

# New nematode *Tahamina indica* gen. nov., sp. nov. (Nematoda, Dorylaimida, Tylencholaimoidea) from the tropical rainforest, Western Ghats

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## Abstract

During a nematological survey in the Western Ghats a new nematode belonging to the superfamily Tylencholaimoidea (Dorylaimida) extracted from the rhizosphere of the soil of grasses, is described and illustrated. *Tahamina indica* gen. nov., sp. nov. is characterized by females with a body length of 1.3–1.4 mm; lip region 8.0 µm wide, approximately one-fourth of the body diameter at the pharyngeal base; amphidial fovea cup-shaped, about one-half as wide as the lip region diameter. Odontostyle 8.0–9.0 µm long, 1.0–1.1 times lip region diameter; guiding ring simple; odontophore rod-like, 10.5–11.5 µm long with basal thickening or minute knobs-like structure; pharynx consisting of a weakly muscular anterior part, expanding abruptly into a cylindrical basal bulb, occupying about two-fifths to one-half of the total pharyngeal length; female genital system monodelphic-opisthodelphic with anterior uterine sac; vulval opening pore-like, and tail elongated with a slight dorsally curved tip. Males not found.

**Key words:** Morphometrics, new genus, new species, SEM, taxonomy



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## Introduction

The Western Ghats is a fascinating biogeographic region located in southern India, and is one of 36 world biodiversity hotspots (Myers 1988; Myers et al. 2000). According to the latest biodiversity survey, the Western Ghats represents about 30% of India's biodiversity, with a significant number of endemic species (CEPF 2016) and is home to 325 threatened species. The average temperature varies from 20 °C in the south to 24 °C in the north, and because of precipitation in some areas, it dips below freezing during winter. The main types of habitats include forests (shola, moist deciduous, dry deciduous, evergreen, semi-evergreen), bamboo breaks, savanna and rocky areas. Rainfall on the Western Ghats's Mountain slopes ranges from 1000 to 7600 mm annually, with an average of 2500 mm (Gadgil 1994, 1996a, 1996b). This is a highly mosaic biogeographic area with rich biodiversity and great environmental variation in topography, soil type, rainfall, and temperature (Gadgil

1996b). Many different invertebrate species are known from this hotspot, including thousands of arthropods, annelids and hundreds of molluscs. However, records of nematofauna from the Western Ghats are scant. Jairajpuri and Ahmad (1982) and Ahmad and Jairajpuri (1985) described two new nematode genera *Aporcedorus* and *Silvallis* respectively from this hotspot. In a series of papers, Ahmad and Jairajpuri (1982–1988), Ahmad et al. (1992), Ahmad and Ahmad (1992), Ahmad and Ahmad (2002), Ahmad (1993) and Dhanam and Jairajpuri (1999) have recorded several species of soil-inhabiting nematodes from this region (Islam and Ahmad 2021a). Recently, Islam and Ahmad (2021–2022) and Islam et al. (2019) in their publications described several new and known species of tylencholaimoid nematodes from the Western Ghats.

In this study, large numbers of soil samples were collected from several localities of the Western Ghats. These samples yielded several species of the superfamily Tylencholaimoidea (Dorylaimida), with most published recently by Islam and Ahmad (l.c.). One sample also contained a nematode taxon with a morphology that is distinct from any known genus. Given the documented morphological distinctiveness, a new genus and species is described in detail herein.

## **Material and methods**

### **Soil sampling and nematode extraction and processing**

Soil samples were collected from the rhizosphere of grasses (*Cynodon dactylon* L.) from Yaraganalu, Shivamoga District, Karnataka State, India. The nematodes were extracted from soil by using Cobb's (1918) sieving and decantation and modified Baermann's funnel methods. Then the specimens were fixed in hot triethanolamine-glycerol fixative and transferred to a glycerine-alcohol solution in a small cavity block which was then kept in a desiccator containing anhydrous calcium chloride for slow dehydration and mounted in anhydrous glycerin. The paraffin wax ring method was used to make permanent mounts (De Maeseneer and d'Herde 1963).

### **Light microscopy study**

An ocular micrometer was used to take measurements, a drawing tube was used to make line drawings, and a Nikon DS digital camera connected to a Nikon Eclipse 80i microscope was used to capture photographs.

### **Scanning Electron Microscopy (SEM) study**

The nematodes were fixated in 4% formaldehyde, transferred to distilled water for 2 hours and then dehydrated in a graded ethanol-acetone series (25% for 24 hours and 25, 30, 50, 70, 80, 90, 95 and 100% for 2 hours each step) and followed by acetone (100% for 1 hour). Following dehydration, samples were dried using critical point drying (Leica-EM CPD 300), coated with a gold sputter (Jeol-JFC 1600), and then observed with a Jeol-JSM 6510 LV SEM.

## Taxonomy

### *Tahamina* gen. nov.

<https://zoobank.org/66077C36-1977-4004-89BF-B2AE34774B4B>

Tylencholaiminae Filipjev, 1934 (Tylencholaimidae Filipjev, 1934).

**Diagnosis.** Large sized nematode, 1.3–1.4 mm long body. Cuticle dorylaimoid, outer cuticle thin with very fine transverse striation, inner layer thicker than the outer, distinctly striated. Lateral chords narrow. Lip region cap-like, offset by constriction, 8.0 µm wide or one-fourth of the body diameter at pharyngeal base, labial papillae raised, lips slightly separated. Cheilostom a truncate cone. Amphidial fovea cup-shaped, aperture slit-like, occupying about one-half as wide as lip region diameter. Odontostyle short, robust, spindle-shaped, 8.0–9.0 µm long, 1.0–1.1 times lip region diameter. Odontophore simple, rod-like, 10.5–11.5 µm long with thickening or minute knobs-like structure at the base. Guiding ring simple. Pharynx consists of a slender and weakly muscular anterior part expanding abruptly into a cylindrical basal bulb, separated by constriction, occupying about two-fifths to one-half (42–50%) of the total pharyngeal length. Female genital system monodelphic-opisthodelphic with anterior uterine sac. Vulva pore-like. Tail long, elongated with a slight dorsally curved tip. Male not known.

**Type and only species.** *Tahamina indica* gen. nov., sp. nov.

**Relationships.** In the presence of a short odontostyle with a distinct lumen, odontophore with minute knobs-like structure and longer pharyngeal expansion, the new genus well fits under the subfamily Tylencholaiminae Filipjev, 1934 of the family Tylencholaimidae (Tylencholaimoidea).

*Tahamina indica* gen. nov. can be separated from *Tylencholaimus* de Man, 1876 in having a dorylaimoid cuticle, radial elements absent (vs. tylencholaimoid cuticle, radial elements present); vulva pore-like (vs. vulva transverse); differently shaped tail (tail long, elongated vs. tail short, rounded to conoid). The new genus differs from the *Heynsnema* Peña-Santiago, Guerrero & Ciobanu, 2008 in having female genital system mono-opisthodelphic (vs. amphidelphic); vulva pore-like (vs. vulva transverse) and differently shaped tail (tail long, elongated vs. tail short, rounded to conoid).

Based on the shape and size of the odontostyle and odontophore, the expanded part of the pharynx and tail, the new genus comes close to *Discomyctus* Thorne, 1939 and *Wasimellus* Bloemers & Wanless, 1996 but differs from the former in having the inner cuticle dorylaimoid (vs. tylencholaimoid); labial disc absent and labial papillae raised (vs. labial disc present and labial papillae not raised); odontophore with thickened base or minute basal knobs-like structure (vs. odontophore with distinct large basal knobs); female genital system mono-opisthodelphic (vs. mono-prodelphic); vulva pore-like (vs. transverse) and tail elongated (vs. tail elongated to tail long filiform). It differs from *Wasimellus* in having a guiding ring single (vs. guiding ring double), the expanded part of the pharynx comparatively long (42–49 vs. 35–42% of total neck length), pharyngeal expansion abrupt (vs. gradual expansion); vulva pore-like without sclerotized pieces (vs. vulva transverse, with sclerotized pieces); female genital system mono-opisthodelphic (vs. amphidelphic), tail elongated (vs. tail filiform).

In the presence of a dorylaimoid cuticle, narrow lateral chord, and weakly muscular expanded part of the pharynx, the new genus resembles *Dorylaimoides* Thorne & Swanger, 1936, which belongs to the family Mydonomidae but differs in having a differently shaped odontostyle (odontostyle spindle-shaped, symmetrical vs. odontostyle simple, asymmetrical) and odontophore (odontophore simple rod-like with basal thickening or very minute knobs-like structure vs. odontophore angular or arcuate without basal thickening or knobs); longer pharyngeal expansion (about two-fifths to one-half vs. one-fifth to one-third of total neck length); and vulva pore-like (vs. vulva transverse).

Based on the shape of the stylet, lip region and tail pattern, the new genus is comparable to two non-tylencholaimid members *Mitoaxonchium* Yeates, 1973 and *Hulqus* Siddiqi, 1981, both belonging to the subfamily Hulqinae of the family Qudsianematidae (Dorylaimoidea) but it differs from the former in having a longer body size ( $L = 1.3\text{--}1.4$  vs.  $0.46\text{--}0.61$  mm); shorter pharynx ( $b = 4.9\text{--}5.2$  vs.  $2.4\text{--}3.2$ ); the position of dorsal esophageal gland nuclei more anteriorly (12–16 vs. 30% of expanded part of pharynx from its expansion), female genital system mono-opisthodelphic (vs. amphidelphic) and differently shaped vulval opening (pore-like vs. transverse). The new genus differs from *Hulqus* in having a longer body ( $L = 1.3\text{--}1.4$  vs.  $0.86\text{--}1.10$  mm); shorter pharynx ( $b = 4.9\text{--}5.2$  vs.  $2.4\text{--}3.0$ ); the position of dorsal esophageal gland nuclei more anteriorly (12–16 vs. 35–41% of expanded part of pharynx from its expansion), pharyngeal expansion abrupt, separated by constriction (vs. expansion gradual, without constriction) and differently shaped vulval opening (pore-like vs. transverse).

***Tahamina indica* sp. nov.**

<https://zoobank.org/D76FB896-7ADD-4B6A-9529-3FE31353E335>

Figs 1–4

**Material examined.** Seven females and two juveniles from Yaraganalu, Shivamoga District, Karnataka State, India;  $13^{\circ}48'04.3''\text{N } 75^{\circ}34'23.4''\text{E}$ ; 17 November 2018. Female **holotype** (slide number AMUZDNC *Tahamina indica* gen. nov. sp. nov. /1), six female **paratype** specimens (slide numbers AMUZDNC *Tahamina indica* gen. nov. sp. nov. /2–5) and two juveniles (J2) (slide number AMUZDNC *Tahamina indica* gen. nov. sp. nov. /6) are deposited with the nematode collection in the Department of Zoology, Aligarh Muslim University, India.

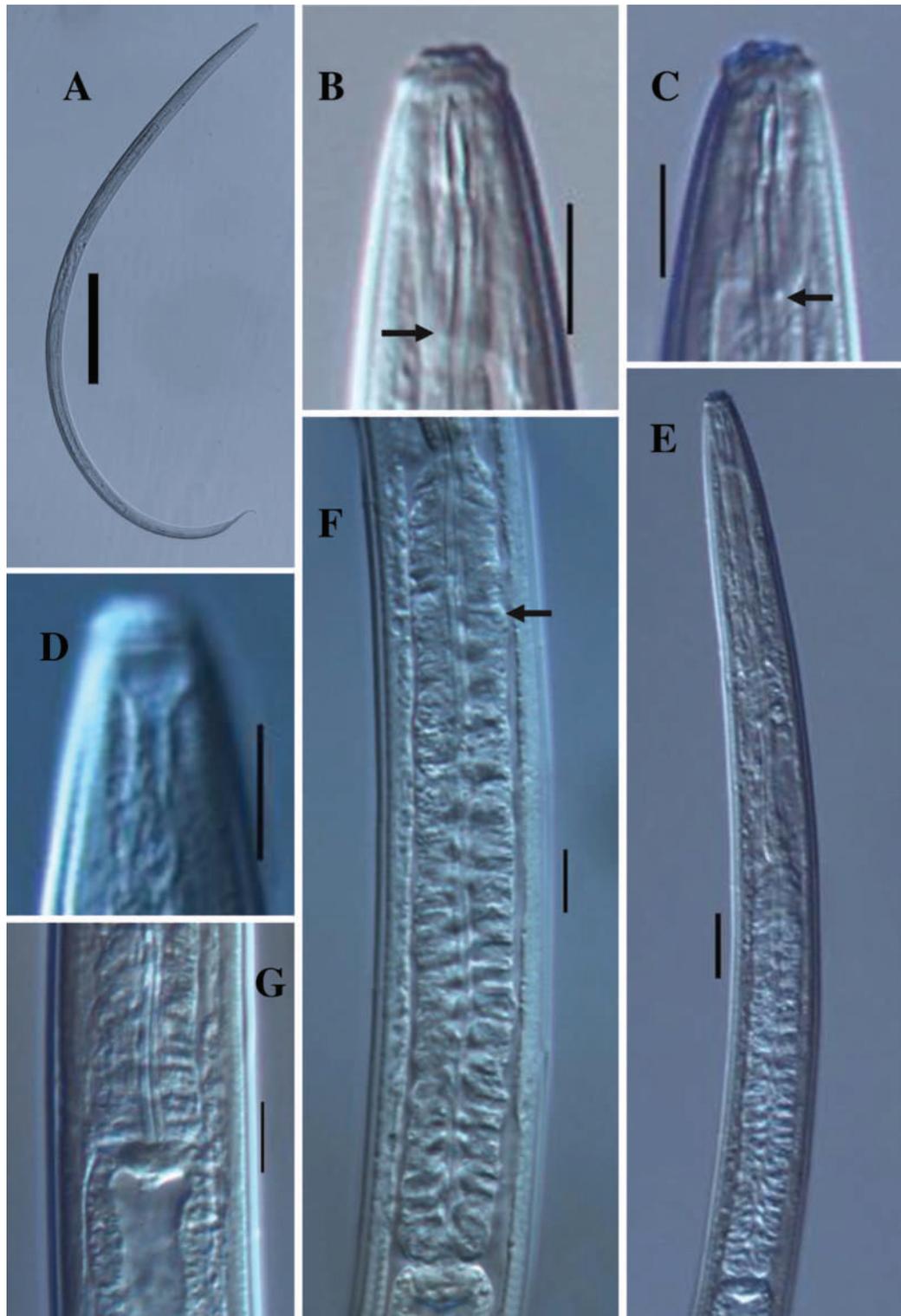
**Measurements.** See Table 1.

**Description. Female.** Slender nematodes of large sized, 1.3–1.4 mm long body, slightly curved ventrally upon fixation; body cylindrical, tapering gradually towards at the anterior end and posteriorly narrowing to form an elongated tail. Cuticle with two distinct layers, 1.0–1.5  $\mu\text{m}$  thick at anterior region, 2.0–2.5  $\mu\text{m}$  at midbody, and 3.0–4.0  $\mu\text{m}$  on the tail. The outer cuticle is thin, with fine transverse striation, the inner layer is thicker than outer, distinctly striated. Lateral chord 5.0–7.0  $\mu\text{m}$  at midbody, occupying about one-sixth to one-four (18–24%) of corresponding body diameter. Lateral, dorsal and ventral body pores are indistinct. Lip region cap-like, offset from the body by constriction, 2.0–2.2 times as wide as high or about one-fourth of the body diameter at the pharyngeal base. Under SEM (Fig. 3A), lips are rounded, and slightly separated, inner portion somewhat amalgamated forming a hexagonal disk-like structure, offset

**Table 1.** Morphometrics of *Tahamina indica* gen. nov., sp. nov. All measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  SD (range).

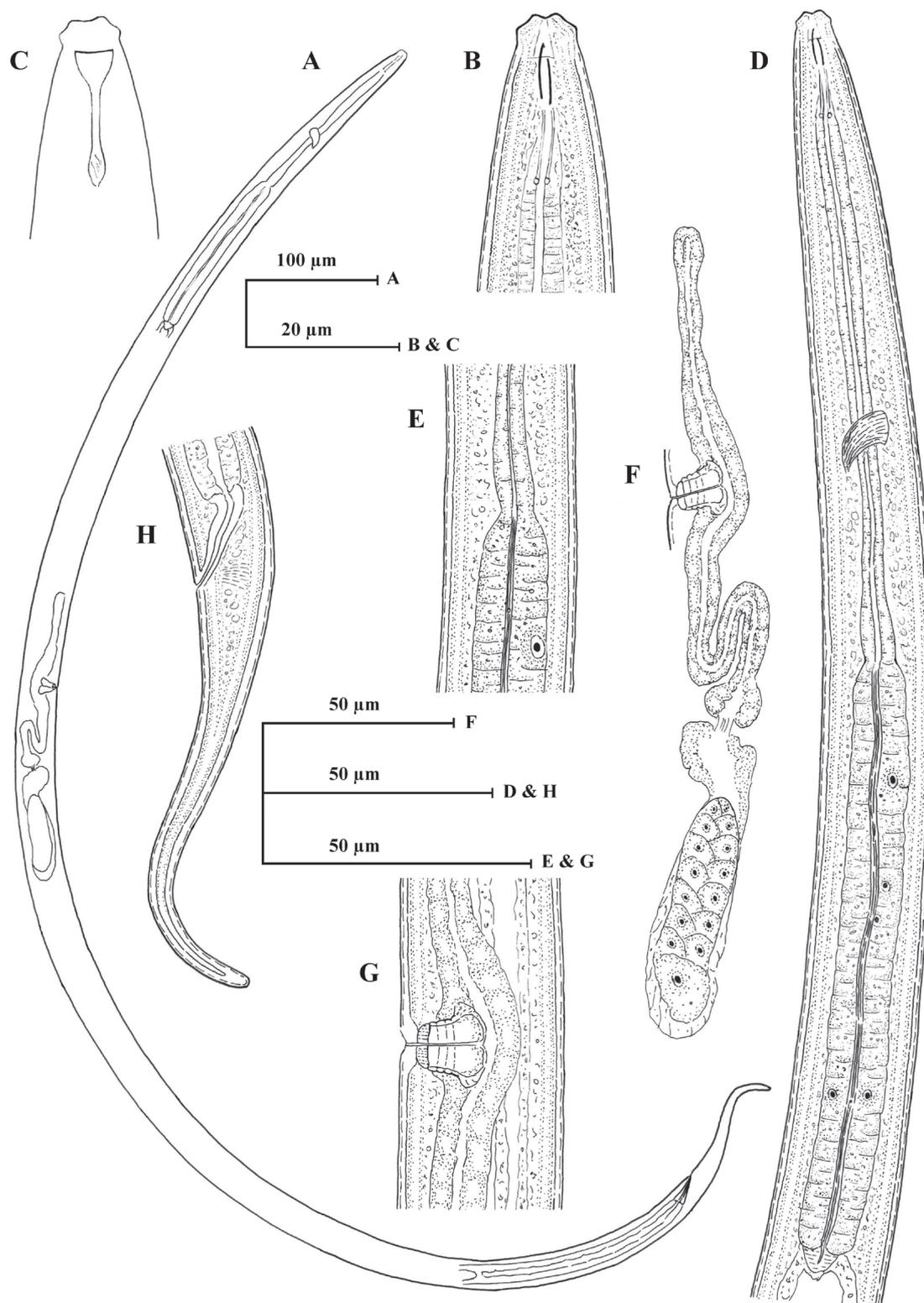
Characters	Holotype female	Paratype females	Juveniles
n	1	6	2
L	1413	1379.8 $\pm$ 34.9 (1332–1430)	1022, 1077
Body diameter at neck base	27	28.1 $\pm$ 0.68 (27–29)	21.5, 22.5
Body diameter at mid body	30	31 $\pm$ 0.48 (30–31)	21.5, 22.5
Body diameter at anus	16.5	17.3 $\pm$ 0.44 (16.5–17.5)	15.0, 15.5
a	46.5	43.8 $\pm$ 1.2 (43.1–46.7)	47.4, 47.8
b	5.2	5.01 $\pm$ 0.10 (4.9–5.2)	4.2, 4.4
c	15.3	15.2 $\pm$ 1.0 (13.7–17.0)	12.9, 13.7
c'	5.5	5.0 $\pm$ 0.42 (4.5–5.8)	5.4, 5.0
V	39.5	39.2 $\pm$ 1.1 (38.3–41.9)	–
G1	4.9	3.1 $\pm$ 0.82 (2.4–3.4)	–
G2	13.8	12.6 $\pm$ 0.90 (11.5–14.2)	–
Lip region diameter	8.0	8.0	7.5, 7.0
Lip region height	4.0	3.7 $\pm$ 0.24 (3.5–4.0)	3.5, 3.0
Amphid aperture	5.0	4.4 $\pm$ 0.43 (4.0–5.0)	4.0, 4.0
Odontostyle length	8.5	8.4 $\pm$ 0.31 (8.0–9.0)	7.0, 6.5
Replacement odontostyle length	–	–	8.0, 7.5
Odontophore length	10.5	10.7 $\pm$ 0.31 (10.5–11.5)	11, 10.5
Total stylet length	19	19.1 $\pm$ 0.45 (19–20)	18, 17.5
Guiding ring from anterior	6.0	5.7 $\pm$ 0.18 (5.5–6.0)	5.5, 5.5
Nerve ring from anterior	93	93.2 $\pm$ 0.62 (92–94)	83, 90
Neck length	271	270.2 $\pm$ 4.9 (259–276)	243, 246
Expanded part of pharynx	127	125.8 $\pm$ 4.7 (115–131)	110, 115
Cardia length	6.0	6.4 $\pm$ 0.88 (5.0–7.0)	7.5, 6.0
Anterior genital branch	69	40.1 $\pm$ 4.4 (34–48)	–
Posterior genital branch	195	178.7 $\pm$ 10.1 (168–195)	–
Vaginal length	14.0	14.4 $\pm$ 0.44 (13.5–14.5)	–
Vulva from anterior	558	553.5 $\pm$ 13.2 (539–579)	–
Prerectum length	157	156.6 $\pm$ 13.0 (129–176)	121, 139
Rectum length	20.5	21.5 $\pm$ 1.2 (19–23)	18.5, 19.5
Tail length	92	89.3 $\pm$ 9.3 (79–103)	79, 78

from the rest of the lip region; labial and cephalic papillae button-like, slightly protruding over the lip surface. Amphidial fovea cup-shaped, aperture slit-like, 4.0–5.0  $\mu\text{m}$  wide or occupying about one-half to three-fifths of lip region diameter. Stoma a truncate cone. Odontostyle short, robust, spindle-shaped with a wide lumen, 1.0–1.1 times the lip region diameter long or 0.54–0.63% of total body length, its aperture about one-fourth of the odontostyle length. Odontophore simple, rod-like with slightly thickened base or minute knobs-like structure (Fig. 1B, C), 1.2–1.4 times the odontostyle length. Guiding ring simple refractive, at 0.7–0.8 times lip region diameter from anterior end. Pharynx consisting of a slender and weakly muscular anterior part, expanding abruptly into a cylindroid bulb, separated by slight constriction, occupying about 42–49% of total pharyngeal length. Dorsal pharyngeal gland nuclei located at 12–16% of expanded part of pharynx from its expansion. Pharyngeal gland nuclei and



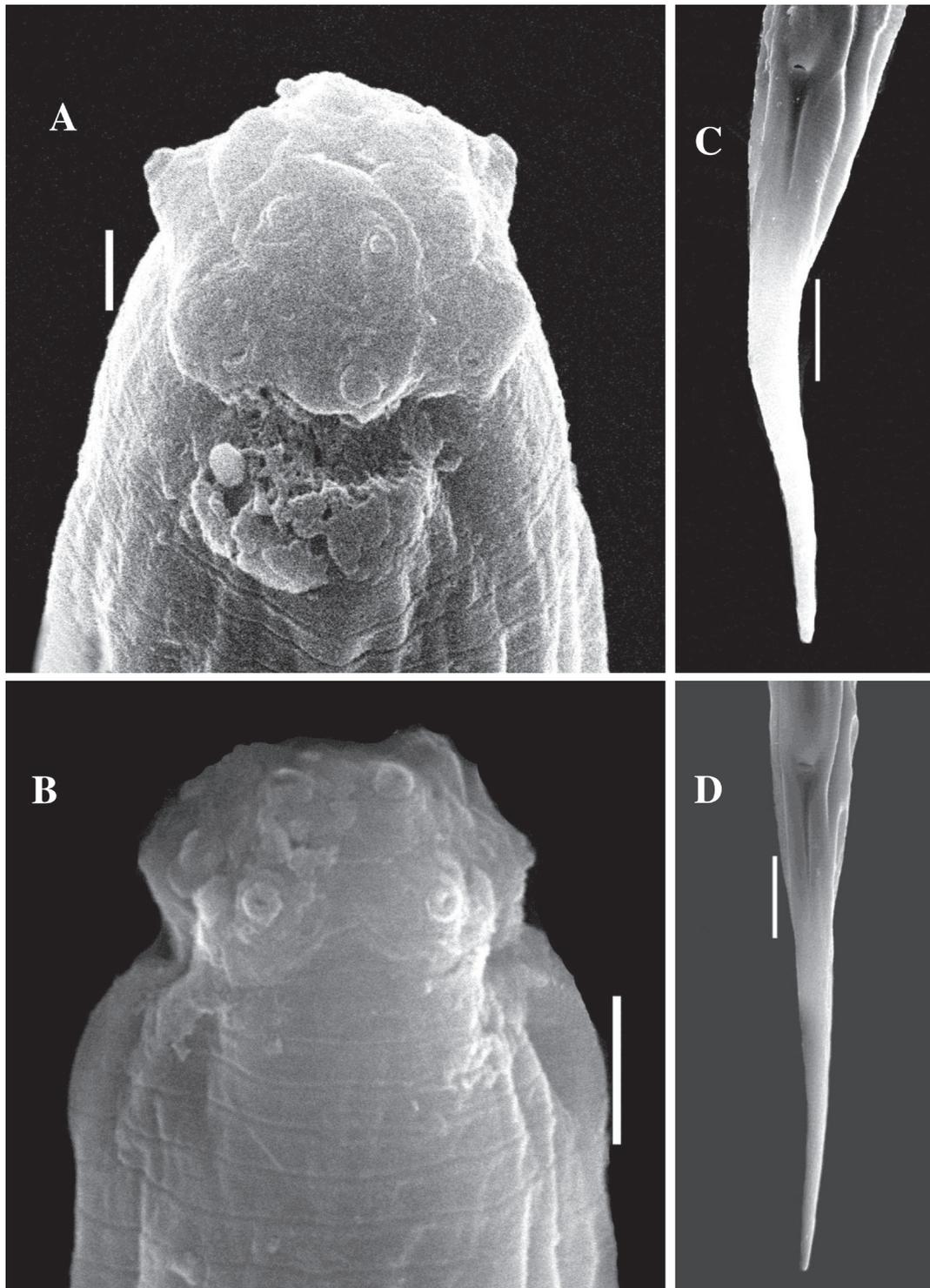
**Figure 1.** *Tahamina indica* gen. nov., sp. nov. female: **A** entire **B, C** anterior region (arrow showing knobs-like structure) **D** anterior region showing amphid **E** pharyngeal region **F** expanded part of pharynx (arrow showing dorsal pharyngeal gland nuclei) **G** pharyngo-intestinal junction. Scale bars: 100 µm (**A**); 10 µm (**B–D, F–G**); 20 µm (**E**).

their orifices are located as follows: DO=58–60, DN=60–62, DO–DN=1.5–2.5, S1N1=74–76, S1N2=79–81, S2N2=88–90, S2O=90–91. Nerve ring at 34–35% of the pharyngeal length from anterior end. Cardia is short, conoid, about one-fifth to one-fourth of the corresponding body diameter long.



