0.038	0.015	0.021	0.065	0.035

Continued.

	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
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18	0.000	-																				
19	0.000	0.000	-																			
20	0.000	0.000	0.000	-																		
21	0.000	0.000	0.000	0.000	-																	
22	0.041	0.041	0.041	0.041	0.041	-																
23	0.044	0.044	0.044	0.044	0.044	0.029	-															
24	0.041	0.041	0.041	0.041	0.041	0.029	0.023	-														
25	0.035	0.035	0.035	0.035	0.035	0.035	0.029	0.018	-													
26	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.021	0.026	-												
27	0.021	0.021	0.021	0.021	0.021	0.026	0.038	0.032	0.032	0.012	-											
28	0.041	0.041	0.041	0.041	0.041	0.044	0.050	0.038	0.044	0.035	0.038	-										
29	0.035	0.035	0.035	0.035	0.035	0.047	0.047	0.041	0.047	0.026	0.032	0.032	-									
30	0.023	0.023	0.023	0.023	0.023	0.026	0.032	0.032	0.032	0.023	0.018	0.041	0.032	-								
31	0.050	0.050	0.050	0.050	0.050	0.038	0.038	0.035	0.038	0.041	0.041	0.059	0.050	0.035	-							
32	0.044	0.044	0.044	0.044	0.044	0.038	0.038	0.029	0.038	0.029	0.035	0.053	0.038	0.035	0.012	-						
33	0.050	0.050	0.050	0.050	0.050	0.044	0.044	0.041	0.044	0.041	0.041	0.070	0.056	0.035	0.023	0.023	-					
34	0.047	0.047	0.047	0.047	0.047	0.041	0.041	0.038	0.041	0.038	0.038	0.067	0.053	0.032	0.021	0.021	0.003	-				
35	0.035	0.035	0.035	0.035	0.035	0.029	0.035	0.035	0.035	0.032	0.026	0.056	0.041	0.021	0.021	0.021	0.026	0.023	-			
36	0.035	0.035	0.035	0.035	0.035	0.029	0.035	0.035	0.035	0.032	0.026	0.056	0.041	0.021	0.021	0.021	0.026	0.023	0.006	-		
37	0.035	0.035	0.035	0.035	0.035	0.029	0.035	0.035	0.035	0.032	0.026	0.056	0.041	0.021	0.021	0.021	0.026	0.023	0.006	0.000	-	