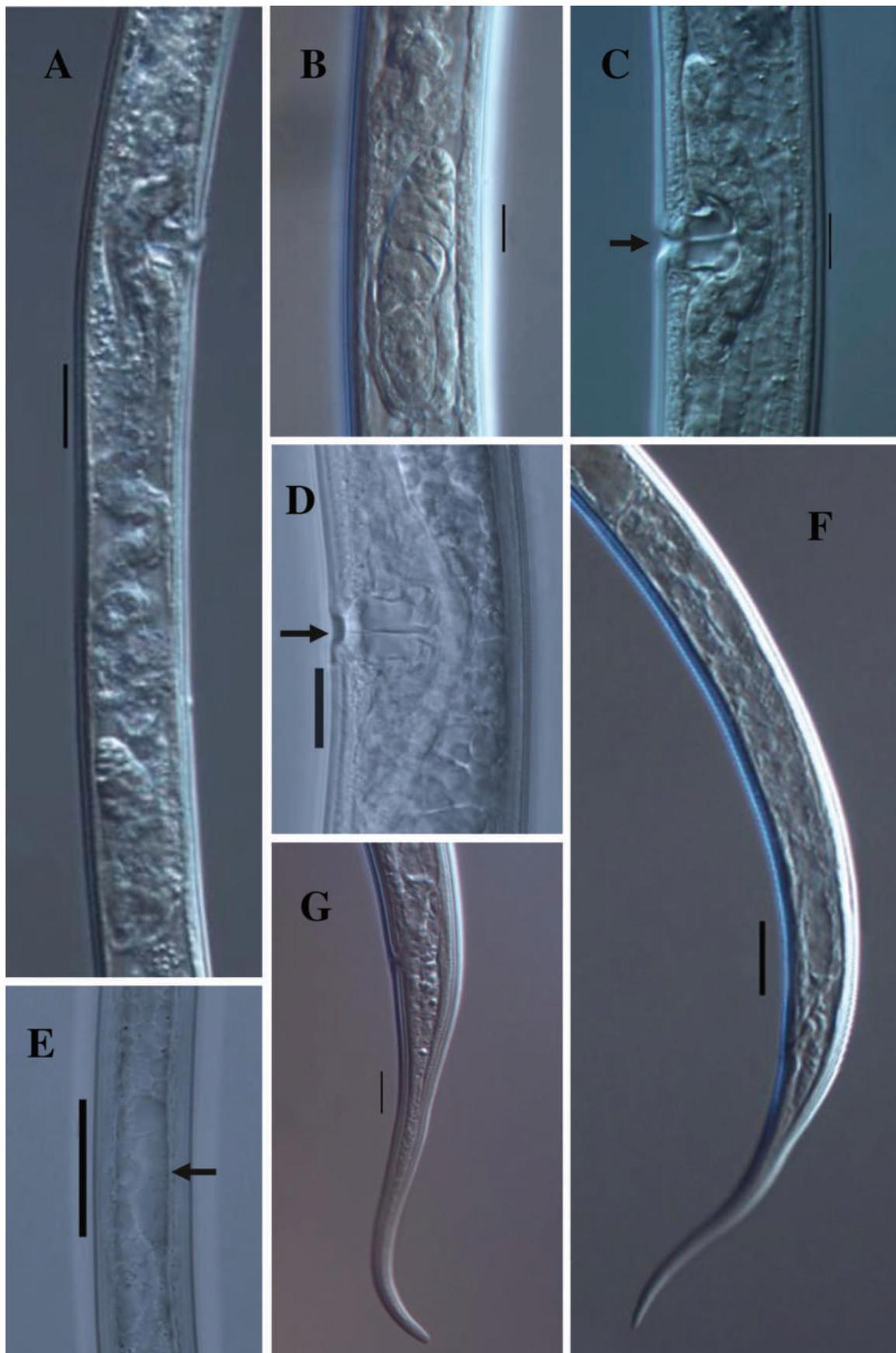
**Figure 2.** *Tahamina indica* gen. nov., sp. nov. female: **A** entire **B** anterior region **C** anterior region showing amphid **D** pharyngeal region **E** pharyngeal expansion **F** genital system **G** Vulval region **H** posterior end.

Genital system mono-opistho-ovarian, didelphic. Ovary reflexed, measuring 50–78  $\mu\text{m}$  long, not reaching oviduct-uterus junction; oocytes arranged in a single row except the near tip. Oviduct joining the ovary subterminally, measuring 57–82  $\mu\text{m}$ , its consisting of a slender distal portion and a well-developed



**Figure 3.** *Tahamina indica* gen. nov., sp. nov. female (SEM): **A** anterior region frontal view **B** anterior region lateral view **C, D** posterior end showing anal opening. Scale bars: 1  $\mu\text{m}$  (**A**); 2  $\mu\text{m}$  (**B**); 10  $\mu\text{m}$  (**C, D**).

*pars dilatata*. Oviduct-uterus junction marked by the well-developed sphincter. Uterus long well differentiated, tripartite, proximal part short well-developed muscular, median part long, convoluted tube and distal part short, somewhat spheroid, measuring 96–109  $\mu\text{m}$ . The anterior genital branch reduced to a simple sac, 34–69  $\mu\text{m}$  or about 1.0–2.2 times midbody diameters long. Vagina cylindrical, extending inwards, 13.5–14.5  $\mu\text{m}$  or about one-half (45–48%) midbody



**Figure 4.** *Tahamina indica* gen. nov., sp. nov. female: **A, B** genital system **C, D** vulval region **E** arrow showing genital primordia in juvenile **F** posterior region **G** posterior end. Scale bars: 20  $\mu$ m (**A, F**); 10  $\mu$ m (**B-D, E-G**).

diameter; *pars proximalis vaginae* 10–12 × 6.0–8.0 µm, its wall encircled by circular muscles; *pars distalis vaginae* 2.5–3.5 µm with slightly curved walls; *pars refringens* absent. Vulva pore-like. Sperm cell absent. Prerectum long, 7.3–10.0 and rectum 1.1–1.4 anal body diameter long. Tail elongated, tapering gradually and its distal part slightly bent dorsally with a rounded terminus, 4.3–5.8 times anal body diameter long with a pair of caudal pores on each side.

**Male.** Not found.

**Juvenile (J2).** General morphology is similar to that of adults, but with the following differences. Medium sized nematodes, 1.0 mm long. Lip region cap-like, 2.1–2.3 times as broad as high or about one-third (29–34%) of body diameter at the pharyngeal base. Functional odontostyle 0.90 times the lip region diameter long, 0.80 times the replacement odontostyle length. Replacement odontostyle 1.0 times the lip region diameter long, with its base located at 45–48 µm from the anterior end. Position of odontostyle forming cell nucleus at 48–49 µm from the anterior end and at 26–28 µm from the odontophore base. Odontophore simple, rod-like, 1.5–1.6 times of functional odontostyle length. Guiding ring simple and refractive, at 0.7 times the lip region diameter from the anterior end. Pharyngeal expansion occupying 45–46% of the total pharyngeal length. Nerve ring at 33–34% of the total pharyngeal length from anterior end. Genital primordia 18–19 µm or 0.8–0.9 times the corresponding body diameter long, located at 442–445 µm or 41–43% of body length from the anterior end.

**Type habitat.** Soil samples collected around the roots of grasses (*Cynodon dactylon* L.) from Yaraganalu, Shivamoga District, Karnataka State, India.

**Etymology.** The new genus and new species named after first author mother's Tahamina and its type locality India.

## Discussion

In the present study, we have compared the new genus with some closely related genera either belong to Tylencholaimoidea (*Tylencholaimus*, *Heynsnema*, *Discomyctus*, *Wasimellus*, *Dorylaimoides*) or Dorylaimoidea (*Mitoaxonchium*, *Hulqus*). The shape and size of the stylet, and the ratio of the expanded part of the pharynx of *Tahamina* displayed a close relation with some members of tylencholaimid nematodes. Similarly, based on especially the lip region and stylet morphology, we cannot ignore a comparison with two interesting members of the subfamily Hulqinae (*Mitoaxonchium*, *Hulqus*). Siddiqi (1981) proposed the subfamily Hulqinae under the family Discolaimidae (Dorylaimoidea) for the new genera *Hulqus* and *Mitoaxonchium* based on the length of the pharynx, more than one-third of body length ( $b = 2.4–3.2$ ) and the position of dorsal pharyngeal gland nuclei at one-third length of expanded part of pharynx from its expansion. Although the new genus is comparable to members of Hulqinae based on the morphology of lip region and stylet, other morphology does not support its position under Hulqinae due to the presence of a shorter pharynx ( $b = 4.9–5.2$ ) and the more anterior position of the dorsal pharyngeal gland nuclei (12–16 vs. 30% of expanded part of pharynx from its expansion). Furthermore, the cuticle of tylencholaimids is of two types: tylencholaimoid type; the inner layer usually thicker than the outer, loose, irregular outline, with distinct radial refractive elements (Tylencholaimidae and Leptonchidae) or dorylaimoid type; the inner layer of cuticle compacts, not loose, lack radial refractive elements but more or less distinctly striated (Tylen-

cholaimellidae, Aulolaimoididae and Mydonomidae). Although, in the absence of radial refractive elements in our newly proposed genus, *Tahamina* is not supported under the family Tylencholaimidae, other morphological characteristics such as the shape and size of the stylet, the weakly muscular pharyngeal expansion, and the position of the dorsal esophageal gland nuclei well support the new genus under the subfamily Tylencholaiminae (Tylencholaimidae, Tylencholaimoidea).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

MNI collected and processed the soil samples, and made all the permanent mount, measurement, comparison, line drawings, and photography of the species. WA designed the work and helped to review the manuscript. Both the authors read, drafted, approved the final version of the manuscript.

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### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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# *Graphidessa jinensis*, a new species of longhorned beetle (Coleoptera, Cerambycidae, Lamiinae, Desmiphorini) from China

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## Abstract

*Graphidessa jinensis* **sp. nov.** is described from Chongqing and Guizhou in Southwest China. The diagnostic morphological characters of the new species are described and illustrated in color plates. The distribution of all species of the genus *Graphidessa* Bates, 1884 is mapped and the key to all species of this genus is updated. The *COI* gene sequence of the new species is also provided.

**Key words:** Chongqing, *COI* gene, genitalia, key, taxonomy



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## Introduction

*Graphidessa* Bates, 1884 is a small genus belonging to the subfamily Lamiinae Latreille, 1825. This genus is characterized by legs covered with abundant yellowish-white pubescence and long black setae, and each elytron has longitudinal haired stripes and a strongly tuberculate base. *Graphidessa* currently contains three described species and one subspecies (Bates 1884; Hayashi 1974; Komiya and Kusama 1974; Fujita 1980), and all of them were recorded only in East Asia. *Graphidessa obliquefasciata* Komiya & Kusama, 1974 and *Graphidessa variegata* Hayashi, 1974 were recorded in Taiwan Island, China (Hayashi 1974; Komiya and Kusama 1974); *Graphidessa venata venata* Bates, 1884 and *Graphidessa venata takakuwai* Fujita, 1980 were recorded in Japan (Bates 1884; Fujita 1980).

## Material and methods

One male specimen and one female specimen used in this study were acquired from the project: Biodiversity along Elevational gradients: Shifts and Transitions (BEST). Malaise traps were used in the project to collect insects along the

elevation gradient of the Jinfoshan National Nature Reserve, Chongqing, China. The other two female specimens used were collected from Guizhou, China. The collected specimens were carefully preserved in 95% alcohol and stored at -20 °C. The whole genomic DNA was extracted from the abdomen of the male specimen using a Mirco Cell/Tissue DNA Kit (Biomarker Technologies), following the revised operation steps of the manufacturer's manual: each sample was pierced with a hole on the abdomen with a fine needle, and incubated in lysis buffer for at least 12 hours on a constant temperature shaker to fully split the DNA; the centrifuge column was kept at room temperature for 3 mins before adding Elution Buffer.

The barcoding region of *COI* (mitochondrial cytochrome c oxidase subunit I) was amplified using the following primers: LCO1490 (5'-GGTCAACAATCATA-AAGATATTG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAT-3') (Folmer et al. 1994). The polymerase chain reaction (PCR) amplifications were performed using a 25 µL reaction volume with the mixture of 1 µL template DNA, 0.5 µL forward and reverse primer (10 µM), 0.25 µL Taq DNA polymerase (5 U/µL), 18.25 µL double distilled H<sub>2</sub>O, 2.5 µL 10× buffer and 2 µL dNTP. The *COI* fragment amplification was carried out with the following conditions: 5 min of initial denaturation at 95 °C, 35 cycles of 20s at 94 °C, 30s at 54 °C (the annealing temperature) and 72 °C at 2 min, and 10 min of final extension at 72 °C. The products of PCR were run in 1% agarose gels and bidirectionally sequenced at Sangon Biotech (Shanghai, China). Geneious R11 (Auckland, New Zealand) was used to check the quality of the chromatograms, and export them as FASTA format. The *COI* sequence was submitted to GenBank under the accession number: OR366841.

The description of the new species was made using a Leica M165C stereo microscope, aided by Leica LED 5000 HDI dome light source. Images were captured using a Leica MC170 HD digital camera attached to the microscope, and subsequent measurements of the specimens, including body length and antennae, were conducted using Leica MC170 software. Serial images were aligned and merged using Zerene Stacker ([www.zerene.com](http://www.zerene.com)) software, while Adobe Photoshop 2021 ([www.adobe.com](http://www.adobe.com)) was employed to refine and enhance the visual clarity of the images. The distributions of *Graphidessa* species were obtained and annotated on the map using the online tool SimpleMapper.

The holotype and one of the paratypes are deposited in the Insect Systematics and Diversity Lab, Fujian Agriculture and Forestry University (FAFU), Fuzhou, China, and the two remaining paratypes are deposited in the School of Biological Science and Technology, Liupanshui Normal University, Liupanshui, Guizhou, China (LPSNU).

## Results

*Graphidessa jinfoensis* sp. nov. is described based on the specimens collected in the Jinfoshan National Nature Reserve of Chongqing and Dongfeng Lake National Wetland Park of Guizhou in Southwest China, which constitute the first specimen records of the genus *Graphidessa* from mainland China. The genus *Graphidessa* now consists of four species (including one species with two subspecies), three of which are distributed in China (Fig. 1).

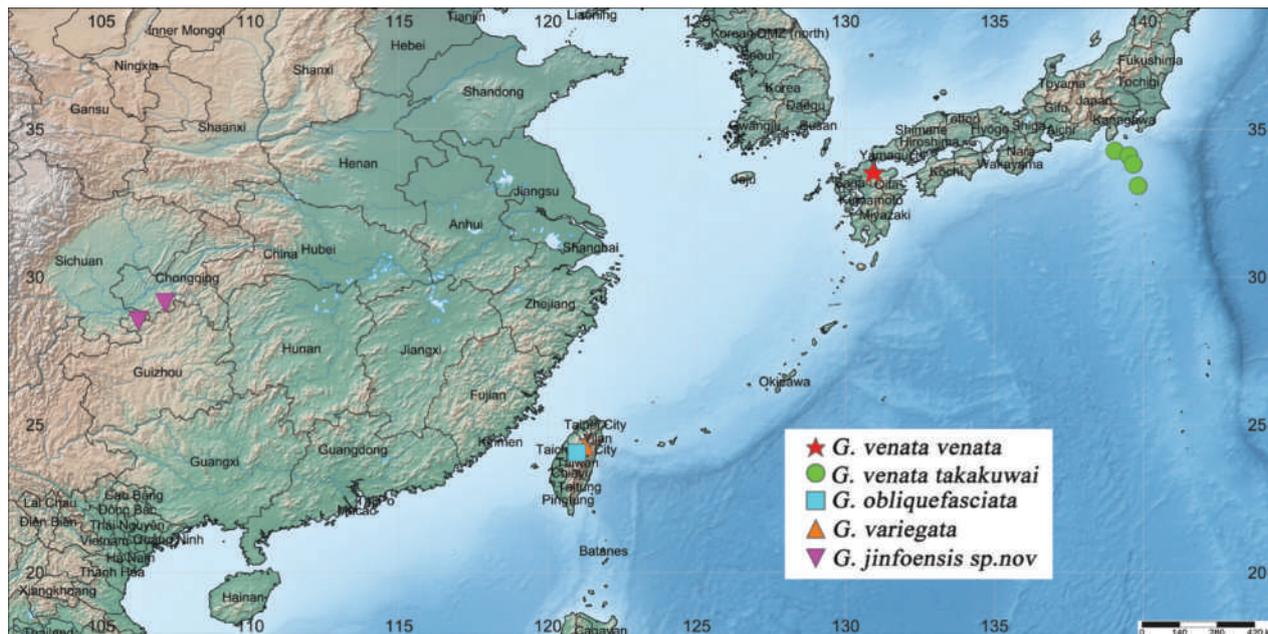


Figure 1. Distribution of *Graphidessa* species.

***Graphidessa jinfoensis* sp. nov.**

<https://zoobank.org/C28A8611-9D3A-4E01-8750-54F638FC47AD>

Figs 1–6, 7D

**Type materials examined.** Specimen code: CQ114714. **Holotype**, ♂, glued on paper point, with genitalia in a separate centrifugal tube. Original label: “中国重庆金佛山国家级自然保护区, 2022年10月30日, 马氏网, 周礼华采” [Jinshoshan National Nature Reserve, Chongqing, China, 2022.X.18, malaise trap, Lihua Zhou leg. (FAFU)], HOLOTYPE / *Graphidessa* / *Jinfoensis* / Xiaolei Huang Geanbank ac-

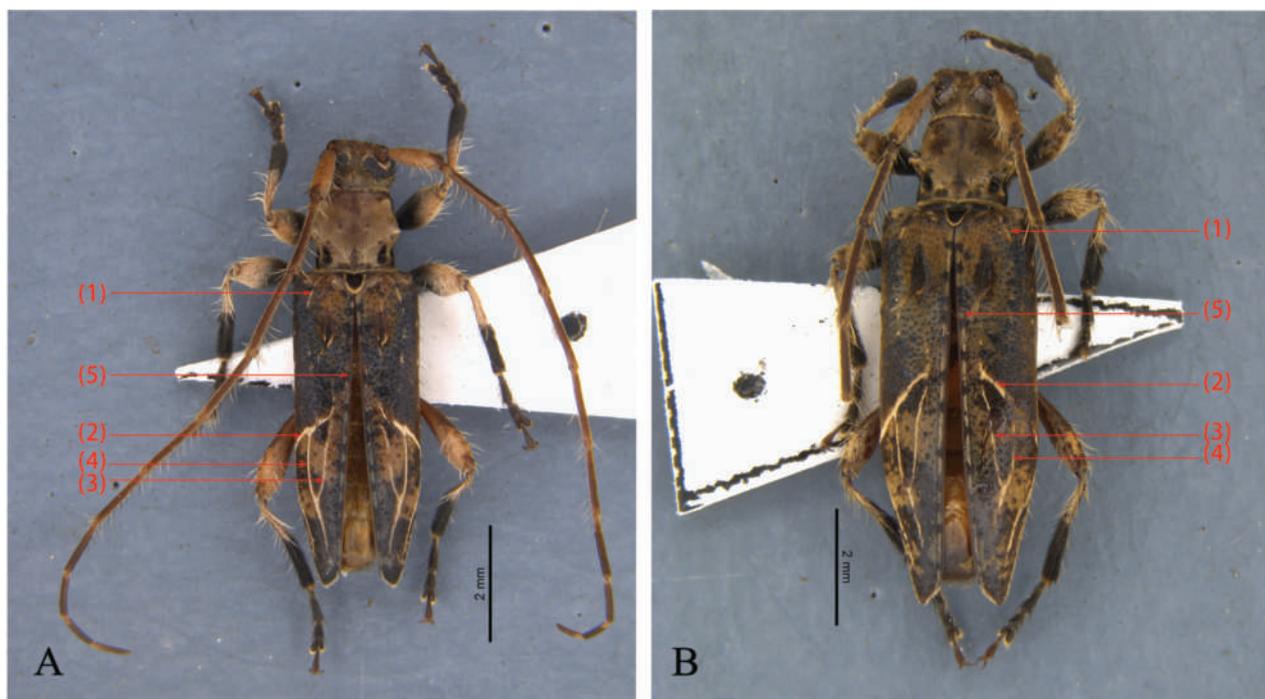


Figure 2. *Graphidessa jinfoensis* sp. nov. habitus, dorsal view **A** holotype, male **B** paratype, female.

cession number: OR366841 [handwritten red label]. **Paratype:** 1♀, Original label: “中国重庆金佛山国家级自然保护区,2022年5月15日,马氏网,周礼华采” [S14, Jin-foshan National Nature Reserve, Chongqing, China, 2022.V.15, malaise trap, Li-hua Zhou leg. (FAFU)]; 2♀♀, Dongfeng Lake National Wetland Park, Xishui County, Zunyi City, Guizhou Province, China, 23.X.2022, leg. Xiudong Huang (LPSNU).

**Diagnosis.** The new species can be distinguished from its closest congeners by the dark brown body, densely covered with short chestnut-like pubescence, and the unique elytral pattern. Head and most of the pronotum are densely covered with short brown hairs, the ventral surface of the prothorax is black, the dorsum of the prothorax is brown with some black patches. Elytra dark brown and covered with short brown pubescence, and black, brown and grayish setae; the white pubescence forming five stripes on each elytron. The bump near elytral base is covered with many long setae, the black setae shorter than the brown setae.

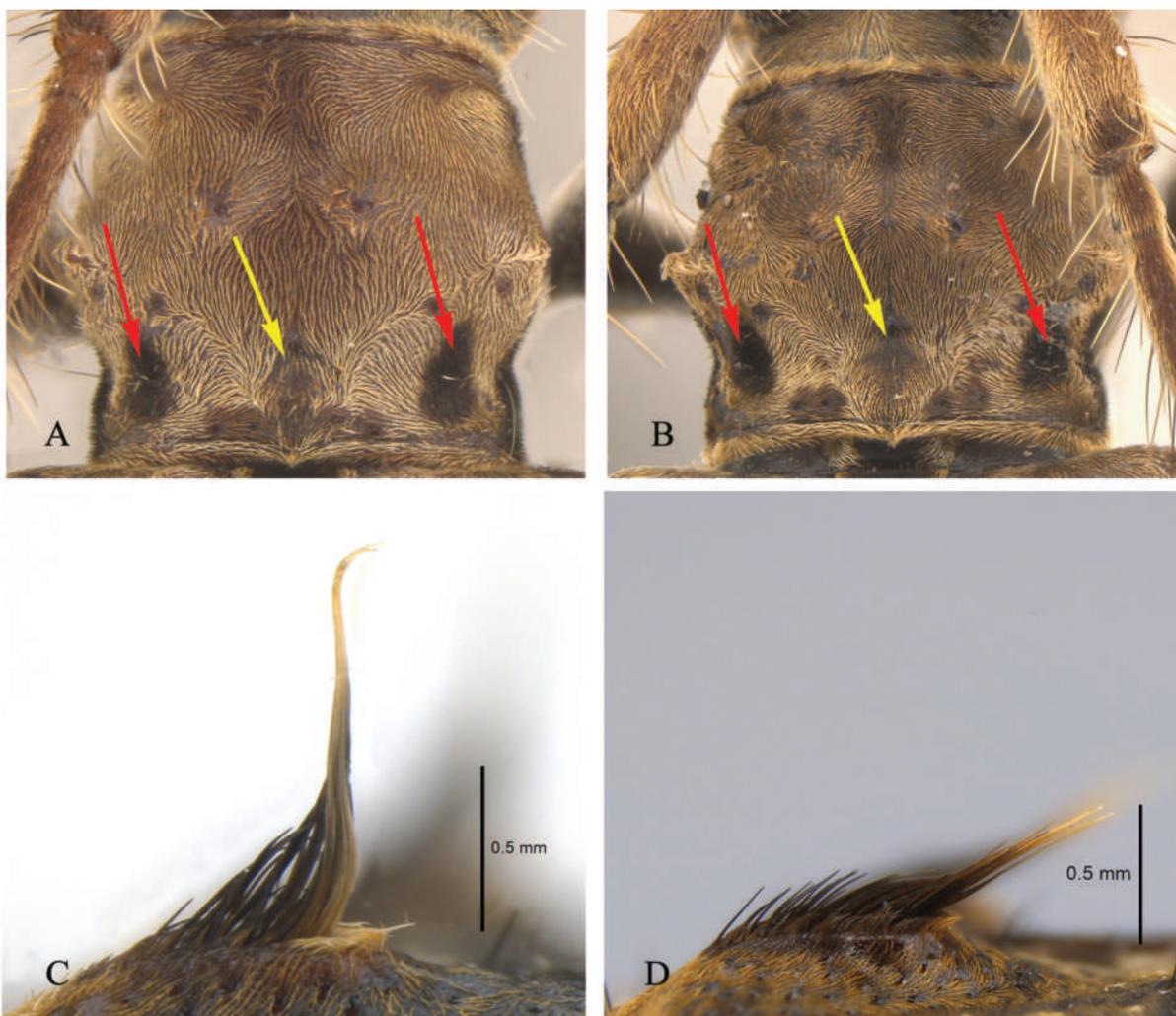
**Description. Male** (Figs 2A, 3A), holotype. **Body length:** 6.2 mm ( $N = 1$ ). **Head:** brown, covered with short brown hairs. Forehead covered with long pale



**Figure 3.** *Graphidessa jinfoensis* sp. nov., habitus, lateral view **A** male (holotype) **B** female (paratype).

brown setae near mouthpart. Scape densely covered with short brown hairs and a few dark brown and yellowish setae; antennomeres II–VII covered with densely short brown hairs and sparsely long black setae beneath; antennomeres VIII–XI covered with short, dense brown hairs. Each antennomere with a brown recumbent seta apically. Length ratio of antennomeres from base to tip: 4.7: 1: 5.4: 6.7: 5.9: 5.5: 5.1: 4.1: 3.4: 3.5: 3.3 (Figs 2A, 3A). **Thorax:** ventral surface of thorax with brown pubescence, interspersed with a few long, erect brownish setae. Two small patches of dark brown short pubescence near the posterior margin of the anterothoracic backplane (two small patches on each side, they are marked with red arrows in Fig. 4A) three petal-like patches of light brown short pubescence precede peltate (three petal-like patches are between the two patches. Three petal-like patches are marked with yellow arrows in Fig. 4A). Prosternum black. (Figs 2A, 4A).

**Legs:** all legs with abundant yellowish-white pubescence and black setae. The basal half of pro- and mesofemora mostly black, covered with short, dense brown hairs and interspersed with a few long erect brownish setae; the apical half of pro- and mesofemora covered with densely short yellowish-white hairs dorsally and interspersed with a few long erect yellow-



**Figure 4.** *Graphidessa jinfoensis* sp. nov. **A, B** details of pronotum **C, D** details of the bump near base of the elytra **A, C** male (holotype) **B, D** female (paratype), not to scale (**A, B**).



**Figure 5.** *Graphidessa jinfoensis* sp. nov. holotype, male genitalia **A–C** tegmen **D–F** median lobe (**A, D** dorsal view **B, E** lateral view **C, F** ventral view).

ish-white setae. Basal half of pro- and mesotibiae with yellowish-white pubescence, obscuring integument, and some setae. Apical half of tibiae with black pubescence obscuring integument and a few long, erect black setae interspersed. Metatarsomere I 0.97 times as long as II–III together (Figs 2A, 3A). **Elytra:** sparsely covered with erect setae, generally black, with white, golden, and brownish pubescence interspersed. Elytra dark areas more than half, light color areas sparsely covered with golden pubescence, dark areas with fewer golden pubescence. Strongly tuberculate at base covered with long and dense setae, posterior setae longer than anterior ones (bump near

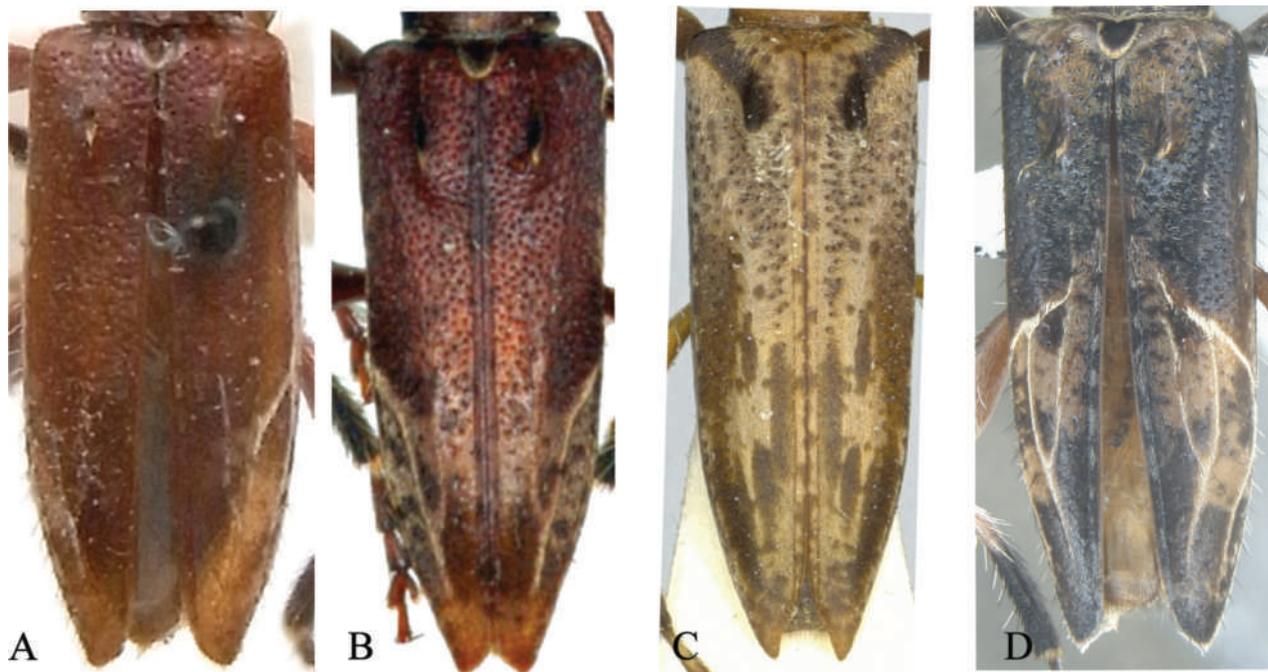


**Figure 6.** *Graphidessa jinfoensis* sp. nov., paratype, female ovipositor **A** dorsal view **B** showing ovipositor dorsal view details **C** lateral view.

elytral base covered with long and dense black setae anteriorly, with dense yellowish-brown setae posteriorly; yellowish-brown setae much longer than black setae, up to 13 mm); disc about 2.71 times as long as humeral width.

Each elytron covered with five longitudinal haired stripes, the first stripe yellowish white, starting from the middle of the basal of elytron, and about one-seventh of elytron length; the second stripe yellowish white, starting at the middle of elytron near suture, extending to the two-thirds of elytron length near outer margin, and ends near outer apical angle; the third yellowish white, starting at the base of the second stripe, extending straight toward outer apical angle, and stops at half of the elytra width; the fourth stripe yellowish white, starting at the intersection of the centerline of elytron and the second stripe, and joins to the third stripe at the middle; a series of short greyish white haired stripes form the fifth stripe, starting from posterior of scutellum and extending along the suture to inner apical angle. Outer margin with some off-white long sub-erect grayish white setae. **Abdomen:** abdominal ventrites covered with long and sparse brown hairs. **Male genitalia** (Fig. 5): tegmen (Fig. 5A–C), lateral lobes near parallel, then gradually narrowing towards apices, rounded and sparsely covered with long brown setae. Median lobe (Fig. 5D–F) moderately curved in profile, median struts about 0.5 times as long as median lobe, ventral plate truncated at apex, dorsal plate narrower than ventral plate, slightly concave at apical middle. **Female genitalia** (Fig. 6A–C): with ovipositor elongate, narrow, apically with short styli.

**Female** (Figs 2B, 3B), paratypes. **Body length:** 7.5–9 mm ( $N = 3$ ) Similar to male, but elytra about 2.64 times as long as humeral width; bump near elytral base covered with shorter yellowish-brown setae up to 8 mm.



**Figure 7.** Dorsal view of elytra **A** *Graphidessa venata venata* Bates, 1884 ♂ holotype **B** *Graphidessa venata takakuwai* Fujita, 1980 ♂ holotype **C** *Graphidessa variegata* Hayashi, 1974 ♂ holotype **D** *Graphidessa jinfoensis* sp. nov. ♂ holotype.

**Etymology.** The scientific name is derived from the Jinfoshan National Nature Reserve, where the holotype was collected.

**Distribution.** China: Chongqing, Guizhou (Fig. 1).

**Remarks.** Left antennomeres V–XI were separated from antennomere IV for one paratype from Guizhou. Right antennomeres IV–XI were missing, the black and yellowish-brown setae on the bump near the elytral base were worn off, right hind leg was separated from body in another paratype from Guizhou.

## Discussion

Information on *Graphidessa obliquefasciata* is notably scarce in online databases like GBIF, Catalogue of Life, Taiwan Encyclopedia of Life and Titan (Tavakilian and Chevillotte 2023). The holotype of this species also cannot be traced in museums worldwide. Moreover, despite multiple attempts, no images of the type specimen of *G. obliquefasciata* could be obtained. Therefore, in this paper, the description of *G. obliquefasciata* was solely derived from the original literature (Komiya and Kusama 1974). In order to obtain more details on the morphology, ecology and bionomy of this species, more extensive sampling should be conducted in the future.

The picture of a male of *G. variegata* in the Atlas of Cerambycidae of Taiwan (Chou, 2008) looks similar to the male of *G. jinfoensis* sp. nov. However, after comparison, we found that the pattern of the elytral stripe (3) and the color of the hind femora of the male specimen shown in Chou's Atlas are very different from *G. jinfoensis* sp. nov. The elytral stripe (3) in that specimen is disconnected, and the color of the hind femora are dark brown, while the overall color of the body is light brown. On the contrary, the elytral stripe (3) in *G. jinfoensis* sp. nov. is continuous, and the hind femora are light brown,

with many yellowish long setae and soft hairs. And the body color of *G. jinfoensis* sp. nov. is dark brown (Fig. 2). Regarding the morphological comparison with the holotypes of the remaining described *Graphidessa* species, there is a reasonable suspicion that the male sample named as *G. variegata* in *The Atlas of Cerambycidae of Taiwan* represents a yet undescribed taxon.

### Key to species of *Graphidessa* Bates, 1884

- 1 Each elytron with less than five stripes..... **2**
- Each elytron with five stripes..... **3**
- 2 Each elytron covered at the base with an oblique short black-haired band basally ..... ***G. variegata***
- Each elytron not covered at the base with an oblique short black-haired band basally..... **4**
- 3 Each elytron with five yellowish stripes, body dark brown.....  
..... ***G. jinfoensis* sp. nov.**
- Each elytron with five blackish stripes, body light reddish brown.....  
..... ***G. obliquefasciata***
- 4 Elytra with relatively sparse yellow-white short hairs ..... ***G. venata venata***
- Elytra with relatively dense yellow-white short hairs..... ***G. venata takakuwai***

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### Additional information

#### Conflict of interest

The authors have declared that no competing interests exist.

#### Ethical statement

No ethical statement was reported.

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### Author contributions

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### Data availability

All of the data that support the findings of this study are available in the main text

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# A new species of shrew moles, genus *Uropsilus* Milne-Edwards, 1871 (Mammalia, Eulipotyphla, Talpidae), from the Wuyi Mountains, Jiangxi Province, eastern China

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## Abstract

Asian shrew moles, genus *Uropsilus*, are the most primitive members of family Talpidae. They are distributed mainly in southwestern China and adjacent Bhutan, Myanmar, and Vietnam. In June 2022, we collected five specimens of *Uropsilus* from Mount Huanggang, Jiangxi Province, eastern China, which is the highest peak of the Wuyi Mountains. We sequenced two mitochondrial (*CYT B* and *12S rRNA*) and three nuclear (*PLCB4*, *RAG1*, and *RAG2*) genes to estimate the phylogenetic relationship of the five shrew moles. We also compared their morphology with recognized species within the genus. Our results show that these specimens collected from Mount Huanggang differ from all named species in *Uropsilus*. We formally describe the species here as *Uropsilus huanggangensis* **sp. nov.** Morphologically, the new species is distinguishable from the other *Uropsilus* species by the combination of dark chocolate-brown pelage, long snout, enlarged first upper incisor, similarly sized lacrimal and infraorbital foramina, and the curved and sickle-like coronoid process. The genetic distances of the cytochrome b (*CYT B*) gene between *U. huanggangensis* and other recognized *Uropsilus* species ranged between 9.3% and 16.4%. The new species is geographically distant from other species in the genus and is the easternmost record of the *Uropsilus*. The divergence time of *U. huanggangensis* was estimated to be the late Pliocene (1.92 Ma, 95% CI = 0.88–2.99).

**Key words:** Mount Huanggang, small mammals, taxonomy, Uropsilinae

## Introduction

The shrew moles of the genus *Uropsilus* Milne-Edwards, 1871 are the sole living genus in the subfamily Uropsilinae in Talpidae (Mckenna et al. 1997; Hutterer 2005). These insectivores primarily inhabit the mountains of southwestern China, as well as adjacent areas in Bhutan and northeastern Myanmar, where they inhabit montane forests at 1,400–3,600 m elevation (IUCN 2015; Hoffmann



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and Lunde 2008). Although the fossil record is sparse, the age of the associated fossils and the timing of the molecular evolution of mammals suggest that the subfamily Uropsilinae would have flourished and spread widely across Eurasia before the Late Miocene (Meredith et al. 2011). In contrast to other moles that have developed adaptive features such as broad front claws and reduced external ears, shrew moles exhibit shrew-like characteristics, including slender front claws, exposed external ears, and long tails almost equal in length to their bodies. All these morphological characteristics suggest that they have retained the terrestrial habits of primitive moles (Allen 1938). Phylogenetic relationships constructed using morphological methods (Motokawa 2004; Sánchez-Villagra et al. 2006) and molecular phylogenetic methods (Douady and Douzery 2003; Shinohara et al. 2003) consistently support that *Uropsilus* forms the basal branch in the phylogenetic tree of the family Talpidae.

The genus *Uropsilus* was first described by Milne-Edwards (1871) based on the specimens from Muping (= Baoxing) in Sichuan, China. The type species of the *Uropsilus* is *U. soricipes* Milne-Edwards, 1871, which has a dental formula of: I 2/1, C 1/1, P 3/3, M 3/3 = 34. Thomas (1912) described two new species belonging to two new genera: *Rhynchonax andersoni* Thomas, 1911 from Mount Omi-san (= Mount Emei), Sichuan, with the dental formula: I 2/2, C 1/1, P 4/3, M 3/3 = 38; and *Nasillus gracilis* Thomas, 1911 from Chin-fu-san (= Jinpo shan), Chongqing, with the dental formula: I 2/1, C 1/1, P 4/4, M 3/3 = 38. Later, Thomas (1922) described *N. investigator* Thomas, 1922, based on larger specimens collected in the Kia-kiang-Salween of Yunnan compared to *N. gracilis*. Additionally, Allen (1923) described two new subspecies of *R. andersoni*: *R. andersoni atronates* Allen, 1923 from Salween drainage, Yunnan; and *R. andersoni nivatus* Allen, 1923 from Lijiang, Yunnan, China. However, the classification of the shrew moles into three genera had been widely disputed. Osgood (1937) considered two genera (*Uropsilus* Milne-Edwards, 1871 and *Nasillus* Thomas, 1911) in the Uropsilinae and merged the genus *Rhynchonax* Thomas, 1911 into the genus *Uropsilus*. Ellerman and Morrison-Scott (1951) assigned all genera to *Uropsilus* and placed the named species in five subspecies of *U. soricipes*: *U. s. soricipes*, *U. s. gracilis*, *U. s. andersoni* (including *atronates*), *U. s. investigator* and *U. s. nivatus*. The proposition of one genus is recognized by most scholars (Cranbrook 1960–1961; Corbet and Hill 1980; Honacki et al. 1982; Hutterer 2005). Hoffmann (1984) conducted a systematic study of this group and recognized three species: *U. soricipes*, *U. gracilis*, and *U. andersoni* under the single genus *Uropsilus*. However, Wang and Yang (1989) believed that *investigator* and *gracilis* were distributed in the same domain, and there was no intermediate transition type, so the *U. s. investigator* should be an independent species. Since then, the view that there are four species of *Uropsilus* has been widely accepted (Hoffmann and Lunde 2008).

Recently, Liu et al. (2013) described *U. aequodonia* Liu et al., 2013 from Puge County, Sichuan, China, which has a dental formula of I 2/2, C1/1, P3 /3, and M 3 /3 = 36. Wan et al. (2013) suggested that *U. nivatus* and *U. atronates* are valid species based on molecular data. Furthermore, they identified seven recognized species and five putative species. Wan (2015) described six new species, but these designations were not recognized because they did not follow the International Code of Zoological Nomenclature (ICZN 2012). Wan et al. (2018) generated gene trees using additional specimens, which phylogenetic analyses revealed that species of *Uropsilus* could be sorted into three distinct lineages. One lineage

includes *U. investigator* from western Yunnan and acts as the basal position of the genus; the second lineage includes *U. aequodonenia*, *U. andersoni*, and *U. nivatus* from Northern Yunnan and western Sichuan; and the third contains *U. soricipes*, *U. gracilis*, *U. atronates*. Additionally, Hu et al. (2021b) used multivariate analyses as well as phylogenetic analyses to describe a new species, *U. dabieshanensis* Hu et al., 2021, from the Dabie Mountains, Anhui Province, eastern China. The phylogenetic results indicate that the lineage of *Uropsilus* has two matriline. More recently, Bui et al. (2023) described a new species, *U. fansipanensis*, from the northwestern Vietnam. Thus, nine taxa are currently recognized as full species under the genus *Uropsilus*: *U. aequodonenia*, *U. andersoni*, *U. gracilis*, *U. investigator*, *U. atronates*, *U. nivatus*, *U. dabieshanensis*, and *U. fansipanensis*. Of all known species, *U. dabieshanensis* is the only one found in eastern China and is considered to have the easternmost distribution of the genus.

During a biodiversity study in June 2022, five shrew mole specimens were collected from the Wuyi Mountains, Jiangxi Province, eastern China (Fig. 1). Our molecular analysis reveals that the five specimens are genetically distinct from all recognized *Uropsilus* species and potentially represents a new species. In this study, we integrate genetic and morphometric approaches to elucidate the taxonomy and phylogeny of these specimens.

## Materials and methods

### Sampling

In June 2022, five *Uropsilus* specimens were collected on Mount Huanggang in Wuyishan National Nature Reserve, Yanshan, Jiangxi Province, eastern China (Fig. 1). Specimens were collected using Sherman and pitfall (plastic buckets

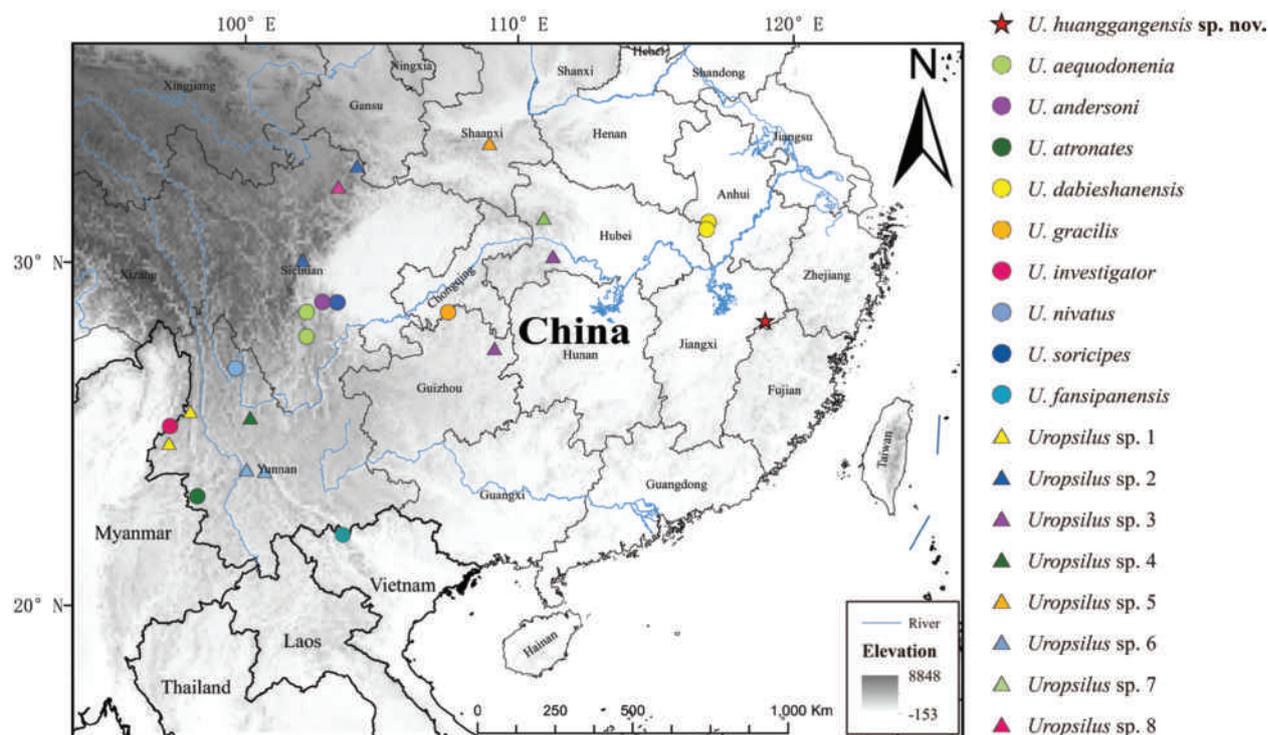


Figure 1. Sampling localities of specimens used in the phylogenetic analysis.

15 cm in diameter and 28 cm in depth) traps. All specimens were euthanized, and muscle or liver tissue was extracted from each and preserved in pure alcohol for subsequent molecular studies. All specimens and tissues were deposited at the Biological Museum of Anhui Normal University (AHNU). Animals were handled in compliance with the animal care and use guidelines of the American Society of Mammologists (Sikes et al. 2016), following the guidelines and regulations approved by the internal review board of AHNU (approval no. AHNU-ET2021002), and with the permissions of local government authorities.

### Phylogenetic analyses

Genomic DNA of the five specimens of *Uropsilus* from Mount Huanggang was extracted from the liver and muscle tissues using a DNA extraction kit (Tiangen DNeasy Blood and Tissue Kit, Beijing, China). Two mitochondrial genes (cytochrome b [*CYT B*], 12S rRNA [*12S*]) and three nuclear genes (phospholipase C beta 4 [*PLCB4*], recombination activating protein 1 [*RAG1*], and recombination activating protein 2 [*RAG2*]) were amplified using the primer pairs outlined in Suppl. material 1. The PCR products were purified and sequenced in both directions using the BigDye Terminator Cycle Kit v. 3.1 (Invitrogen, Waltham, MA, USA) on an ABI 3730xl sequencer (Applied Biosystems, Waltham, MA, USA). The obtained sequences were assembled using SeqMan (DNASTAR, Lasergene v. 7). Corresponding sequences of 38 specimens of nine recognized species and six unrecognized species of *Uropsilus* were downloaded from the GenBank (Suppl. material 2). We downloaded sequences of *Talpa altaica* and *Sorex araneus* as out-group taxa following Wan et al (2018). All sequences were then aligned in MEGA v. 11 (Tamura et al. 2021).

The uncorrected *p*-distance of the *CYT B* gene between species was calculated in MEGA v. 11 (Tamura et al. 2021). We used maximum likelihood (ML) and Bayesian inference (BI) methods to conduct phylogenetic analyses of mitochondrial–nuclear genes (mtDNA + nDNA, 4090 bp) concatenated datasets in PhyloSuite (Zhang et al. 2020). The best-fit partitioning scheme and evolutionary models were selected using PartitionFinder v. 2.0 with the greedy algorithm under the Bayesian information criterion (BIC) (Suppl. material 3) (Lanfear et al. 2012).

### Molecular dating

We used BEAST v. 2.6.6 (Bouckaert et al. 2019) to estimate divergence times based on the Birth-Death model as the tree prior and relaxed lognormal as the clock model prior. Evolutionary models or partition schemes were estimated based on the Bayesian Information Criterion (BIC) in PartitionFinder v. 2.0 (Lanfear et al. 2012). Two fossil calibrations were used following the guide of Wan et al. (2018): (1) the first division of *Uropsilus* at 6.18 Ma (95% HPD: 4.27–8.65 Ma), with a lognormal distribution prior (mean: 6.20, SD: 0.215, offset: 0.02), so the median age was at 6.08 Ma and the 95% CI was 4.27–8.65 Ma; (2) the earliest known *U. soricipes* from the Early Pleistocene 2.0–2.4 Ma, with an exponential distribution prior (offset = 2.0, M = 0.67 [2.0 × 0.333]), so the median age was 2.46 Ma and the 95% CI was 2.03–4.01 Ma. Each analysis was run for 100 million generations, sampling every 10000 generations. The first 10% of the samples were discarded as burn-in. Convergence was assessed using Tracer v. 1.7 (Rambaut et al. 2018).

## Morphological measurements and analyses

We examined and measured the specimens of *Uropsilus* in the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences, and AHNU. A total of 83 specimens were examined and they were assigned to *U. aequodonenia* ( $n = 2$ ), *U. andersoni* ( $n = 6$ ), *U. atronates* ( $n = 25$ ), *U. dabieshanensis* ( $n = 6$ ), *U. gracilis* ( $n = 16$ ), *U. investigator* ( $n = 11$ ), *U. nivatus* ( $n = 7$ ), *U. soricipes* ( $n = 5$ ), and *Uropsilus* sp. nov. ( $n = 5$ ) (Appendix 1).

The body weight (**Wt**) and four external measurements, including head and body length (**HBL**), tail length (**TL**), hindfoot length (**HF**), and ear length (**EL**), were taken from specimen labels or field notes. Twenty-one craniodental measurements were taken with digital calipers to the nearest 0.01 mm, following Yang et al. (2005, 2007). All the craniodental measurements were taken by a single observer. The following measurements were taken:

<b>PL</b>	Profile length;
<b>HB</b>	Height of braincase;
<b>GNB</b>	Greatest neurocranium breadth Cranial breadth;
<b>BS</b>	Basion-Staphylion;
<b>GBSn</b>	Greatest breadth of snout;
<b>BBP<sup>1</sup>–P<sup>2</sup></b>	Maxillary sides P <sup>1</sup> –P <sup>2</sup> Interdental external width;
<b>MPL</b>	Palatal length;
<b>APB</b>	Anterior palatal breadth;
<b>LUTR</b>	Length of upper tooth row;
<b>PPB</b>	Posterior palatal breadth;
<b>P<sup>4</sup>–M<sup>3</sup></b>	Distance from the upper fourth premolar to the upper third molar;
<b>Id–Gol</b>	From infradentale to gonion laterale;
<b>M<sup>1</sup>–M<sup>3</sup></b>	Upper molar row length;
<b>HVR</b>	Oral height of the vertical ramus;
<b>GBUM</b>	Great breadth of upper molars;
<b>Coh–M<sub>3</sub></b>	From the highest point of the Condyle process to the upper third molar;
<b>ML</b>	Mandible length;
<b>GBLM</b>	Greatest breadth of lower molars;
<b>Id–Coh</b>	From infradentale to the high point of the condyle process;
<b>LBO</b>	least breadth between orbits;
<b>LBTR</b>	Length of below tooth row.

We compared morphology of the new species with other species of *Uropsilus*. Comparative morphological characters of these other species were obtained from Thomas (1912, 1922), Allen et al. (1923), Hu et al. (2021b), and Bui et al. (2023), and we followed these authors' terminologies in our morphological description of the new species. Meanwhile, to better distinguish between the different species, we compared the ratio of GNB to PL, as well as the ratio of TL to HBL.

Overall similarities of skulls were assessed first through principal component analyses (PCA) based on the 21 log<sub>10</sub>-transformed craniodental variables. Groups of individuals sharing a comparable morphology were then discriminated through discriminant analysis (DA). To make the results more concise, we limited the PCA and DA analyses to the six taxa with the same dental for-

mula, including: *U. atronates*, *U. dabiieshanensis*, *U. gracilis*, *U. investigator*, *U. nivatus*, and the new species. The PCA and DA were conducted in SPSS v. 22.0 (SPSS Inc., USA). Furthermore, independent sample *t*-tests were conducted to test the variances of measurements highly correlated with PC1 and PC2 (i.e. LUTR, LBTR, GBSn, and GBUM; loading > 0.8) between the new species and the other species.

## Results

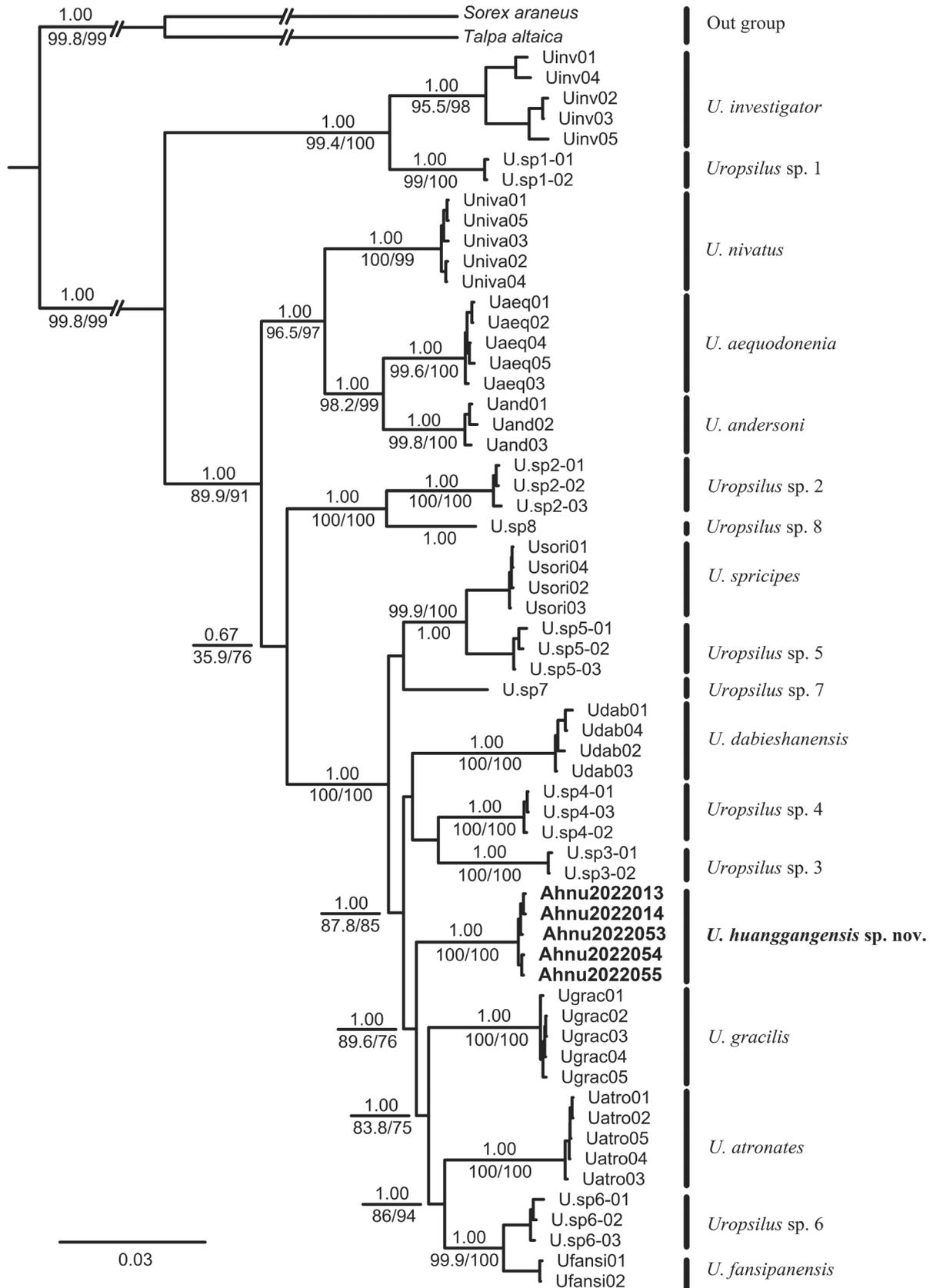
### Phylogenetic analyses

We obtained 4090-bp-long sequences for each voucher specimen, including 2002-bp mitochondrial [*CYT B* (1140 bp) and *12S* (862 bp)] and 2088-bp nuclear [*PLCB4* (330 bp), *RAG1* (1008 bp), and *RAG2* (750 bp)] sequences. All the new sequences were deposited in the GenBank [OQ730193–OQ730207, OQ725651–OQ725655, OR161365–OR161369, Suppl. material 2]. The uncorrected *p*-distance of *CYT B* reveals a high genetic divergence between the new species and all other nominal *Uropsilus* species, ranging from 9.3% (with *U. gracilis*) to 16.4% (with *U. investigator*) (Table 1).

The ML and BI trees recovered similar topologies (Fig. 2). In all phylogenetic trees, sequences of the new species from Mount Huanggang formed a monophyletic clade with high support (SH-aLRT = 100, Utboot = 100, and PP = 1.00). The new species has a sister relationship with the clade that is comprised of *U. atronates*, *Uropsilus* sp. 6, *U. fansipanensis*, and *U. gracilis*; this clade is strongly supported in the BI tree (PP = 1.0), but this relationship only has moderate support in the ML tree (SH-aLRT = 89.6, Utboot = 76). BEAST divergence analyses show that the divergence of the new species from the others was estimated to be at the early Pleistocene (1.92 Ma, 95% CI = 0.88–2.99) (Fig. 3).

### Morphological analyses

All external and skull measurements are given in Table 2. The PCA, which was based on 21 craniodental measurements, produced two axes with eigenvalues exceeding 2.0, which explained 57.24% and 10.53% of the variance (67.77% total) (Table 3). The first principal component (PC1) is positively correlated with LUTR and LBTR (loading > 0.80), indicating it mainly represents tooth row length. The second principal component (PC2) has high positive loadings on GBSn and GBUM (loading > 0.85). The independent-sample *t*-tests further show significant differences of at least two of the four measurements (i.e. LUTR, LBTR, GBSn, and GBUM) between the new species and *U. atronates*, *U. dabiieshanensis*, *U. gracilis*, *U. investigator*, *U. nivatus* ( $p < 0.05$ ; Suppl. material 4). A plot of PC1 and PC2 (Fig. 4) shows *Uropsilus* sp., *U. investigator*, and *U. dabiieshanensis* occupy the positive region of PC1, indicating that these three species have longer tooth rows than *U. atronates*, *U. nivatus*, and *U. gracilis*. The new species plots on the near-origin area of PC2, while *U. investigator* occupies the negative regions and *U. dabiieshanensis* occupies the positive region, suggesting that the snout and upper molars of the new species are relatively narrower than *U. dabiieshanensis* but broader than those in *U. investigator*. The DA shows that 98.60% of the species are



**Figure 2.** Molecular phylogenetic tree of *Uropsilus* based on mitochondrial–nuclear concatenated data and analyzed using maximum likelihood and Bayesian inference analyses. Numbers above branches refer to Bayesian posterior probabilities (PP). Numbers below branches indicate SH-like approximate likelihood ratio test supports (SH-aLRT)/ultrafast bootstrap supports (UFBoot). Scale bars represent substitutions per site.

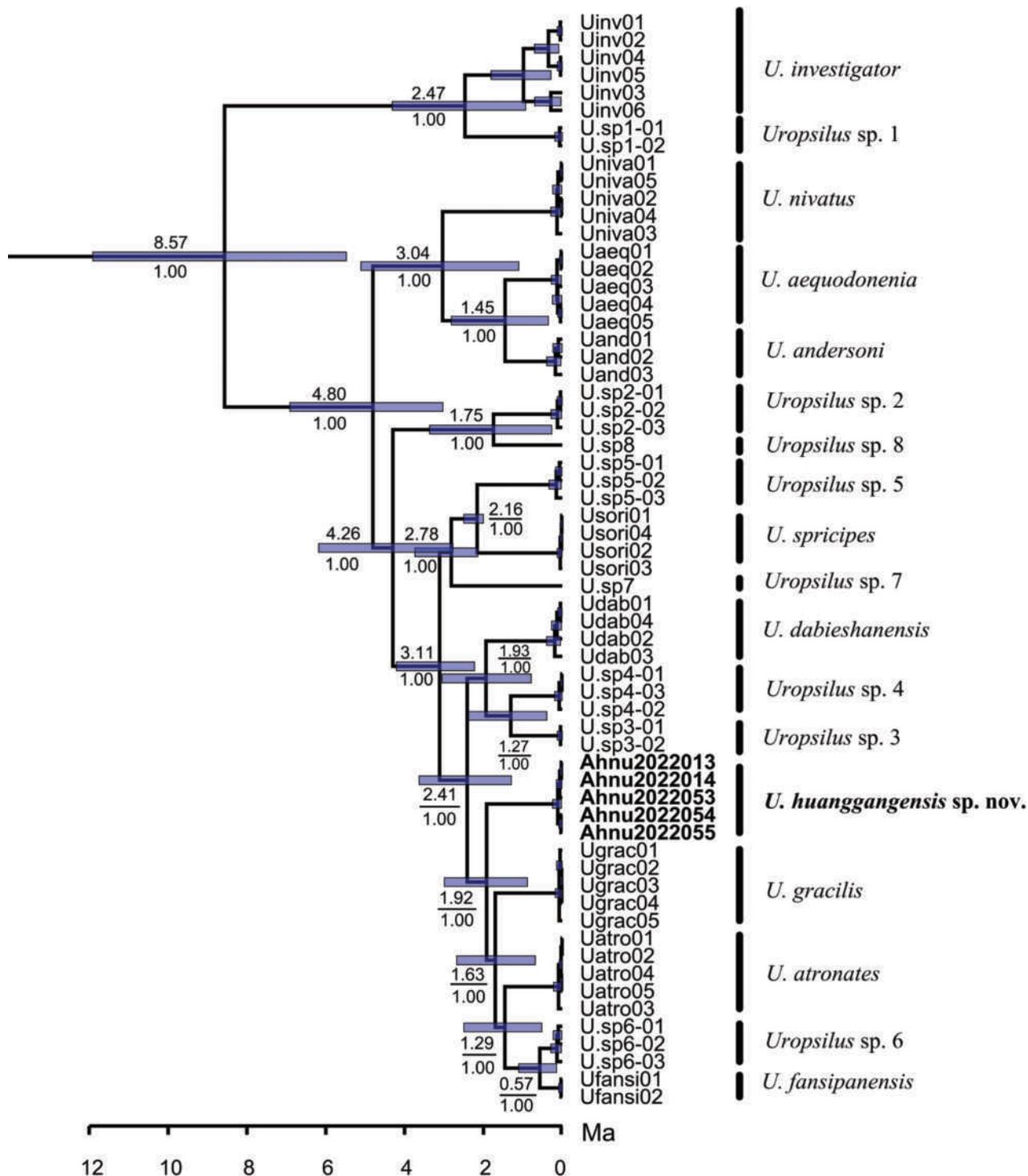


Figure 3. Divergence times estimated using BEAST based on mitochondrial–nuclear concatenated data. Node numbers refer to divergence time in million years (Ma) and Bayesian posterior probabilities (PP).

correctly classified based on the 21 craniodental measurements, with only one specimen labeled as *U. investigator* misclassified as *U. nivatus*. The first two canonical axes (CAN 1–2) explains 63.30% and 19.40% of the total variation, respectively (Table 3). In CAN 1 and CAN 2 plots (Fig. 4), specimens of the new species are separate from the others and remain close to *U. nivatus* and *U. investigator*.

**Table 1.** The uncorrected *p*-distances between *Uropsilus* species/cryptic species based on the *CYT B* gene.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	<i>U. huanggangensis</i> sp. nov.																	
2	<i>U. gracilis</i>	0.093																
3	<i>U. soricipes</i>	0.094	0.084															
4	<i>U. fansipanensis</i>	0.094	0.092	0.091														
5	<i>U. atronates</i>	0.100	0.097	0.095	0.095													
6	<i>U. dabieshanensis</i>	0.104	0.097	0.105	0.108	0.125												
7	<i>U. nivatus</i>	0.133	0.113	0.126	0.130	0.133	0.139											
8	<i>U. aequodomenia</i>	0.138	0.130	0.122	0.143	0.142	0.147	0.103										
9	<i>U. andersoni</i>	0.139	0.134	0.132	0.138	0.144	0.141	0.099	0.079									
10	<i>U. investigator</i>	0.164	0.164	0.155	0.180	0.175	0.158	0.163	0.167	0.165								
11	<i>U. sp. 1</i>	0.161	0.157	0.154	0.172	0.160	0.155	0.157	0.156	0.162	0.097							
12	<i>U. sp. 2</i>	0.134	0.130	0.135	0.133	0.145	0.128	0.133	0.137	0.129	0.160	0.158						
13	<i>U. sp. 3</i>	0.100	0.108	0.104	0.108	0.104	0.114	0.126	0.142	0.139	0.158	0.160	0.135					
14	<i>U. sp. 4</i>	0.091	0.077	0.088	0.083	0.097	0.118	0.116	0.135	0.133	0.164	0.165	0.132	0.089				
15	<i>U. sp. 5</i>	0.097	0.086	0.039	0.094	0.101	0.097	0.127	0.128	0.135	0.154	0.150	0.135	0.101	0.090			
16	<i>U. sp. 6</i>	0.094	0.091	0.091	0.038	0.093	0.110	0.132	0.144	0.141	0.172	0.172	0.130	0.100	0.082	0.090		
17	<i>U. sp. 7</i>	0.088	0.087	0.084	0.088	0.099	0.099	0.127	0.133	0.132	0.157	0.157	0.131	0.096	0.085	0.079	0.095	
18	<i>U. sp. 8</i>	0.139	0.120	0.133	0.137	0.136	0.138	0.121	0.122	0.127	0.169	0.156	0.087	0.139	0.137	0.137	0.139	0.130

**Table 2.** External and skull measurements (mm) used in morphometric analyses of the genus *Uropsilus*, including mean values, standard deviations, range, sample size and the ratio of partial measurements.

	<i>U. huanggangensis</i> sp. nov.	<i>U. dabieshanensis</i>	<i>U. gracilis</i>	<i>U. atronates</i>	<i>U. investigator</i>	<i>U. nivatus</i>	<i>U. aequodonia</i>	<i>U. andersoni</i>	<i>U. soricipes</i>	<i>U. fansipanensis</i>
<b>W</b>	8.80 ± 0.38 8.17–9.19; 5	9.28 ± 1.25 7.63–10.52; 6	8.84 ± 0.95 7.30–10.20; 14	6.93 ± 0.92 5.50–8.80; 15	7.25 ± 0.88 5.90–8.40; 10	8.00 ± 1.38 6.40–10.90; 7	8	9.04 ± 0.57 8.20–9.80; 5	9.52 ± 1.34 7.20–11.70; 10	8.0; 8.0
<b>HBL</b>	72.40 ± 1.34 71.00–74.00; 5	72.67 ± 1.89 69.50–75.00; 6	69.86 ± 4.15 63.00–77.00; 14	65.27 ± 2.76 61.00–69.00; 15	71.65 ± 4.30 66.00–79.00; 10	69.71 ± 2.14 67.00–73.00; 7	75	74.33 ± 3.01 70.00–79.00; 6	73.36 ± 2.69 70.00–79.00; 11	77.5; 74.00
<b>TL</b>	62.60 ± 3.21 57.00–65.00; 5	55.50 ± 3.35 52.50–61.50; 6	63.57 ± 6.11 53.00–75.00; 14	59.73 ± 4.45 52.00–68.00; 15	65.40 ± 4.30 60.00–71.00; 10	68.86 ± 3.98 61.00–73.00; 7	70	65.17 ± 10.68 46.00–73.00; 6	63.18 ± 8.21 46.00–73.00; 11	62.50; 61.00
<b>HF</b>	13.10 ± 0.22 13.00–13.50; 5	11.75 ± 1.21 10.50–13.00; 6	12.96 ± 0.97 10.00–14.00; 14	12.80 ± 0.77 11.00–14.50; 15	13.20 ± 1.40 11.00–15.00; 10	14.14 ± 1.68 11.00–16.00; 7	14	14.75 ± 0.76 14.00–16.00; 6	14.05 ± 0.99 13.00–16.00; 11	13.41; 13.57
<b>EL</b>	9.20 ± 0.45 9.00–10.00; 5	8.33 ± 0.41 8.00–9.00; 6	9.39 ± 1.1 7.00–11.00; 14	8.07 ± 0.86 7.00–9.50; 15	7.95 ± 0.98 6.50–9.50; 10	9.50 ± 0.84 8.00–10.00; 6	11	9.67 ± 0.58 9.00–10.00; 3	9.38 ± 0.74 8.00–10.00; 8	8.51; 8.52
<b>PL</b>	21.44 ± 0.47 21.00–22.18; 5	21.04 ± 0.45 20.32–21.50; 6	20.89 ± 0.41 19.93–21.81; 16	20.12 ± 0.43 18.99–20.79; 25	21.19 ± 0.71 19.97–22.14; 11	20.78 ± 0.29 20.39–21.12; 7	21.91	22.06 ± 0.33 21.59–22.34; 3	21.05 ± 0.23 20.63–21.3; 5	20.4; 20.69
<b>GNB</b>	11.10 ± 0.17 10.95–11.39; 5	11.52 ± 0.29 11.00–11.86; 6	11.27 ± 0.20 10.88–11.63; 16	10.91 ± 0.23 10.62–11.31; 25	11.10 ± 0.34 10.45–11.64; 11	11.31 ± 0.25 10.98–11.66; 7	11.90 ± 0.19	11.66 ± 0.18 11.49–11.91; 3	11.36 ± 0.26 10.94–11.73; 5	10.96; 11.07
<b>GBSn</b>	7.62 ± 0.11 7.47–7.77; 5	8.10 ± 0.34 7.65–8.51; 6	7.57 ± 0.18 7.23–7.89; 16	7.27 ± 0.21 6.84–7.72; 25	7.27 ± 0.28 6.92–7.81; 11	7.82 ± 0.24 7.51–8.15; 7	8.12 ± 0.02	7.87 ± 0.21 7.69–8.26; 6	7.70 ± 0.14 7.52–7.92; 5	7.39; 7.62
<b>MPL</b>	9.85 ± 0.29 9.50–10.21; 5	9.79 ± 0.18 9.46–9.96; 6	9.39 ± 0.17 9.18–9.68; 16	8.90 ± 0.24 8.49–9.33; 25	9.72 ± 0.38 9.19–10.25; 11	9.59 ± 0.22 9.31–9.90; 7	10.11 ± 0.13	10.00 ± 0.15 9.75–10.22; 5	9.72 ± 0.08 9.65–9.85; 5	9.62; 9.75
<b>LUTR</b>	9.47 ± 0.24 9.12–9.76; 5	9.46 ± 0.27 9.03–9.68; 6	9.02 ± 0.17 8.65–9.25; 16	8.65 ± 0.26 8.23–9.32; 25	9.38 ± 0.34 8.96–9.93; 11	9.17 ± 0.20 8.95–9.40; 7	9.61 ± 0.10	9.55 ± 0.15 9.24–9.68; 6	9.24 ± 0.16 9.04–9.49; 5	9.03; 9.07
<b>P<sup>a</sup>-M<sup>3</sup></b>	5.51 ± 0.14 5.35–5.67; 5	5.65 ± 0.24 5.27–6.03; 6	5.33 ± 0.12 5.05–5.55; 16	5.13 ± 0.19 4.79–5.64; 25	5.36 ± 0.21 5.04–5.64; 11	5.37 ± 0.16 5.11–5.55; 7	5.78 ± 0.07	5.75 ± 0.15 5.53–5.98; 6	5.58 ± 0.20 5.26–5.86; 5	
<b>M<sup>1</sup>-M<sup>3</sup></b>	4.53 ± 0.10 4.37–4.62; 5	4.62 ± 0.21 4.29–4.93; 6	4.36 ± 0.13 4.11–4.64; 16	4.17 ± 0.15 3.92–4.62; 25	4.35 ± 0.17 4.15–4.72; 11	4.40 ± 0.13 4.22–4.58; 7	4.51 ± 0.10	4.55 ± 0.2 4.26–4.92; 6	4.39 ± 0.18 4.10–4.58; 5	
<b>GBUM</b>	1.92 ± 0.06 1.82–1.97; 5	2.02 ± 0.08 1.91–2.14; 6	1.84 ± 0.06 1.76–1.95; 16	1.76 ± 0.07 1.62–1.88; 25	1.71 ± 0.07 1.63–1.84; 11	1.83 ± 0.07 1.77–1.94; 7	1.82 ± 0.03	1.89 ± 0.05 1.80–1.97; 6	1.82 ± 0.11 1.72–1.95; 5	
<b>ML</b>	13.84 ± 0.26 13.46–14.12; 5	14.07 ± 0.22 13.80–14.41; 6	13.53 ± 0.27 12.96–13.92; 16	12.98 ± 0.36 12.36–13.75; 25	13.73 ± 0.54 12.85–14.34; 11	13.67 ± 0.18 13.41–13.87; 7	14.35 ± 0.08	14.27 ± 0.28 13.94–14.73; 6	13.81 ± 0.2 13.45–14.02; 5	13.52; 13.77

	<i>U. huanggangensis</i> sp. nov.	<i>U. dabieshanensis</i>	<i>U. gracilis</i>	<i>U. atronates</i>	<i>U. investigator</i>	<i>U. nivatus</i>	<i>U. aequodonemia</i>	<i>U. andersoni</i>	<i>U. soricipes</i>	<i>U. fansipanensis</i>
<b>Id-Coh</b>	12.40 ± 0.35 11.99–12.74; 5	12.32 ± 0.32 11.82–12.74; 6	12.55 ± 0.19 12.28–12.94; 16	12.04 ± 0.33 11.44–12.77; 25	12.42 ± 0.65 11.34–13.26; 11	12.21 ± 0.26 11.86–12.60; 7	12.87 ± 0.41 12.46; 13.28; 2	12.82 ± 0.38 12.38–13.41; 6	12.44 ± 0.46 11.8–13.17; 5	
<b>LBTR</b>	8.10 ± 0.12 7.93–8.20; 5	8.10 ± 0.21 7.84–8.42; 6	7.69 ± 0.21 7.17–8.01; 16	7.46 ± 0.23 7.04–7.84; 25	8.04 ± 0.37 7.50–8.54; 11	7.80 ± 0.19 7.57–8.05; 7	8.18 ± 0.06 8.12; 8.24; 2	8.01 ± 0.12 7.84–8.17; 6	7.87 ± 0.22 7.67–8.22; 5	
<b>LBO</b>	5.51 ± 0.12 5.32–5.65; 5	5.42 ± 0.26 5.05–5.77; 6	5.33 ± 0.12 5.12–5.58; 16	5.46 ± 0.18 5.12–5.79; 25	5.54 ± 0.16 5.23–5.86; 11	5.6 ± 0.14 5.31–5.77; 7	5.63 ± 0.07 5.56; 5.70; 2	5.82 ± 0.11 5.64–5.97; 5	5.47 ± 0.14 5.20–5.61; 5	5.51; 5.80
<b>HB</b>	7.06 ± 0.15 6.92–7.35; 5	6.84 ± 0.40 6.08–7.22; 6	7.15 ± 0.26 6.65–7.65; 16	6.70 ± 0.23 6.24–7.17; 25	7.16 ± 0.31 6.62–7.68; 11	7.03 ± 0.15 6.77–7.17; 7	7.13 7.13; 1	7.37 ± 0.28 7.08–7.75; 3	7.38 ± 0.29 6.8–7.52; 5	6.90; 6.91
<b>BS</b>	7.57 ± 0.16 7.33–7.76; 5	7.48 ± 0.24 7.16–7.73; 6	7.36 ± 0.22 6.97–7.96; 16	7.27 ± 0.29 6.68–7.8; 25	7.34 ± 0.19 6.88–7.59; 11	7.32 ± 0.13 7.12–7.54; 7	7.57 7.57; 1	7.57 ± 0.36 7.07–7.93; 3	7.50 ± 0.09 7.40–7.65; 5	
<b>BBP<sup>1</sup>-P<sup>2</sup></b>	2.96 ± 0.11 2.77–3.05; 5	3.08 ± 0.17 2.84–3.38; 6	2.82 ± 0.09 2.67–2.94; 16	2.68 ± 0.10 2.41–2.88; 25	2.77 ± 0.09 2.63–2.94; 11	2.80 ± 0.09 2.63–2.88; 7	2.99 ± 0.06 2.93; 3.05; 2	2.96 ± 0.05 2.90–3.03; 6	2.93 ± 0.08 2.80–3.03; 5	
<b>APB</b>	2.58 ± 0.10 2.45–2.72; 5	2.55 ± 0.13 2.35–2.77; 6	2.36 ± 0.10 2.26–2.67; 16	2.33 ± 0.20 2.00–3.03; 25	2.50 ± 0.10 2.31–2.65; 11	2.45 ± 0.09 2.30–2.58; 7	2.51 ± 0.06 2.45; 2.57; 2	2.34 ± 0.13 2.16–2.47; 6	2.37 ± 0.08 2.28–2.48; 5	
<b>PPB</b>	3.23 ± 0.09 3.10–3.32; 5	3.29 ± 0.19 2.93–3.54; 6	3.13 ± 0.10 2.94–3.31; 16	3.06 ± 0.14 2.68–3.35; 25	3.13 ± 0.13 2.91–3.34; 11	3.34 ± 0.18 3.13–3.59; 7	3.50 ± 0.03 3.47; 3.52; 2	3.27 ± 0.09 3.18–3.40; 6	3.20 ± 0.04 3.12–3.24; 5	
<b>Id-Gol</b>	12.96 ± 0.05 12.90–13.03; 5	13.14 ± 0.28 12.66–13.54; 6	12.06 ± 0.33 11.46–12.68; 16	11.43 ± 0.43 10.79–12.38; 25	12.85 ± 0.47 12.18–13.58; 11	12.69 ± 0.20 12.43–12.98; 7	13.09 ± 0.10 12.99; 13.18; 2	13.07 ± 0.13 12.89–13.3; 6	12.83 ± 0.19 12.61–13.18; 5	
<b>HVR</b>	6.31 ± 0.15 6.10–6.57; 5	6.39 ± 0.31 5.91–6.90; 6	6.32 ± 0.12 6.09–6.49; 16	6.00 ± 0.19 5.67–6.49; 25	6.22 ± 0.29 5.77–6.61; 11	6.32 ± 0.09 6.19–6.49; 7	6.73 ± 0.02 6.71; 6.74; 2	6.63 ± 0.15 6.44–6.84; 6	6.44 ± 0.17 6.30–6.75; 5	
<b>Coh-M<sub>3</sub></b>	5.79 ± 0.05 5.72–5.87; 5	5.56 ± 0.29 5.18–6.00; 6	5.68 ± 0.25 5.15–6.11; 16	5.36 ± 0.26 4.81–5.98; 25	5.81 ± 0.32 5.25–6.29; 11	5.78 ± 0.17 5.52–6.00; 7	6.45 6.45; 1	6.07 ± 0.33 5.67–6.55; 6	5.90 ± 0.23 5.57–6.22; 5	
<b>GBLM</b>	1.19 ± 0.05 1.10–1.25; 5	1.25 ± 0.06 1.18–1.33; 6	1.14 ± 0.04 1.07–1.21; 16	1.09 ± 0.05 1.02–1.19; 25	1.09 ± 0.04 1.03–1.16; 11	1.14 ± 0.05 1.08–1.23; 7	1.19 ± 0.03 1.16; 1.22; 2	1.20 ± 0.05 1.13–1.27; 6	1.11 ± 0.06 1.04–1.19; 5	
<b>TL/HBL</b>	86.46%	76.37%	91.00%	91.51%	91.28%	98.78%	93.33%	87.68%	86.12%	83.50%
<b>GNB/PL</b>	51.80%	55.74%	53.94%	54.24%	52.40%	54.43%	53.45%	52.87%	53.96%	53.61%

**Table 3.** Character loadings, eigenvalues, and percent variance explained on the first two components of a principal components analysis and the five canonical axes discriminant function analyses of the genus *Uropsilus*.

Variables	PCA		DA				
	1	2	1	2	3	4	5
LUTR	0.832	0.313	0.243	-0.079	0.275	-0.457	-1.450
LBTR	0.807	0.256	0.353	-0.398	-0.934	0.619	0.266
PL	0.781	0.283	1.259	-0.271	-0.119	-0.180	0.735
Id-Gol	0.770	0.276	0.952	-0.544	0.215	-0.133	-0.329
ML	0.667	0.302	0.625	1.142	-0.602	-0.854	-0.317
MPL	0.647	0.198	-0.754	-0.556	0.599	1.207	0.877
P <sup>4</sup> -M <sup>3</sup>	0.625	0.549	-0.374	0.322	-0.680	-0.106	-0.944
M <sup>1</sup> -M <sup>3</sup>	0.604	0.598	0.003	0.058	0.666	0.184	0.848
APB	0.592	0.228	-0.092	-0.052	-0.302	0.220	0.267
GBUM	0.138	0.892	0.264	0.459	-0.083	0.641	0.649
GBSn	0.211	0.854	-0.276	0.421	0.475	-1.268	0.024
GBLM	0.288	0.741	-0.079	-0.062	0.249	0.249	0.341
BBP <sup>1</sup> -P <sup>2</sup>	0.368	0.698	0.591	0.459	-0.474	0.373	-0.416
PPB	0.252	0.603	-0.339	-0.393	0.229	0.047	0.356
Id-Coh	0.321	0.067	-1.660	0.156	0.770	0.207	-0.678
HVR	0.354	0.402	-0.601	0.166	-0.025	0.164	-0.487
Coh-M <sub>3</sub>	0.484	-0.006	-0.291	-0.622	0.580	0.164	1.254
GNB	0.111	0.524	0.759	0.270	0.023	-0.253	-0.493
HB	0.293	0.112	0.047	-0.434	0.608	-0.251	-0.004
BS	0.179	0.218	0.195	0.256	-0.424	0.515	0.104
LBO	0.184	-0.070	-0.203	-0.254	-0.585	-0.497	0.476
Eigenvalues	12.020	2.211	8.426	2.829	0.973	0.563	0.263
Percent variance explained (%)	57.24%	10.53%	64.70%	21.70%	7.20%	4.30%	2.00%

Both the molecular and morphological analyses indicate that *Uropsilus* sp. nov. is diagnosable from all other recognized species of the genus *Uropsilus*. Based on the diagnosis and monophyly-based phylogenetic species concept (Mayden 1997; Gutierrez and Garbino 2018), we recognize it as a new species, which we formally describe below.

### Taxonomic account

#### *Uropsilus huanggangensis* Chen, Jiang & Ren, sp. nov.

<https://zoobank.org/160BAE4A-EBEB-4177-8A50-ADF35E932D4C>

Figs 4, 5

**Suggested common name.** Huanggang shrew mole; Chinese common name: 黄岗鼯鼠.

**Type materials. Holotype:** AHNU 2022013, an adult male collected by Zhongzhen Chen in June 2022 from Mount Huanggang, Wuyishan National Park, Jiangxi Province, China (27°58'53"N, 117°47'2.4"E, altitude 2061 m a.s.l.). The dried skin and cleaned skull are deposited in ANHU. **Paratypes:** AHNU 2022014, AHNU 2022053, AHNU 2022054, and AHNU 2022055; 4 adult specimens collected from Mount Huanggang, Wuyishan National Park, Jiangxi Province, China at elevations between 1830 and 2060 m a.s.l. The specimens are deposited in ANHU.

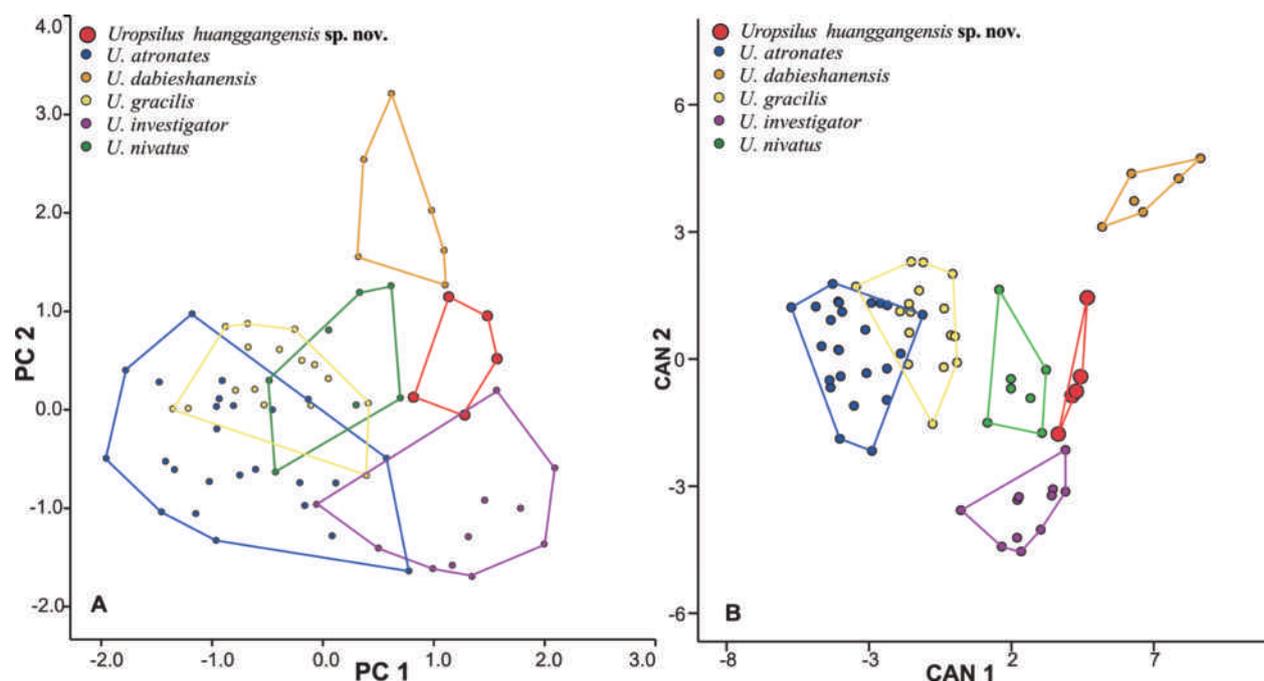
**Etymology.** The specific name *huanggangensis* is derived from Mount Huanggang, the type locality of the new species; the Latin adjectival suffix *-ensis* means "belonging to".

**Diagnosis.** The dorsal pelage of *U. huanggangensis* is dark chocolate-brown. The snout is the longest of any species in the genus. The first incisor  $I^1$  is wide and shows an enlargement at the apex of the rostrum, with a visible gap to  $I^2$ .  $C_1$  is larger than  $P_1$ , and  $P_1$  and  $P_3$  are similar in size. Tail is slim and relatively short, averaging 86% of head and body length. The tufts at the tail tip are short. The lacrimal foramen and infraorbital foramen are similar in size. The coronoid process is pointed and converges more upward with an incisive tip. The dental formula is  $I\ 2/1, C1/1, P\ 4/4, M\ 3/3 = 38$ .

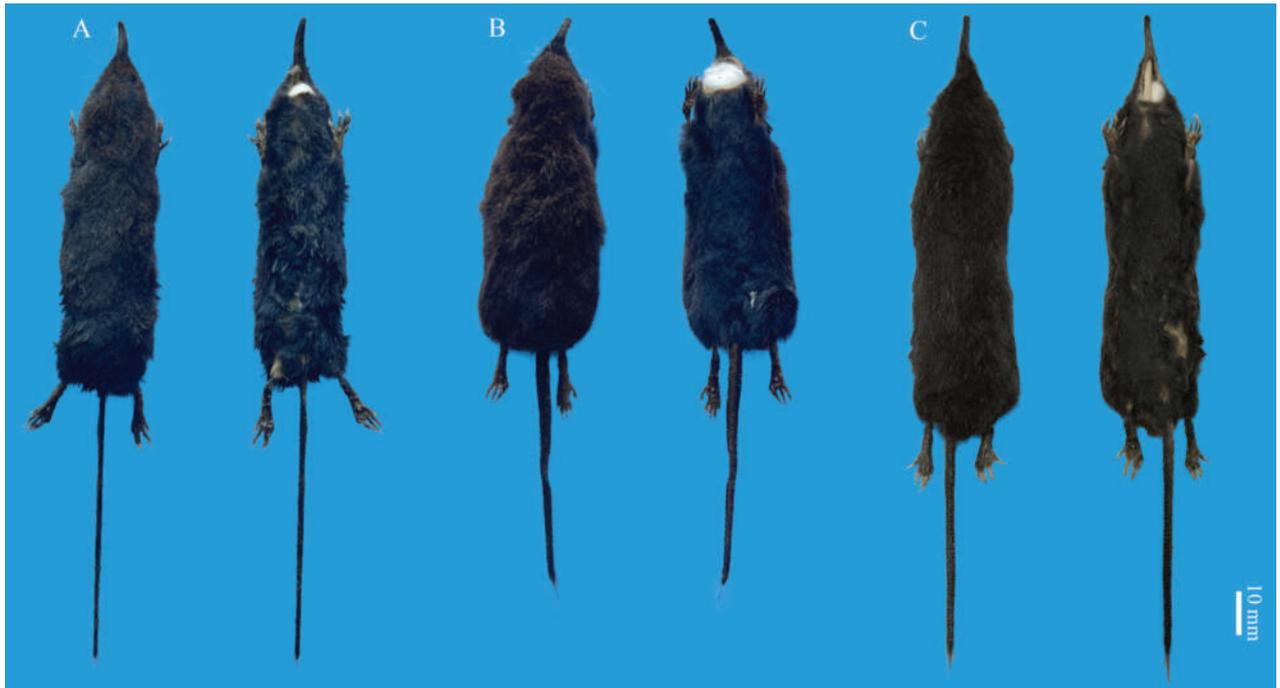
**Description.** *Uropsilus huanggangensis* is a medium-sized species of *Uropsilus* (HBL =  $72 \pm 1$  mm, PL =  $21.44 \pm 0.47$  mm; Table 2). The dorsal pelage is dark chocolate-brown, consisting of brown fur with a light grey base; the ventral fur is slightly paler. The snout is very long, at about 12 mm, and is the longest in the genus. The tail is slim and relatively short (TL =  $63 \pm 3$  mm, 57–65 mm), about 86% of the combined head and body length. The tail is black above and slightly paler below, with a sparse tuft of short hair at its tip. The hind foot is covered with short black hair; its length is 13–14 mm and constitutes approximately 18% of the combined head and body length.

The outlines of the skull are rounded, and there is a complete zygomatic arch. The rostrum is relatively long, the braincase is narrow, and the proportion of GNB and PL is 51.8%, which is the smallest of any species in the genus (GNB/PL > 52.4% in other species). The zygomatic arches are stout and only slightly bow outward. The lacrimal foramen and infraorbital foramen are similar in size.

The dental formula is  $I\ 2/1, C1/1, P\ 4/4, M\ 3/3 = 38$ .  $I^1$  is large and wide, causing the enlargement at the apex of the rostrum.  $I^1$  is bigger than  $I^2$ , and there is a visible gap between them.  $C^1$  is almost equal to  $P^1$ , while  $P^3$  is smaller.  $P^2$  is larger than  $P^1$  and  $P^3$ . The first upper molar  $M^1$  and second upper molar  $M^2$  are large, and have well-developed, W-shaped lateral cusps. In contrast, the third upper molars  $M^3$  are reduced.



**Figure 4.** Results of **A** principal component analysis (PCA) **B** discriminant function analysis (DA) for partial species with the same dental formula within the genus *Uropsilus*.



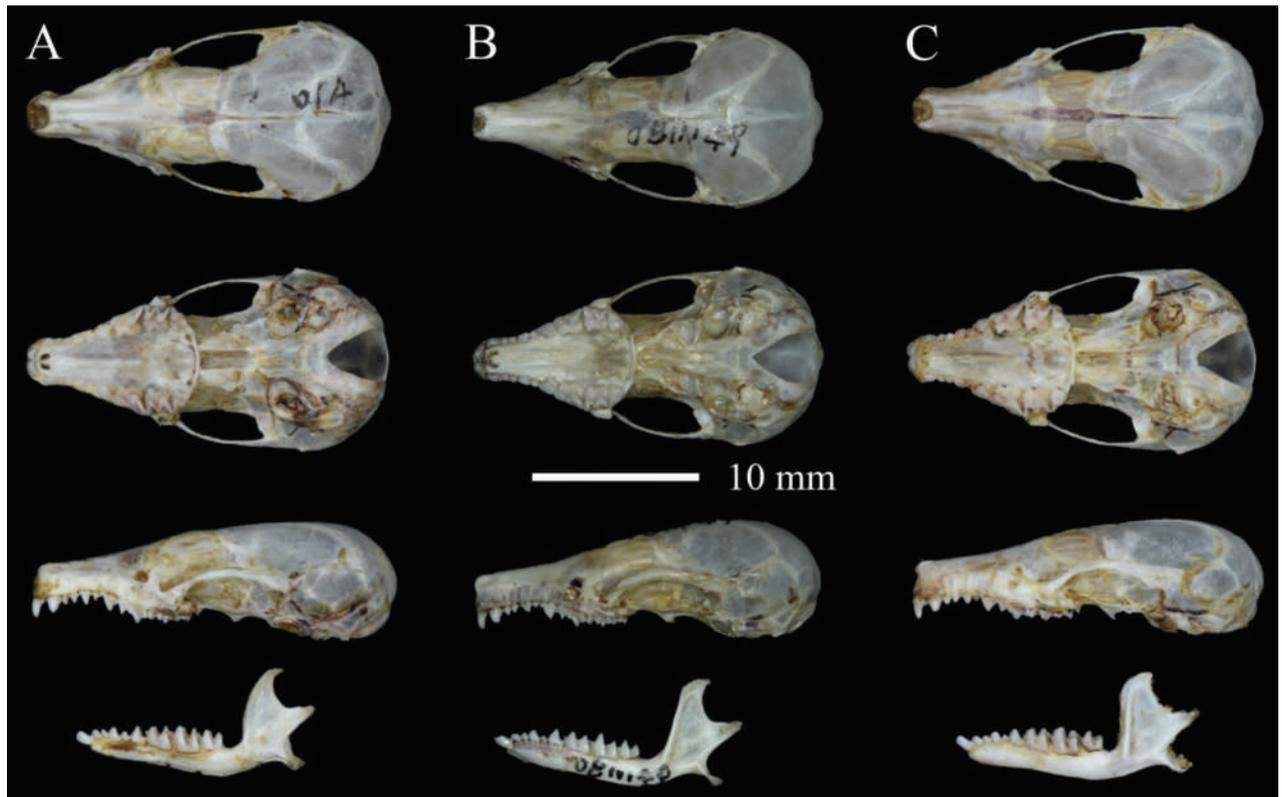
**Figure 5.** Dorsal and ventral views of three *Uropsilus* species **A** *U. huanggangensis* sp. nov. **B** *U. gracilis* **C** *U. dabieshanensis*.

The body of the mandible is long and slender. The coronoid process is high, pointed, and curved to the posterior, with an incisive tip pointing straight to the posterior, resembling the outline of a sickle. The angular process is long, rounded, and points downward at roughly 45°. The first lower premolar ( $P_1$ ) is slightly smaller than the lower canine  $C_1$ .  $P_1$  and  $P_3$  are similar in size.  $M_2$  is W-shaped and larger than  $M_1$  and  $M_3$ .  $M_3$  is slightly smaller than  $M_1$  (Fig. 6).

**Comparison.** Among other *Uropsilus* species, *U. huanggangensis* is morphologically most similar to *U. dabieshanensis* and *U. gracilis*. However, the new species can be distinguished from them by many characteristics.

Compared to *U. dabieshanensis*, *U. huanggangensis* has darker fur, a relatively longer and slimmer tail, and a much larger hindfoot and ear, despite that the heads and body lengths of the two species are almost the same (Table 2; Fig. 5). The tail of *U. huanggangensis* (TL =  $63 \pm 3$  mm) is relatively longer than *U. dabieshanensis* (TL =  $56 \pm 3$  mm). Most individuals of *U. huanggangensis* (4 of 5) have a tail length of more than 63 mm, while most individuals of *U. dabieshanensis* (5 of 6) have a tail length less than 57 mm. The hairs on the tail (bristle hairs) of *U. huanggangensis* are shorter and sparser than those in *U. dabieshanensis*, and the tufts at the tail tip of *U. dabieshanensis* appear much longer. The skull of *U. huanggangensis* is much slenderer than in *U. dabieshanensis* (Fig. 6), and the proportion of GNB and PL (CB / GLS = 53.96%) in *U. dabieshanensis* is greater than that in *U. huanggangensis* (GNB / PL = 51.80%). The coronoid process is pointed and curved to the posterior in *U. huanggangensis*, while the coronoid process of *U. dabieshanensis* is high and straight, with a squared tip.

Compared to *U. gracilis*, the dorsal pelage of *U. huanggangensis* is much darker. The snout of *U. huanggangensis* is longer, and the incisor is larger than that of *U. gracilis*. The tail of *U. huanggangensis* (TL/HBL = 86%) is relatively shorter than *U. gracilis* (TL / HBL = 91%) in proportion, and the tufts at the tail tip of *U. huanggangensis* are much shorter than those in *U. gracilis*. In terms of



**Figure 6.** Dorsal, ventral, and lateral views of the skull and lateral views of the mandible of three *Uropsilus* species **A** *U. huanggangensis* sp. nov. **B** *U. gracilis* **C** *U. dabieshanensis*.

body size, *U. huanggangensis* is relatively larger than *U. gracilis* for most external and craniomandibular measurements (Table 2). In particular, the range of Id-Gol (*U. huanggangensis* 12.90–13.03 mm vs *U. gracilis* 11.46–12.68 mm) between the two species does not overlap. The coronoid process of *U. gracilis* is high and squared, similar to that of *U. dabieshanensis*, but differs from that of *U. huanggangensis*.

Compared to *U. atronates* and *U. nivatus*, the dark chocolate-brown dorsal pelage of *U. huanggangensis* differs from the chestnut red of *U. atronates* and the black-gold pelage of *U. nivatus*. Meanwhile, *U. huanggangensis* is larger than both *U. atronates* and *U. nivatus* for most external and craniomandibular measurements (Table 2).

The pelage color of *U. huanggangensis* is dark chocolate-brown, which is much lighter than the black pelage of *U. investigator*. The ears of *U. huanggangensis* are relatively larger (EL =  $9.20 \pm 0.45$  mm, range 9.00–10.00 mm) than that of the *U. investigator* (EL =  $7.95 \pm 0.98$  mm, range 6.50–9.50 mm). The color of *U. huanggangensis* is uniform compared to the bicolored tail of *U. investigator*. Also, the  $P_1$  of *U. investigator* is larger than  $P_3$ , while  $P^1$  and  $P_3$  of *U. huanggangensis* are similar in size.

Compared to the upward orbital process of *U. fansipanensis*, the orbital process of *U. huanggangensis* is downward. The lacrimal foramen of *U. fansipanensis* is larger than infraorbital foramen, while the two are of similar size in *U. huanggangensis*.

The dental formula of *U. huanggangensis* is I 2/1, C1/1, P 4/4, M 3/3 = 38, which can be easily distinguished from *U. soricipes* (dental formula I 2/1, C 1/1,

P 3/3, M 3/3 = 34), *U. andersoni* (dental formula I 2/2, C1/1, P 4/3, M 3/3 = 38), and *U. aequodonenia* (dental formula I 2/2, C 1/1, P 3/3, M 3/3 = 36).

**Distribution and ecology.** *Uropsilus huanggangensis* is currently known only from the type locality on Mount Huanggang, Wuyishan National Park, Jiangxi Province, eastern China, where specimens were collected at elevations between 1830 and 2060 m a.s.l. Coniferous forests and shrub meadows, with abundant rocks on the ground, dominate the habitat in this area.

## Discussion

For a long time, it was believed that the genus *Uropsilus* was only distributed in the mountains of southwestern China and adjacent Myanmar (Wan et al. 2013; Kryštufek and Motokawa 2018). Hu et al. (2021b) expanded the known distribution of the genus by reporting the presence of *U. dabiешanensis* on Dabie Mountain, Anhui, eastern China, which represents the easternmost distribution of genus. In the present study, through integrating morphological and molecular approaches, we demonstrate that the isolated population on Mount Huanggang is distinct from all nominal species of *Uropsilus* and recognize it as a new species, *U. huanggangensis*.

In line with previous studies (Wan et al. 2013; Hu et al. 2021b), our phylogenetic analyses reveal that *Uropsilus* species can be sorted into two distinct lineages. One lineage includes *U. investigator* and *U. sp. 1*, occupying the basal position of the genus, and the other lineage exhibits a widespread distribution throughout China. Although *U. huanggangensis* consistently forms a monophyletic group with strong support in all phylogenetic trees, it is worth noting that the phylogenetic trees of *Uropsilus* species display considerable instability, as previously reported in the literature. Additionally, eight putative new species (*Uropsilus* sp. 1–8) have been identified but not yet officially described; there is a high level of cryptic diversity and extensive cryptic diversification within the genus. Broader sampling, in-depth gene sequencing, and morphological analysis are needed to improve the understanding of the genus.

As the easternmost occurring species of *Uropsilus*, our discovery of *U. huanggangensis* significantly expands our knowledge of the geographic distribution of the genus and contributes to our understanding of its macroevolution. The divergence of *U. huanggangensis* is estimated in the early Pleistocene (1.98 Ma, 95% CI = 0.88–2.99). Global cooling and drying events during this period (Qiu and Li 2005; Ge et al. 2013), as well as the isolation effects of Wuyi Mountain and Yangtze River, may have been critical in the divergence of *U. huanggangensis*, and Mount Huanggang may have provided a refuge for *U. huanggangensis* during the ice age. Recently, several new small mammal species have been described in eastern China, such as *Chodsigoa dabiешanensis* Chen et al., 2022, *Crocidua dongyangjiangensis* Liu et al., 2020, and *Typhlomys huangshanensis* Hu et al., 2021, indicating that biodiversity in the region is severely underexplored (Hu et al. 2021a; Chen et al. 2022). The description of *U. huanggangensis* in the Wuyi Mountains region highlights the overlooked biodiversity of the mountains of eastern China. It is therefore crucial to conduct further comprehensive investigations and taxonomic studies on small mammals in this region to gain a deeper understanding of the biodiversity of this region.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Formal analysis: YX, XR. Funding acquisition: ZC. Investigation: XJ, ZC, XR, YF, HY, LC, WZ. Methodology: ZC. Resources: XJ, YL, LC, ZC, WZ. Software: YX, ZC, XR. Supervision: ZC. Writing – original draft: XR. Writing – review and editing: ZC, YX, LK, XR.

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### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Appendix 1

Specimens examined for this study. Abbreviations: KIZ, Kunming Institute of Zoology, Chinese Academy of Sciences; AHNU, Anhui Normal University.

*Uropsilus huanggangensis* sp. nov. ( $n = 5$ ): Mount Huanggang, Jiangxi (AHNU 2022013-014, AHNU 2022053-055).

*U. aequodonenia* ( $n = 2$ ): Mount Daliangshan, Yuexi, Sichuan (KIZ 0906075, KIZ SC2110638).

*U. andersoni* ( $n = 6$ ): Mount Erlang, Tianquan, Sichuan (KIZ SC2110004, KIZ SC2110014-015, KIZ Z201505738-739, KIZ Z201505755).

*U. atronates* ( $n = 25$ ): Mount Laobieshan, Yongde, Yunnan (KIZ 0212145, KIZ 0212185, KIZ 0212187, KIZ 0212190, KIZ 0212309, KIZ 0212314, KIZ

- 0212332, KIZ 0212334, KIZ 0212339, KIZ 0212381-382, KIZ 0212413); Caojian, Yunlong, Yunnan (KIZ 0904362, KIZ 0904420-403); Laowo, Lushui, Yunnan (KIZ 2012121120); Wayao, Baoshan, Yunnan (KIZ H2004, KIZ H2007, KIZ H2013-14, KIZ H2040, KIZ H2052, KIZ H2054, KIZ H2073, KIZ H2090).
- U. dabieshanensis* ( $n = 6$ ): Dabie Mountain, Anhui (AHNU 202109002, AHNU 202109115, AHNU 202109302, AHNU 202109337, AHNU 202109409, AHNU 202109457).
- U. gracilis* ( $n = 16$ ): Mount Jinfoshan, Nanchuan, Chongqing (KIZ Y204002, KIZ Y204043); Mount Jiaozishan, Dongchuan, Yunnan (KIZ 0810003, KIZ 0810137, KIZ0810159-160, KIZ 0810191-192, KIZ 0810245, KIZ 0810274, KIZ 0810488, LIZ 0810504); Mount Jiaozishan, Luquan, Yunnan (KIZ 0811149, KIZ 0811175-176); Mount Wumeng, Zhaotong, Yunnan (KIZ 201309117).
- U. investigator* ( $n = 11$ ): Mount Gaoligong, Gongshan, Yunnan (KIZ PM1311422, KIZ PM1312467, KIZ PM1312511, KIZ PM1312570, KIZ PM1312600, KIZ 201211136, KIZ 201211160, KIZ 201211575, KIZ GLGS1945).
- U. nivatus* ( $n = 7$ ): Mount Diancang, Dali, Yunnan (KIZ DL1110269); Mount Cangshan, Dali, Yunnan (KIZ H0122); Mount Yunling, Deqin, Yunnan (KIZ DQ1204010, KIZ DQ1204049); Mount Yunling, Weixi, Yunnan (KIZ WX1204157, KIZ WX1204284); Mount Wushan, Changyang, Hubei (KIZ CY2010353).
- U. soricipes* ( $n = 5$ ): Beichuan, Sichuan (KIZ 0905021); Shawan, Leshan, Sichuan (KIZ 0905283, KIZ 0905292, KIZ 0905308); Mount Daxiangling, Shimian, Sichuan (KIZ 0905409).

## Supplementary material 1

### PCR amplification primer sequences for *CYT B*, *12S*, *RAG1*, *RAG2*, and *PLCB4*

Authors: Xueyang Ren, Yifan Xu, Yixian Li, Hongfeng Yao, Yi Fang, Laxman Khanal, Lin Cheng, Wei Zeng, Xuelong Jiang, Zhongzheng Chen

Data type: xlsx

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Link: <https://doi.org/10.3897/zookeys.1186.111592.suppl1>

## Supplementary material 2

### Samples and sequences used for molecular analyses

Authors: Xueyang Ren, Yifan Xu, Yixian Li, Hongfeng Yao, Yi Fang, Laxman Khanal, Lin Cheng, Wei Zeng, Xuelong Jiang, Zhongzheng Chen

Data type: xlsx

Explanation note: New sequences generated in this study are shown in bold.

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## Supplementary material 3

### Partitioning schemes and molecular evolution model used in mitochondrial–nuclear concatenated gene tree estimations

Authors: Xueyang Ren, Yifan Xu, Yixian Li, Hongfeng Yao, Yi Fang, Laxman Khanal, Lin Cheng, Wei Zeng, Xuelong Jiang, Zhongzheng Chen

Data type: xlsx

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Link: <https://doi.org/10.3897/zookeys.1186.111592.suppl3>

## Supplementary material 4

### Independent-sample *t*-test

Authors: Xueyang Ren, Yifan Xu, Yixian Li, Hongfeng Yao, Yi Fang, Laxman Khanal, Lin Cheng, Wei Zeng, Xuelong Jiang, Zhongzheng Chen

Data type: xlsx

Explanation note: Independent-sample *t*-test of the variances of measurements highly correlated with PC1 and PC2 between *Uropsilus* sp. nov. and *U. atronates*, *U. dabieshanensis*, *U. gracilis*, *U. investigator*, and *U. nivatus*.

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# Another new species of the genus *Habrophlebia* Eaton, 1881 (Ephemeroptera, Leptophlebiidae) from the Maghreb

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## Abstract

A new species belonging to the genus *Habrophlebia* Eaton, 1881 is described at the nymphal stage from the Rif Mountains of Morocco. This species presents unique features, such as the chorionic arrangement of the egg and the ornamentation of the posterior margin of abdominal tergites. It is compared to all west European *Habrophlebia* species and a table with discriminating characters is given. A phylogenetic reconstruction based on COI sequences fully supports the hypothesis of a new species in the Rif Mountains, with possible further distribution in southern Spain.

**Key words:** COI, *Habrophlebia dakkii* sp. nov., mayflies, Morocco, Rif Mountains, Spain, systematics, West Palaearctic



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## Introduction

Affiliated to the subfamily Habrophlebiinae (Leptophlebiidae), *Habrophlebia* Eaton, 1881 is a Holarctic genus, represented by a single species in the Nearctic (Peters 1979) and eight in the West Palearctic (Jacob and Sartori 1984; Alba-Tercedor 2000; Bauernfeind and Soldán 2012; Barber-James et al. 2013; Benhadji et al. 2018; Kechemir et al. 2020). So far, five species of this genus have been reported from North Africa: *Habrophlebia vaillantorum* Thomas, 1986 (Thomas et al. 1999) found in the Moroccan High Atlas; *H. consiglioi* Biancheri, 1959, described from Sardinia and recently reported from Tunisia (Zrelli et al. 2011, 2016); two species discovered in northern Algeria, *H. hassainae* Benhadji & Sartori, 2018 and *H. djurdjurenensis* Kechemir, Sartori & Lounaci, 2020; the presence of *H. fusca* (Curtis, 1834) in the Maghreb (Dakki and El Agbani 1983; Giudicelli and Dakki 1984; Dakki 1987; El Agbani et al. 1992; Lounaci et al. 2000) remains doubtful (Thomas 1998; Thomas et al. 1999) and unconfirmed (El Alami 2002; Benhadji et al. 2018; Kechemir et al. 2020; El Alami et al. 2022a). In fact, in northern Morocco, a review of the ephemeropteran collection from the Rif Mountains, whose specimens come from various streams, revealed the absence of the species in this biogeographical area (El Alami et al. 2022a).

With the exception of *H. fusca*, *H. lauta* McLachlan, 1884 and *H. eldae* Jacob & Sartori, 1984, the majority of species have a very restricted distribution. *Habrophlebia antoninoi* Alba-Tercedor, 2000, is a southern Iberian endemic, *H. vaillantorum* is a Moroccan High Atlas endemic, *H. hassainae* and *H. djurdjurenensis* are Algerian endemics and *H. consiglioii* was collected only in Sardinia and Tunisia.

The isolation of populations in West Palearctic rivers and streams due to geographical barriers has favored speciation within the genus and contributed to an increase in the endemism rate within its biogeographical zone (Dakki 1987; Cheylan 1990; El Alami et al. 2022a).

The Rif is the most northerly mountain range in Morocco. It is characterized by a number of features that give its aquatic fauna a certain originality (Bennas 2002; El Alami 2002; Blondel et al. 2010; Hajji 2013; Errochdi et al. 2014; El Bazi et al. 2017; Khadri et al. 2017). Although the climate is typically Mediterranean, the western part is subject to Atlantic disturbances and is much wetter than the eastern part. In addition, the geological history of the Rif presents two distinctive features: the dominance of schistose or marly soils, with a limestone formation that extends mainly to the west (Thauvin 1971a, b; El Gharbaoui 1981), and the generally steep relief resulting in valleys that are often deep. The connections between the Rif and the Iberian Peninsula over their geological history have enabled a fauna exchange between the African and European continents and enriched the region's populations (Bonada et al. 2009; Jaskuła 2015; Poulakakis et al. 2015; Mabrouki et al. 2017; Múrria et al. 2017; Bennas et al. 2018; Slimani 2018; Taybi et al. 2020; El Alami et al. 2022a).

Ongoing research on mayflies from northern Morocco has revealed that the *Habrophlebia* specimens are not related to either *H. vaillantorum* or *H. fusca*. They belong in fact to a new species that has been cited as *Habrophlebia* sp. in numerous works on the Rif (El Alami 2002; El Bazi et al. 2017; Khadri et al. 2017; Guellaf et al. 2021; El Alami et al. 2022a).

The main objective of the present study is to describe this species based on Mrs El Alami's collection and from material freshly collected by Ms El Yaagoubi. Morphological and molecular data (COI sequences) were combined to separate the nymphs of the Rif populations from other western Palearctic species. We also provide preliminary information on its distribution and ecological preferences.

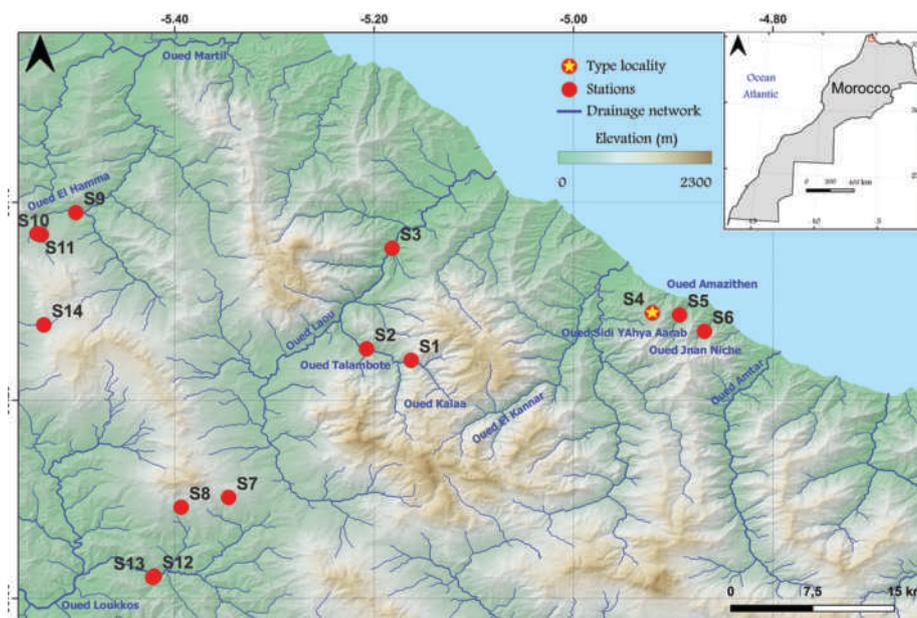
## Materials and methods

Samplings were performed by the LESCOB team between 1997 and 2023 (Fig. 1). They were subsequently preserved in 70% or 95% ethanol for description and DNA extraction.

Pictures of nymphal habitus were made using a Canon EOS 6D camera and the Visionary Digital Passport imaging system (formerly available and distributed by Dun Inc., Virginia), and processed with Adobe Photoshop Lightroom and Helicon Focus ver. 5.3.

Nymphal dissection was performed in Cellosolve or in 10% KOH, and specimens were mounted on slides with Euparal medium, or the dissected parts of the nymphs were mounted directly in Hoyer's liquid (Alba-Tercedor 1988).

Microscopic pictures were taken using an Olympus BX51 microscope coupled with an Olympus SC50 camera; pictures were enhanced with the stacking



**Figure 1.** The sampling site localization of *Habrophlebia dakkii* sp. nov. in the Rif domain.

software Olympus Stream Basic ver. 2.3.2. and Adobe Photoshop ver. 21.2.2. Alternatively, pictures were taken using an Olympus CX41 microscope.

### Molecular analysis

To complement our morphological investigations, we sequenced a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI hereafter) for specimens of the new species and other *Habrophlebia* species collected in the Maghreb. For this, the DNA extraction method described in Vuataz et al. (2011) was used to ensure non-destructive extraction. The Polymerase Chain Reaction (PCR), purification and sequencing steps followed the method outlined in El Alami et al. (2022b). Forward and reverse sequencing reads were assembled and edited in CodonCode Aligner ver. 10.0.2 (Codon-Code Corporation, Dedham, MA). To augment our molecular dataset, we initially downloaded all COI sequences associated with *Habrophlebia* available on the GenBank database as of 27 August 2023, totaling 69 records. Additionally, we obtained *Habrophlebia* sequences from the BOLD SYSTEMS data portal on the same date and selectively retained only those not shared with GenBank, yielding an additional set of six sequences. We then manually excluded GenBank/BOLD sequences obtained from specimens collected outside the western Palearctic region or shorter than 250 bp. This selection process was conducted after confirming that the COI sequences of the new species were clearly distinct from the removed sequences (data not shown). We also included sequences from the Freshwater Diversity Identification for Europe (FREDIE) project (unpublished; <https://wp.fredie.eu/>). A total of 63 sequences remained for further analyses, comprising 18 newly generated sequences (Table 1), 38 sequences from GenBank [five from Cardoni et al. (2015); ten from Gattolliat et al. (2015); 15 from Morinière et al. (2017); three from Behrens-Chapuis et al. (2021); five unpublished International Barcode of Life (iBOL) data releases], five from BOLD (BG-MAY026-10, BGMAY092-11, BGMAY446-11, DTNHM444-21, TRSKA4318-20),

**Table 1.** Newly sequenced specimens (nymphs) for the present study, with collection information, GenBank accession numbers and nomenclature details.

Specimen catalogue nb	Species	Country	Stage	Locality	GPS coordinates	Date	GenBank ID	GenSeq Nomenclature
GBIFCH01144259	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Sidi Yahia Aarab	35°17.179'N, 4°53.625'W	27.xi.2021	OR570530	genseq-2 COI
GBIFCH01144258	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	El Ouesteyine	35°17.299'N, 4°55.267'W	1.ix.2021	OR570531	genseq-2 COI
GBIFCH01144262	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Beni idder	35°22.102'N, 5°32.283'W	16.vii.2021	OR570532	genseq-2 COI
GBIFCH00970948	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Tanaqoub	35°5.533'N, 5°23.604'W	31.iii.2021	OR570533	genseq-2 COI
GBIFCH01144257	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Mezine village	35°6.133'N, 5°20.767'W	31.iii.2021	OR570534	genseq-2 COI
GBIFCH00970944	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Jbel Laalam	35°23.387'N, 5°29.953'W	20.iv.2021	OR570535	genseq-2 COI
GBIFCH00970947	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Souk El Had	35°1.283'N, 5°25.300'W	11.iv.2021	OR570536	genseq-2 COI
GBIFCH00970945	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Tzroute	35°16.583'N, 5°31.883'W	2.v.2021	OR570537	genseq-2 COI
GBIFCH01144261	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Tzroute	35°16.583'N, 5°31.883'W	2.v.2021	OR570538	genseq-2 COI
GBIFCH00970946	<i>Habrophlebia dakkii</i> sp. nov.	Morocco	Nymph	Hammadesh	35°22.033'N, 5°32.033'W	20.iv.2021	OR570539	genseq-2 COI
GBIFCH00970949	<i>Habrophlebia</i> sp. 2	Morocco	Nymph	Afeska	35°10.184'N, 5°13.105'W	2.iv.2021	OR570540	genseq-4 COI
79JJ30_B07	<i>Habrophlebia hassainae</i>	Algeria	Nymph	El Ourit	34°51'57"N, 1°15'54"W	1.i.2016	OR570541	genseq-4 COI
79JJ30_G06	<i>Habrophlebia hassainae</i>	Algeria	Nymph	El Ourit	34°51'57"N, 1°15'54"W	5.ii.2016	OR570542	genseq-4 COI
GBIFCH00673196	<i>Habrophlebia djurdjurenensis</i>	Algeria	Nymph	Tirourda	36°29.431'N, 4°21.693'E	9.vii.2019	OR570543	genseq-4 COI
GBIFCH00673194	<i>Habrophlebia djurdjurenensis</i>	Algeria	Nymph	Echemlili	36°28.267'N, 3°59.84'E	25.v.2018	OR570544	genseq-4 COI
GBIFCH00673195	<i>Habrophlebia djurdjurenensis</i>	Algeria	Nymph	Echemlili	36°28.267'N, 3°59.84'E	25.v.2018	OR570545	genseq-4 COI
GBIFCH00673199	<i>Habrophlebia djurdjurenensis</i>	Algeria	Nymph	Ouadhias	36°29.279'N, 4°07.362'E	9.vii.2019	OR570546	genseq-4 COI
GBIFCH01211557	<i>Habrophlebia djurdjurenensis</i>	Algeria	Nymph	Tala Rana Selloum 2	36°26.902'N, 4°18.820'E	28.iv.2021	OR570547	genseq-4 COI

and two from FREDIE: MO008a\_SR4H12 (Morocco, Rif, wadi Farda at Imizzar) and ES035a\_SR4E11 (Spain, Cordilleras Béticas, Barranco de los Madroñales near Otivar). Two sequences from specimens of *Habroleptoides* Schönemund, 1929 were downloaded from GenBank and included in the dataset as outgroups. All sequences were aligned using MAFFT (Katoh et al. 2019) with default settings as implemented in Jalview ver. 2.11.2.7 (Waterhouse et al. 2009). The number of parsimony-informative sites of the alignment was calculated in Mega ver. 10.2.4 (Kumar et al. 2018; Stecher et al. 2020).

To explore and visualize the COI evolutionary divergence, we employed pairwise genetic distances and gene tree approaches. COI pairwise distances were calculated using the `dist.dna` function from the `ape` 5.7-1 package (Paradis and Schliep 2019) in R ver. 4.3.1 (R Core Team 2023), selecting the raw model and the `pairwise.deletion` option, corresponding to uncorrected p-distances (see Sriwathsan and Meier 2012) with missing data removed in a pairwise way. Mean, minimum, and maximum distances within and between putative COI species, referred to as Molecular Operational Taxonomic Units (MOTUs) hereafter, were calculated using the `ddply` function from the `plyr` ver. 1.8.8 package (Wickham 2011). The assignment of COI sequences to MOTUs was determined based on the results of the species delimitation analyses (as described below). Prior to reconstructing the COI gene tree, the best evolutionary model (GTR+ $\Gamma$ ) was selected based on the second-order Akaike information criterion (AICc; Hurvich and Tsai 1989) implemented in `JmodelTest` ver. 2.1.10 (Darriba et al. 2012) with five substitution schemes, six gamma categories and default values for other parameters. To account for different substitution rates among COI codon positions, we analyzed our data set in two partitions, one with first and second codon

positions, and the other with third positions (1 + 2, 3). Bayesian inference analysis was performed using BEAST ver. 1.10.4 (Suchard et al. 2018) on the CIPRES Science Gateway ver. 3.3 (Miller et al. 2010). The input BEAST file was generated in BEAUTi ver. 1.10.4 (Suchard et al. 2018), incorporating the selected evolutionary model and partition scheme described above. A relaxed molecular clock model (uncorrelated lognormal) and a UPGMA starting tree were used, with default settings for other parameters. Two independent Markov chain Monte Carlo (MCMC) analyses were run for 50 million generations, logging parameters every 1000 generations. Convergence of the MCMC runs was visually verified in Tracer ver. 1.7.2 (Rambaut et al. 2018). The log and tree files from the independent runs were combined using LogCombiner ver. 1.10.4 (Suchard et al. 2018), after discarding the initial 10% of trees as burn-in, ensuring that all parameters reached effective sample size values > 200. The maximum clade credibility tree was obtained using TreeAnnotator ver. 1.10.4 (Suchard et al. 2018) with default settings and then visualized and edited in iTOL ver. 6.8 (Letunic and Bork 2021).

Finally, we applied three contrasting single-locus species delimitation methods to our COI dataset: the distance-based ASAP (Assemble Species by Automatic Partitioning; Puillandre et al. 2020), and the tree-based GMYC (General Mixed Yule-Coalescent; Pons et al. 2006; Fujisawa and Barraclough 2013) and mPTP (multi-rate Poisson Tree Processes; Kapli et al. 2017) approaches. ASAP, an improved version of the ABGD (Automatic Barcode Gap Discovery; Puillandre et al. 2012) approach, was employed using the ASAP webserver (<https://bio-info.mnhn.fr/abi/public/asap/asapweb.html>), computing the genetic distances from our COI alignment under simple p-distances with all other settings set to default. The GMYC model, which requires a time-calibrated ultrametric tree as input, implements a maximum likelihood (ML) approach that defines a threshold separating the branches modelled under speciation events (Yule process) from those described by allele neutral coalescence. The ultrametric tree used as input for GMYC was generated in BEAST, following the same procedure described earlier. However, a reduced dataset was utilized, in which outgroups were excluded and haplotypes were pruned (see Talavera et al. 2013) using Collapsetypes ver. 4.6 (Chesters 2013). MCMC chains were run here for a total of 30 million generations. GMYC was run in R using the SPLITS package 1.0–20 (Ezard et al. 2009). We favored the single-threshold version of the GMYC model because it was shown to outperform the multiple-threshold version (Fujisawa and Barraclough 2013). The mPTP approach, an extension of the PTP method by Zhang et al. (2013), also exploits phylogenetic differences within and between species, but with the advantage of directly using the number of substitutions from a phylogenetic tree, eliminating the need for time calibration. The input ML tree for mPTP was generated in RAxML-NG ver. 1.1.0 (Kozlov et al. 2019), selecting the all-in-one (ML search + bootstrapping) option and MRE-based bootstrap convergence criterion. The best model of evolution and the partition scheme specified above, as well as 50 random and 50 parsimony starting trees, were implemented.

## Abbreviations

- MZL** Muséum Cantonal des Sciences Naturelles, Lausanne (Switzerland);  
**LESCB** Laboratoire d'Ecologie, Systématique et Conservation de la Biodiversité (Morocco).

## Results

### Systematics

#### *Habrophlebia dakkii* El Alami, Sartori & Vuataz, sp. nov.

<https://zoobank.org/D4B71565-041B-417A-8575-8348702DCF73>

Figs 2–8

*Habrophlebia* sp. in El Alami 2002; Khadri et al. 2017; El Bazi et al. 2017.

*Habrophlebia* sp.1 in El Alami et al. 2022a.

**Material examined. Holotype:** one nymph in ethanol (GBIFCH01133087), MOROCCO, Chefchaouen Province, S4 Oued Amazithen, Loc. El Ouesteyine; 35°17.299'N, 4°55.267'W; alt. 483 m; 2.IX.2021; S. El Yaagoubi leg.; MZL.

**Paratypes.** MOROCCO, Chefchaouen Province, same data as holotype; 9 nymphs in ethanol (GBIFCH01133086); 1 nymph on slide (GBIFCH01144258-DNA); MZL; same locality as holotype; 19.IX.2014; Khadri leg.; 2 nymphs in ethanol (GBIFCH01133083); MZL • Chefchaouen Province, S7 Oued Harakat, Loc. Mezine village; 35°6.133'N, 5°20.767'W; alt. 740 m; 31.III.2021; S. El Yaagoubi leg.; 6 nymphs in ethanol (GBIFCH01133085); 1 nymph on slide (GBIFCH01144257-DNA); same locality, 29.II.2020; 1 nymph on slide (GBIFCH00970950); MZL • Chefchaouen Province, S5 Oued Sidi Yahia Aarab, Loc. Sidi Yahia Aarab; 35°17.179'N, 4°53.625'W; alt. 347 m; 18.VI.2014; M. El Alami leg.; 2 nymphs in ethanol (GBIFCH01133079); MZL; 4 nymphs in ethanol; 1 nymph on slide; LESCOB; same locality, 27.XI.2021; S. El Yaagoubi leg.; 1 nymph in ethanol (GBIFCH01133080); 1 nymph on slide (GBIFCH01144259-DNA); MZL • Chefchaouen Province, S6 Oued Jnane Nich, Loc. Jnane Nich; 35°16.1856'N, 4°52.128'W; alt. 215 m; 12.VIII.2021; S. El Yaagoubi leg.; 3 nymphs in ethanol; LESCOB • Chefchaouen Province, S8 Oued Ima sunna, Loc. Tanaqoub; 35°5.533'N, 5°23.604'W; alt. 684 m; 31.III.2021; S. El Yaagoubi leg.; 2 nymphs in ethanol (GBIFCH01133084); 1 nymph on slide (GBIFCH00970948-DNA); MZL; 1 nymph in ethanol; 1 nymph on slide; LESCOB • Chefchaouen Province; S1 Oued Kelâa, Loc. Akchour; 35°14.333'N, 05°10.144'W; alt. 460 m; 17.IV.2008; M. El Alami leg.; 3 nymphs in ethanol (GBIFCH01133088); MZL; 13 nymphs in ethanol, 1 nymph on slide; LESCOB • Chefchaouen Province, S2 Oued Talambote, Loc. usine électrique; 35°16.665'N, 5°13.46.171'W; alt. 129 m; 2.VI.2021; S. El Yaagoubi leg.; 20 nymphs in ethanol; LESCOB • Chefchaouen Province, S3 Oued Laou, Loc. Afertane; 35°20.924'N, 5°11.241'W; alt. 55 m; 4.VI.2022; S. El Yaagoubi leg.; 20 nymphs in ethanol; LESCOB.

• Tetouan Province, S9 Oued El Hamma, Loc. Jbel Laalam; 35°23.387'N, 5°29.953'W; alt. 200 m; 20.IV.2021; S. El Yaagoubi leg.; 6 nymphs in ethanol (GBIFCH01133076); 1 nymph on slide (GBIFCH00970944-DNA); MZL; same locality; 20.II.2022; S. El Yaagoubi leg.; 9 nymphs in ethanol; 1 nymph on slide; LESCOB • Tetouan Province, S10 Oued Tisgris, Loc. Hammadesh; 35°22.033'N, 5°32.033'W; alt. 505 m; 20.IV.2021; S. El Yaagoubi leg.; 8 nymphs in ethanol (GBIFCH01133077); 1 nymph on slide (GBIFCH00970946-DNA); MZL; same data; 7 nymphs in ethanol; LESCOB • Tetouan Province, S11 Oued Taida, Loc. Beni idder; 35°22.102'N, 5°32.283'W; alt. 507 m; 16.VII.2021; S. El Yaagoubi leg.; 7 nymphs in ethanol (GBIFCH01133081); 1 nymph on slide (GBIFCH01144262-DNA); MZL; same locality; 15.V.2017; M. El Alami leg.; 4 nymphs in ethanol; LESCOB.

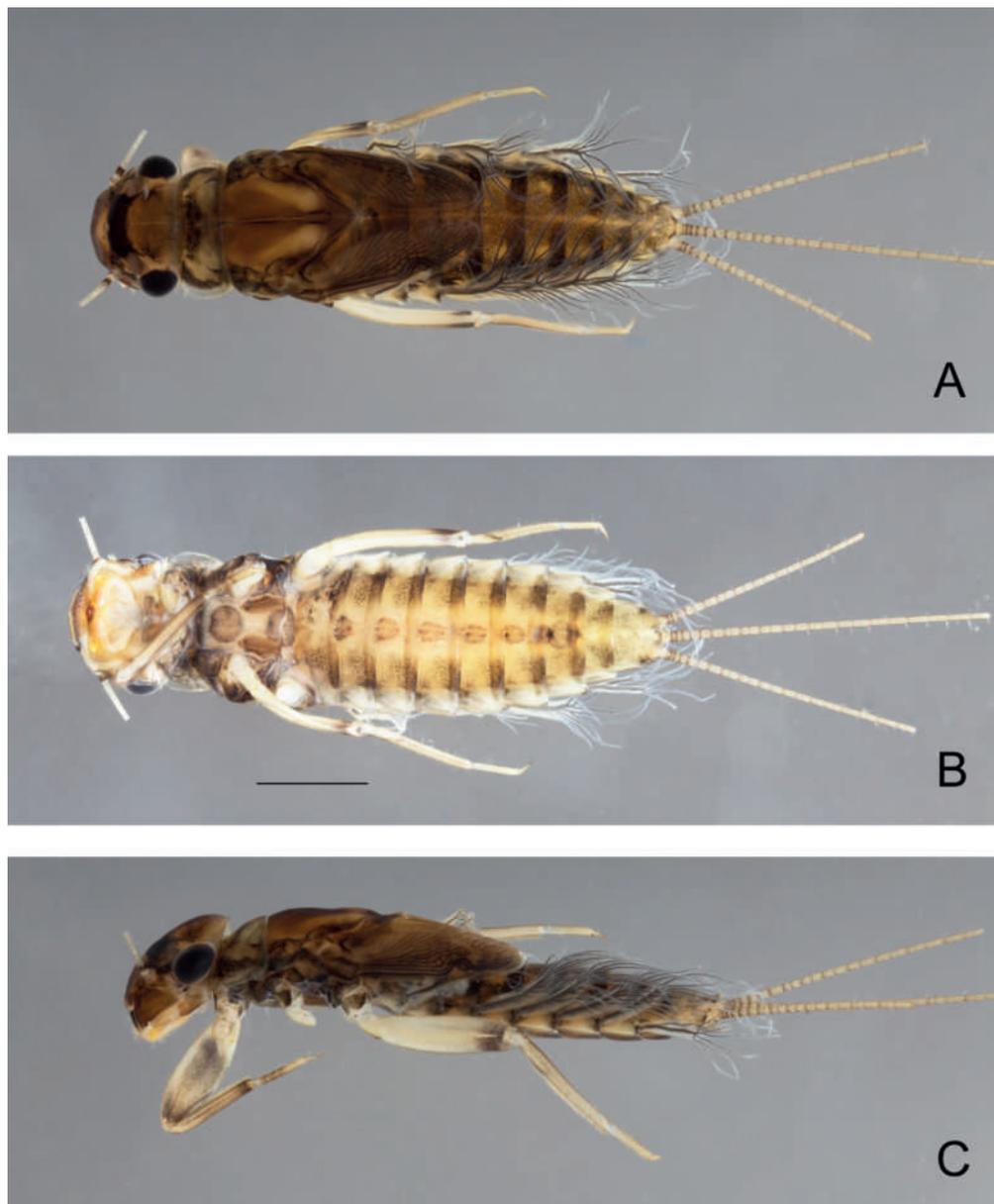
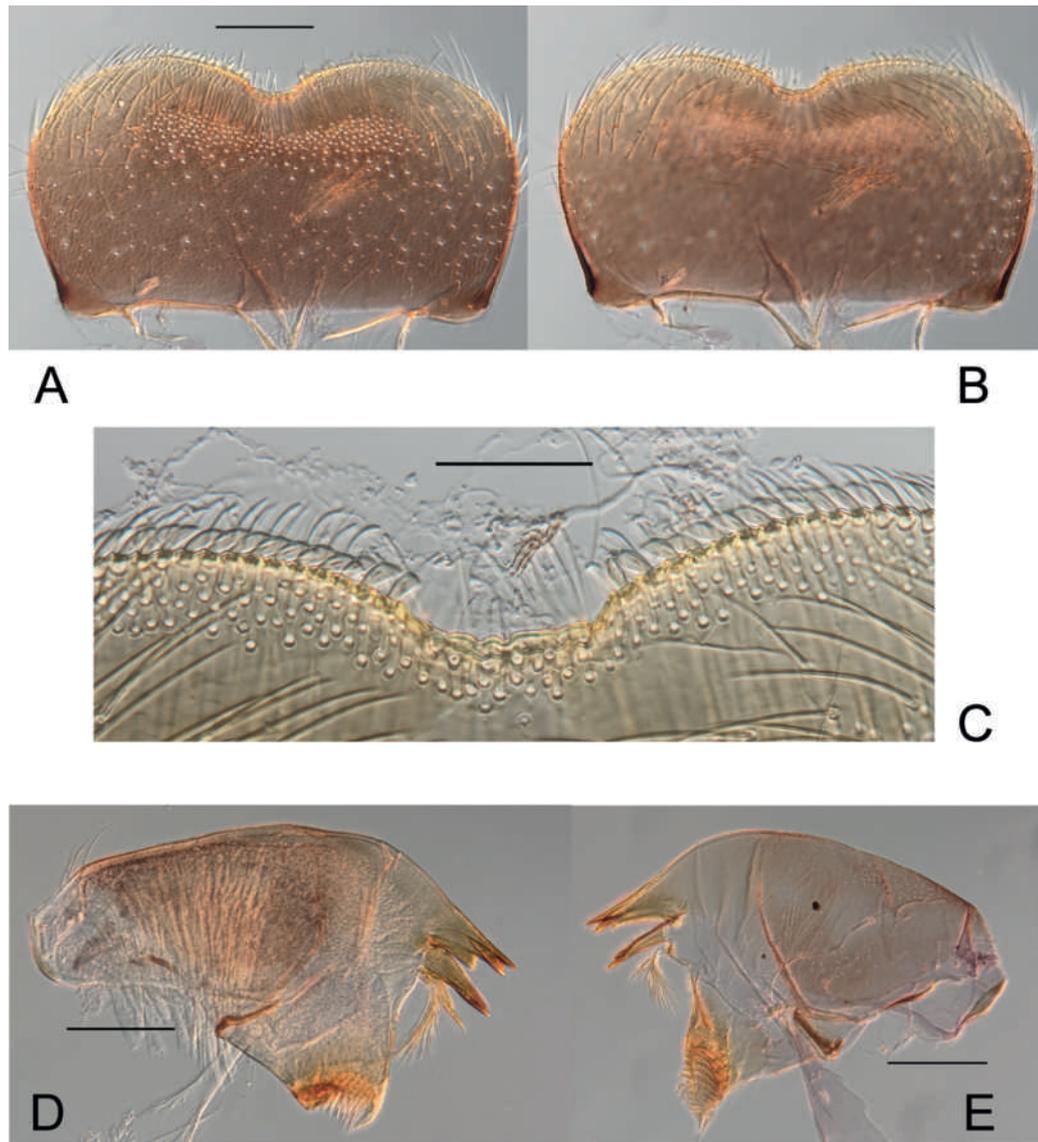


Figure 2. *Habrophlebia dakkii* sp. nov., habitus of the nymph **A** dorsal view **B** ventral view **C** lateral view. Scale bar: 1 mm.

• Larache Province, S14 Oued Stah, Loc. Tzroute; 35°16.583'N, 5°31.883'W; alt. 766 m; 2.V.2021; S. El Yaagoubi leg.; 16 nymphs in ethanol (GBIFCH01133078); 2 nymphs on slide (GBIFCH00970945-DNA, GBIFCH01144261-DNA); MZL; same data; 8 nymphs in ethanol; 2 nymphs on slide; LESCOB; same locality; 18.VI.2022; S. El Yaagoubi leg.; 5 nymphs in ethanol; 1 nymph on slide; LESCOB.

• Ouezzane Province, S13 Oued Qoub, Loc. Souk El Had; 35°1.283'N, 5°25.300'W; alt. 143 m; 11.IV.2021; S. El Yaagoubi leg.; 5 nymphs in ethanol (GBIFCH01133082); 1 nymph on slide (GBIFCH00970947-DNA); MZL; same data; 4 nymphs in ethanol; LESCOB; same locality; 3.VI.2022; S. El Yaagoubi leg.; 2 nymphs in ethanol; LESCOB • Ouezzane Province, S12 Oued Loukkos, Loc. Souk El Had; 35°1.350'N, 5°25.233'W; alt. 140 m; 11.IV.2021; S. El Yaagoubi leg.; 2 nymphs in ethanol; LESCOB.

**Description. Nymph. Coloration and dimensions.** Body length of final instar, excluding caudal filaments, 5.2 to 6.5 mm for male and 5.5 to 8 mm for female.



**Figure 3.** *Habrophlebia dakkii* sp. nov., nymphal mouthparts **A** labrum, dorsal view **B** labrum, ventral view **C** emargination of the labrum **D** left mandible **E** right mandible. Scale bars: 100  $\mu\text{m}$  (**A**, **B**), 50  $\mu\text{m}$  (**C**), 200  $\mu\text{m}$  (**D**, **E**).

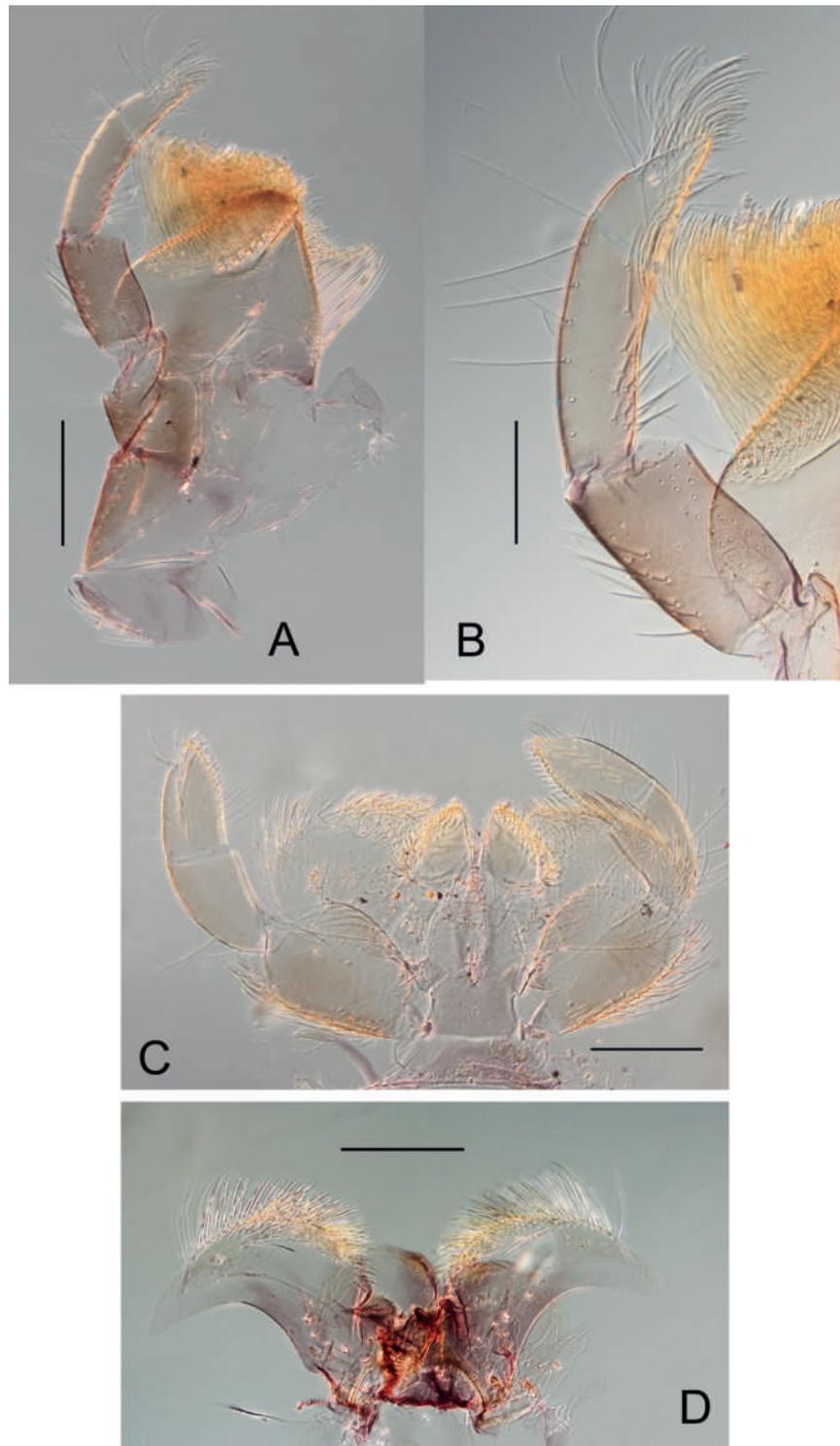
Cerci as long as body length. General dark brown coloration with light brown to yellowish markings mainly on abdominal terga. The whole cuticle is shagreened.

**Head.** General coloration light brown; paler area between compound eyes and lateral ocelli; between ocelli, a dark brown mark not reaching the clypeus distally, and extending laterally in front of the compound eyes; vertex sutures yellowish, well visible (Fig. 2). Upper portion of male eyes reddish-brown. Antenna with pedicel greyish brown, scape and filament yellowish.

**Labrum** rectangular (Fig. 3A, B), ca 2 $\times$  wider than long; dorsal surface covered distally with scattered stout setae, proximally with long and thin setae; anterior margin with a row of stout, long and spatulate setae medially; emargination narrow, U-shaped with six flat denticles (Fig. 3C); ventral surface with two bunches of stout setae medially.

**Mandibles** similar to other *Habrophlebia* species (Fig. 3D, E).

**Maxilla** (Fig. 4) stocky, subapical row of 6 or 7 pectinate setae (Fig. 4A); maxillary palp with three segments, segment 1 and 2 subequal in length, and longer

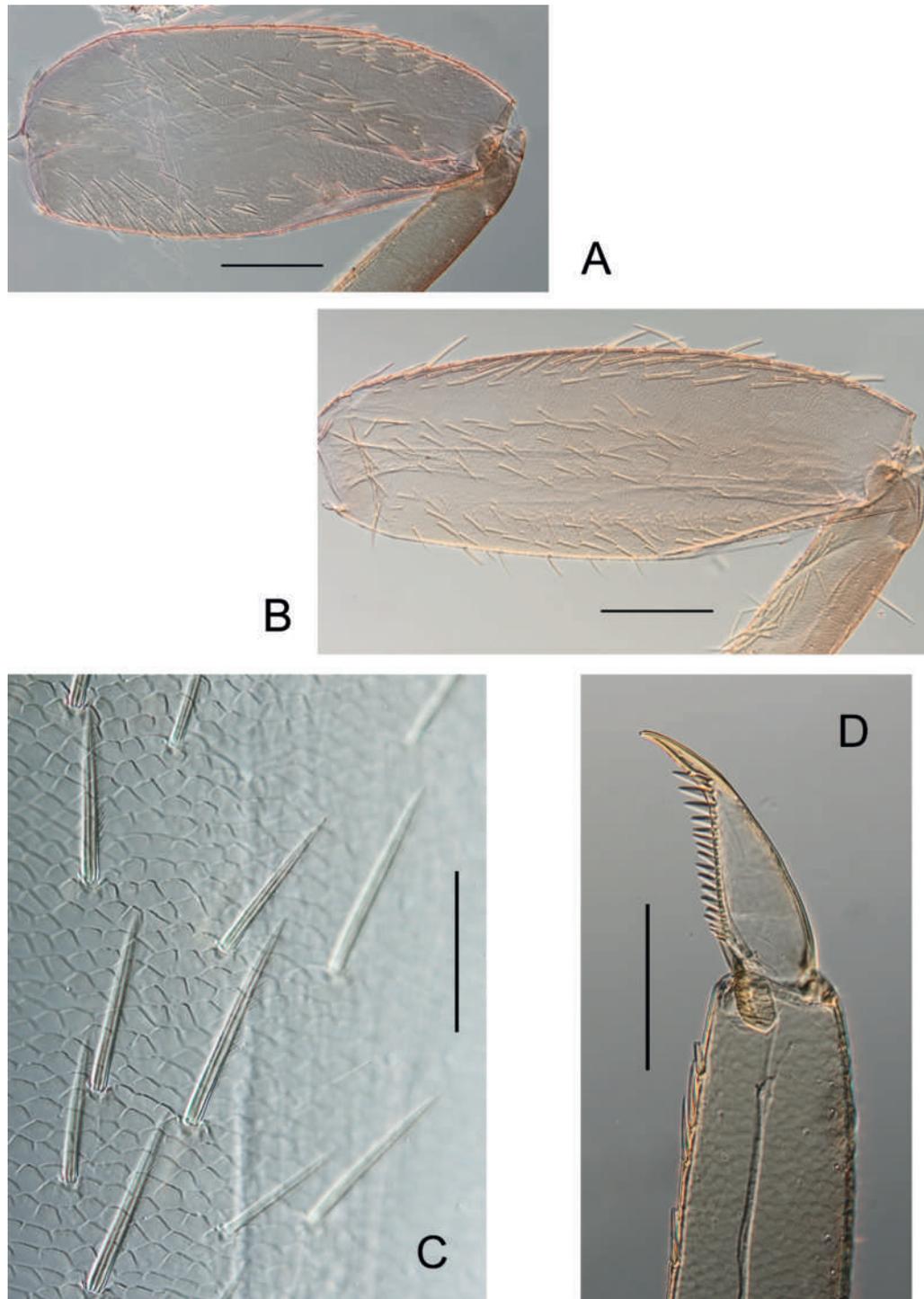


**Figure 4.** *Habrophlebia dakkii* sp. nov., nymphal mouthparts **A** maxilla **B** maxillary palp **C** labium **D** hypopharynx. Scale bars: 200 µm (**A, C, D**), 100 µm (**B**).

than segment 3; segment 3 triangular a little bit 1.5× longer than wide at base; all setae on palp segments stout and entire (Fig. 4B).

**Hypopharynx** with highly developed superlinguae terminated by a membranous digitation (Fig. 4D).

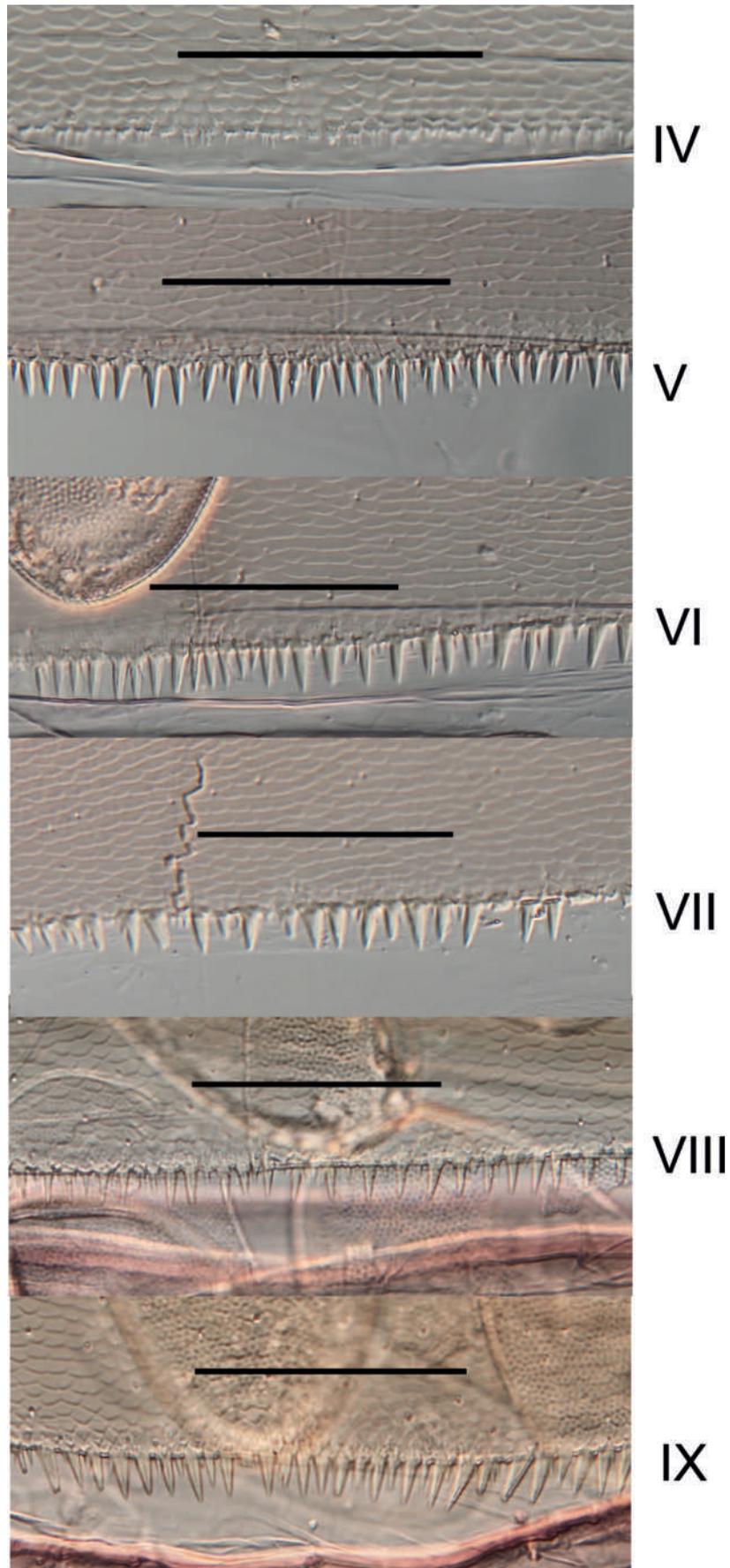
**Labium** (Fig. 4C) with rhomboid glossae, outer margin and apex covered by short, broad setae; paraglossae enlarged laterally, covered with fine and long se-



**Figure 5.** *Habrophlebia dakkii* sp. nov., nymphal legs **A** fore femur **B** hind femur **C** setae on upper face of hind femur **D** claw. Scale bars: 200 µm (**A**, **B**); 50 µm (**C**); 100 µm (**D**).

tae on the dorsal surface; with long, thick setae on the outer margin; labial palp with three segments, inner margin of segment 1 highly dilated near the middle, about 0.8× longer than the maximum width, segments 2 as long as segment 3, ca 0.7× length of segment 1; segment 3 ca 1.6× longer than wide at base, conical shape and the inner margin with one row of longer and robust setae.

**Thorax.** Pro- and mesonotum yellowish to light brown, with greyish brown maculae, on medium and lateral margins (Fig. 2A).



**Figure 6.** *Habrophlebia dakkii* sp. nov., posterior margin of abdominal tergites IV to IX of the nymph. Scale bar: 100  $\mu$ m.

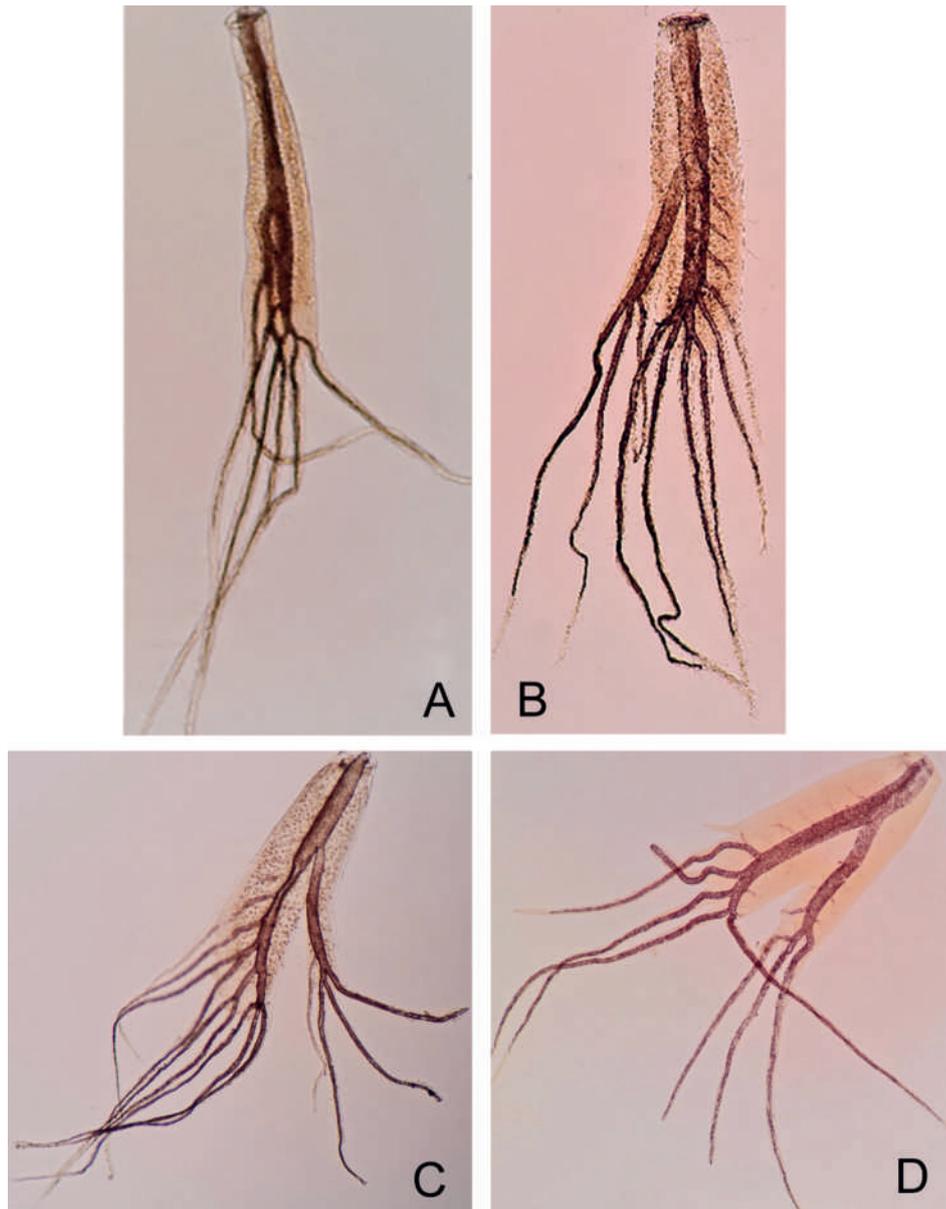


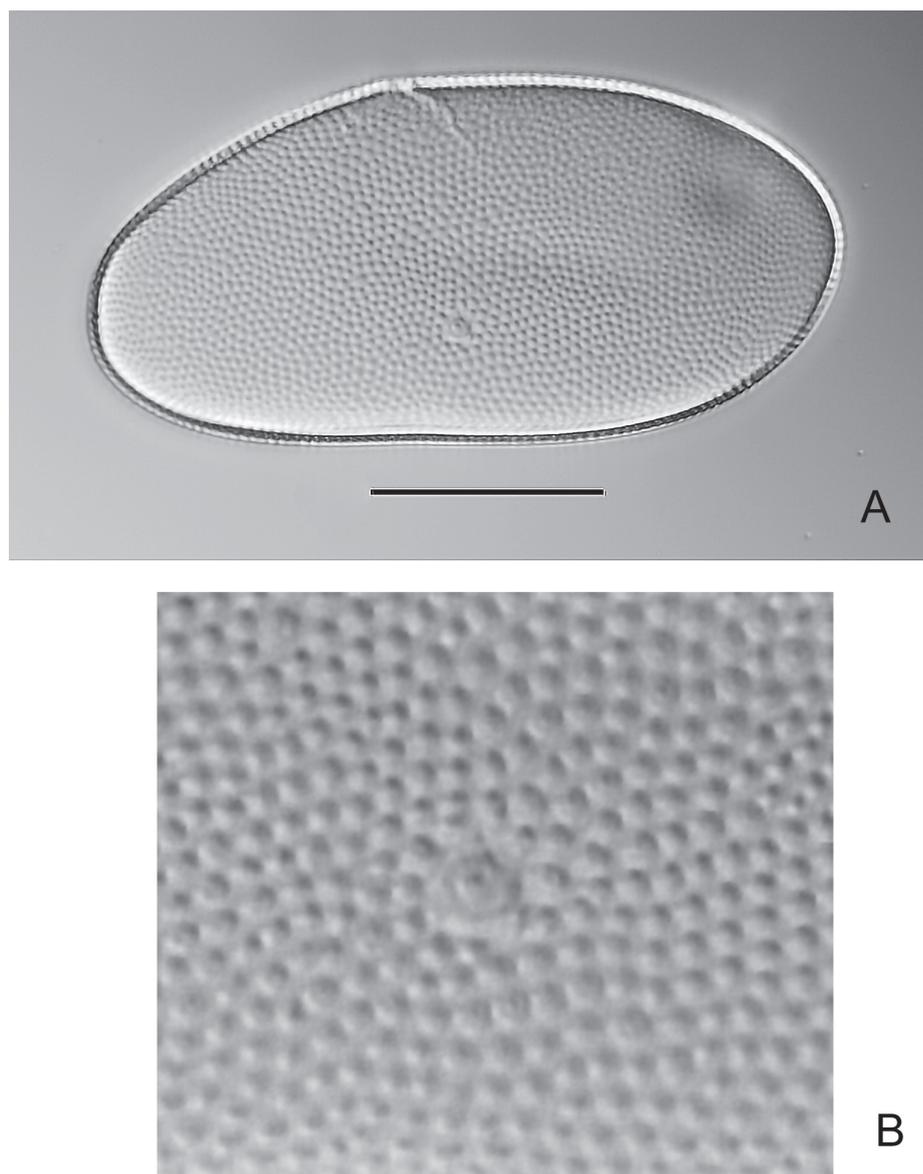
Figure 7. *Habrophlebia dakkii* sp. nov., nymphal gills A gill I B gill III C gill IV D gill VII.

**Legs** light to medium brown; dorsal surface of femora almost entirely washed with greyish brown macula; tarsi and tibiae generally lighter, except sometimes in mature nymphs.

**Fore legs** (Fig. 5A) femora elongated, ca 1.2× longer than wide, upper surface covered with long, entire and pointed setae; fore tibiae subequal in length to femora, outer margin with thin and long setae, inner margin with several rows of long stout and feathered setae especially near apex; tarsi 0.4× length of tibiae, outer margin with long and thin setae, inner margin with long and pointed feathered setae.

**Middle legs** similar to fore legs, femora ca 2.5× longer than wide, dorsal surface of femora with more numerous and slightly longer stout and pointed setae; tibiae and femora of subequal length; tarsi 0.5× length of tibiae.

**Hind legs** (Fig. 5B) with femora 2.8× longer than wide, dorsal surface covered with stout, long, pointed and feathered setae (Fig. 5C); ventral surface with few



**Figure 8.** *Habrophlebia dakkii* sp. nov., egg **A** overview of the chorionic structure **B** detail of the chorion with micropyle. Scale bar: 50  $\mu$ m.

feathered setae; hind tibiae as long as hind femora, outer margin with scattered stout, pointed setae; inner margin with stout, pointed, scattered setae; tarsi 0.4 $\times$  length of tibiae, outer margin with long and thin setae, inner margin with long, stout, pointed setae. Claws (Fig. 5D) of all legs slightly hooked, with 15 to 18 long, thin, pointed denticles that decrease in size from the apex to the tarsus.

**Abdomen.** Grey to dark brownish terga with characteristic light markings (Fig. 2A). Tergites I-II with a dark brown color, terga III-IX with two sublateral elongated dark brown maculae, joining on the posterior margin, leaving two lateral light areas pronounced on tergites III-VIII; posterior marking sometimes absent on tergites IX-X. Sternite I entirely greyish brown (Fig. 2B); sternites II-VIII yellowish with lateral, anterior and posterior bands brown, and two parasagittal nervous ganglia greyish browns; sternite IX lighter brown, in male nymphs with genitalia well visible and styliiger plate dark brown. Posterolateral expansions only on segments VIII and IX. Posterior margin of tergite IX with

well-developed narrow and pointed spines, ca 2–3× longer than wide at base (Fig. 6); shape and size of posterior spines on abdominal segments V to VIII similar, a little bit smaller than those on segment IX; tergites I–IV with barely visible spines (Fig. 6).

**Gills** present on segments I–VII; all gills long and large; first gill (Fig. 7A) with dorsal lamella bearing 3 or 4 filaments, ventral lamella with 2–3 filaments, gills II–VII (Fig. 7B–D) with 3–4 and 6–9 filaments on the ventral and dorsal lamella respectively. Cerci and paracercus yellowish brown, medium brown in mature nymphs (Fig. 2A–C).

**Eggs** ovoid, ca 155 µm x 80 µm, without ribs; chorion surface regularly decorated by numerous and small granulations (Fig. 8).

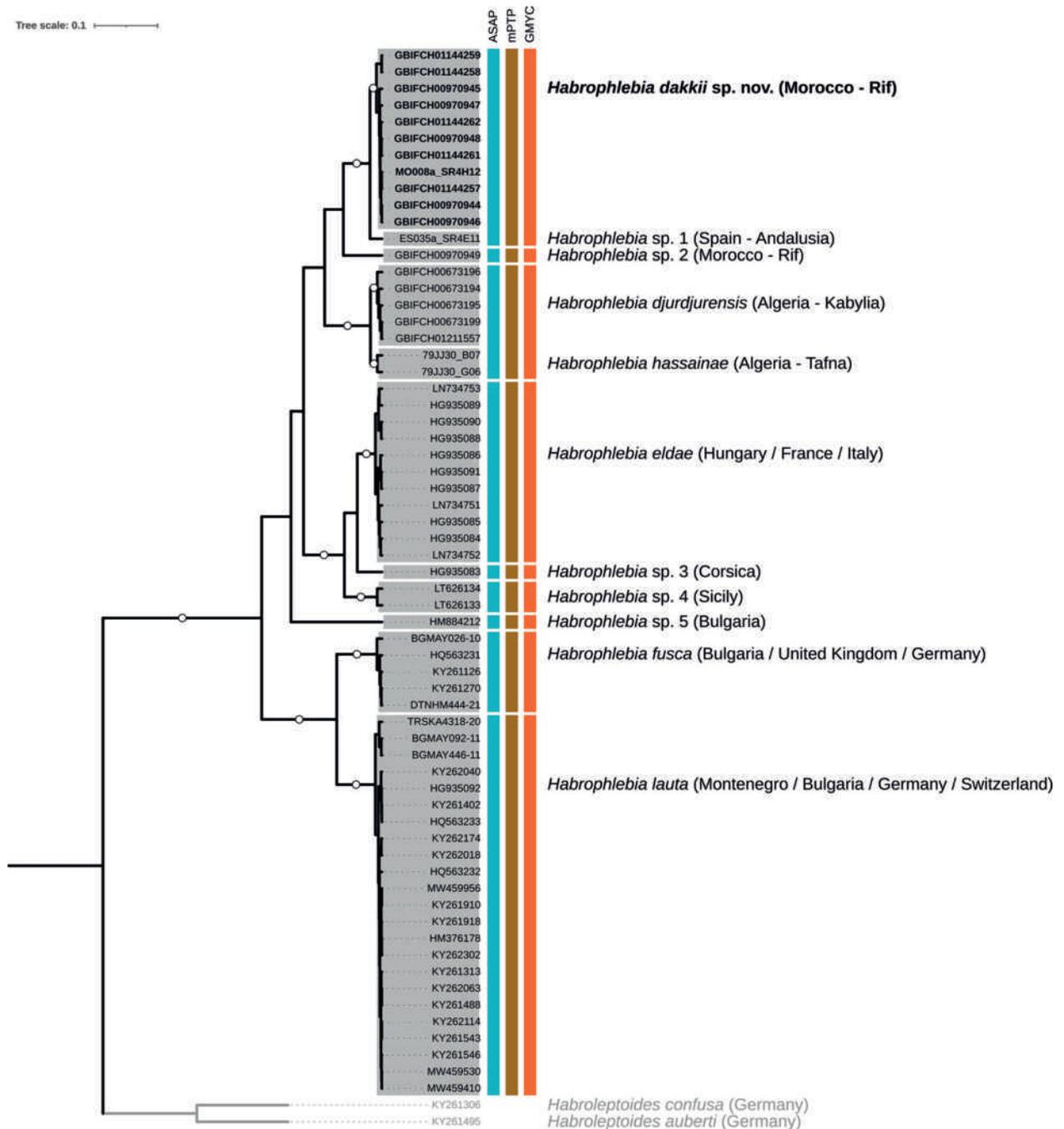
**Imago.** Unknown.

**Etymology.** The first author dedicates this species to her former mentor, Professor Dakki Mohamed. He contributed significantly to her training and specialization in the hydrobiological study of Moroccan streams.

**Molecular analysis.** The COI data set was > 97% complete and included 26% of parsimony informative sites. Pairwise COI distances across all sequences ranged from 0% to 18.2%. All species delimitation methods were fully congruent in delimiting nine *Habrophlebia* MOTUs (Fig. 9). Among these, the 11 sequences from the new species formed a strongly supported monophyletic clade, closely related to the sequence of *H. sp. 1* from Spain (Andalusia) from which it exhibits a minimal p-distance of 0.9%. According to our species delimitation methods, both lineages were grouped into a single MOTU. Similarly, the sequences of *H. djurdjurenensis* and *H. hassainae* formed two distinct, well-supported, sister clades, that were merged into the same MOTU (Fig. 9). The overall mean p-distance within MOTUs was 1.0% (mean range: 0.4%–1.9%), while the overall mean p-distance between MOTUs was 12.9% (mean range: 7.0%–16.9%). The maximum p-distance within MOTUs varied from 1.3% (*H. sp. 4*) to 4.2% (*H. djurdjurenensis* + *H. hassainae*). The minimum p-distance between MOTUs ranged from 6.7% (*H. sp. 3*–*H. eldae*) to 12.3% (*H. fusca*–*H. lauta*). The maximum p-distance within the new species was 1.5%, whereas it was 1.8% for both *H. djurdjurenensis* and *H. hassainae*. The minimum p-distance between the new species and *H. sp. 1* was 0.9%, and between *H. djurdjurenensis* and *H. hassainae* was 2.1%.

## Discussion

Until the late 1970s, only two species of Ephemeroptera were known from the Rif, due to the works of Eaton (1899), Navás (1922, 1929, 1935), Lestage (1925) and Kimmins (1938). This fauna corresponded to two Baetidae recorded in the regions of Tétouan and Chefchaouen [*Baetis rhodani* (Pictet, 1843) and *Proclonon cf. concinnum*]. Since then, a large number of surveys have been carried out to augment this initial list with an additional 35 species, resulting in a total species richness of 37 taxa, which constitutes more than 68% of Morocco's Ephemeroptera (Dakki and El Agbani 1983; Giudicelli and Dakki 1984; El Alami and Dakki 1998; El Alami et al. 2000, 2022a; Kaltenbach et al. 2022; Gattolliat et al. 2023; El Alami et al. 2023; El Yaagoubi et al. 2023). These investigations have collected no less than three new species from the Rif, including *Habrophlebia dakkii* sp. nov.



**Figure 9.** Bayesian (BEAST) maximum clade credibility COI tree of the genus *Habrophlebia* in the West Palearctic. Branch ends labelled with GBIF or 79JJ30 codes indicate newly sequenced specimens; the other codes correspond to sequences obtained from various sources (see material and methods). Colored vertical boxes indicate species delimitation hypothesis (MOTUs) according to the ASAP, mPTP, and GMYC methods. For each MOTU, the corresponding species names (where available) and the country (-region) of origin is provided, with the newly described species and associated GBIF codes specified in bold. Circles on branches indicate Bayesian posterior probabilities > 0.9. The outgroup branches are presented in grey, along with their corresponding labels and species names.

### Morphology characteristics

The main characters used to distinguish the hitherto known species from the new one, are presented in Table 2. At the egg stage, *Habrophlebia dakkii* sp. nov. presents a unique chorionic arrangement among *Habrophlebia* species,

composed of fine granulations evenly distributed on the whole surface, without any other attachment structures (ribs, polar caps). *Habrophlebia dakkii* sp. nov. is most similar to *H. eldae*, especially concerning the abdominal spines on the posterior margin of abdomen, although those of *H. eldae* are larger than those of *H. dakkii*. The latter mainly differs from the former by the number of gill filaments. *Habrophlebia dakkii* can be easily separated from the other Maghrebian species; from *H. hassainae* by the number of denticles on the claw, by the number of gill filaments, and by the shape of posterior abdominal spines on tergites V; from *H. djurdjurenensis* also by the shape of posterior abdominal spines on tergites V-VIII; and from *H. vaillantorum* by the number and the size of denticles on the tarsal claw, the number of gill filaments, and by the shape of posterior abdominal spines on tergites V-IX.

### Genetic characteristics

While the 11 sequences from the new species group together in a well-supported COI clade (Fig. 9), they are sister to the sequence of *H. sp. 1* from Spain (Andalusia) obtained from a young nymph, showing a minimal p-distance of 0.9% between both lineages. Such a genetic distance is typically found within species, as reported in insects in general (e.g., Virgilio et al. 2010) and especially in mayflies (e.g., Ball et al. 2005; Morinière et al. 2017). This observation is further corroborated by the results obtained from the three species delimitation methods. To conclusively determine whether these lineages represent distinct species and potentially establish the new species as a Moroccan endemic or if they constitute a single species with an extended geographic range, additional investigations in the Iberian Peninsula are imperative with new material. A similar situation occurred with the recently described baetid species *Baetis rifensis* El Yaagoubi, Vuataz & Gattolliat, 2023 from Morocco. Its closest COI sequences were from three specimens sampled in the Iberian Peninsula, but in this case, the minimum p-distance between the Moroccan and Iberian lineages was higher (3.2%; El Yaagoubi et al. 2023).

Also interesting is the close genetic relationship between *H. hassainae* and *H. djurdjurenensis*; both species are only separated by a minimum genetic distance of 2.1%, which suggests they could belong to the same species. Morphologically however, both species differ by a number of characters (Table 2), among which are the shape and size of spines on the posterior margin of abdominal tergites, the number of gill filaments and the number of denticles on the claw. Such a low COI divergence between species, possibly indicating recent reproductive isolation, has been sporadically documented in animals (Hebert et al. 2003), including mayflies (e.g., Morinière et al. 2017), and appears to be relatively frequent among closely related species pairs of stoneflies (Vuataz pers. obs.)

Our COI analyses have identified a new *Habrophlebia* lineage in the Rif region, labeled as *H. sp. 2* (Fig. 9). Initial morphological examinations suggest it may be a new species, which will be further investigated in a future study. Additionally, we could not assign names to several other COI clades. While *H. sp. 3* from Corsica and *H. sp. 4* from Sicily were previously identified as *H. eldae* in Gattolliat et al. (2015) and Tenchini et al. (2018), respectively, the COI distances between those three lineages (see Fig. 9) are more indicative of distinct species (with minimum p-distance of *H. eldae*–*H. sp. 3*: 6.7%; *H. eldae*–*H. sp. 4*: 8.5%; *H. sp. 3*–*H. sp. 4*:

**Table 2.** Taxonomic criteria differentiating nymphs and eggs of Western Palaearctic *Habrophlebia* species: (0) Present study; (1) Thomas et al. 1999; (2) Wagner et al., 2007; (3) Mazzini and Gaino 1985 (*H. eldae* sub. nom. *H. fusca*); (4) Zrelli et al. 2011; (5) Alba-Tercedor 2000; (6) Benhaji et al. 2018; (7) Kechemir et al. 2020; (8) Biancheri 1959; (9) Kimmins 1954; (10) Landa 1969; (11) M. Sartori, pers. obs.; (12) Jacob and Sartori 1984; (13) Bauernfeind and Soldán 2012; (14) Thomas and Bouzidi 1986; (15) Righetti 2020; (16) Belfiore and Gaino 1985.

Character	<i>H. fusca</i>	<i>H. lauta</i>	<i>H. eldae</i>	<i>H. consiglioi</i>	<i>H. antoninovi</i> (5)	<i>H. vaillantorum</i>	<i>H. hassainae</i> (6)	<i>H. djurdjurenensis</i> (7)	<i>H. dakkil</i> sp. nov. (0)
Egg chorionic structure	ribs long, punctuated (1)	ribs long, not punctuated (2)	ribs long, slightly punctuated (3)	ribs barbed, long, punctuated (3,4)	ribs forming a reticulated mesh	ribs long, not punctuated (1)	ribs short, not punctuated	ribs long, not punctuated	without ribs, entirely covered with small granulations
Position of the costal process of hind wing	middle (9)	middle (10)	middle (11)	middle (8)	distal	middle (1)	middle	middle	middle
Bristles on upper face of hind femur	truncated, entire (12)	pointed, entire (12)	pointed, fringed (12)	pointed, fringed (12)	?	pointed, entire (13)	pointed, fringed	pointed, fringed	pointed, fringed
Number of denticles on claws	11–13 (11)	14–16 (11)	14–17 (11, 15)	15–17 (11)	?	13–16 (1, 14)	18–22	15–18	15–18
Size of distal denticles on claws	normal (11)	normal (11)	normal (11)	normal (11)	?	reduced (1, 14)	normal	normal	normal
Number of filaments on dorsal (costal) and ventral (anal) lamellae of gills II–VI	7–8; 3–4 (13)	5–7; 4–5 (13)	3–7; 2–4(13)	3–6; 1–3 (16)	?	5–6; 4–5 (1, 14)	9–12; 5–8	8–11; 4–7	6–9; 3–4
Shape and size of posterior spines on abdominal segment IX	truncated, wider than long (1, 2, 16)	triangular, as long as wide at base (2, 16)	triangular, 2–3x longer than wide at base (2, 16)	triangular, 1.5–2x longer than wide at base (16)	?	minute, needle-shaped (1)	triangular, 2–3x longer than wide at base	lanceolate, 2–3x longer than wide at base	triangular, 2–3x longer than wide at base
Shape and size of posterior spines on abdominal segment VIII	truncated, wider than long (16)	triangular, as long as wide at base (16)	triangular, 2–3x longer than wide at base (16)	triangular, 1.5–2x longer than wide at base (16)	?	? (probably minute, needle-shaped)	triangular, 2–3x longer than wide at base	minute, needle-shaped	triangular, 2–3x longer than wide at base
Shape and size of posterior spines on abdominal segment VII	truncated, wider than long (16)	triangular, as long as wide at base (16)	triangular, 2–3x longer than wide at base (16)	triangular, 1.5–2x longer than wide at base (16)	?	minute, needle-shaped (1)	triangular, 2x longer than wide at base	minute, needle-shaped	triangular, 2–3x longer than wide at base
Shape and size of posterior spines on abdominal segment V	truncated, wider than long (16)	triangular, as long as wide at base (16)	triangular, 2–3x longer than wide at base (16)	triangular, as long as wide at base (16)	?	? (probably minute or absent)	minute, needle-shaped	minute, needle-shaped	triangular, 2–3x longer than wide at base

7.7%), as pointed out by Tenchini et al. (2018). Similarly, *H. sp. 5* from Bulgaria, presently labeled as *H. fusca* in GenBank, clearly differs from *H. fusca* and all other lineages in our dataset. The p-distance to its nearest neighbors, *H. eldae* and *H. lauta* (12.2%), strongly supports *H. sp. 5* as a distinct, new species. These results emphasize the need for more taxonomic research on this genus in Europe.

### **Distribution and ecology**

This species is widely distributed in the Rif, where it occupies a large range of biotope types, spanning from sea level up to an altitude of 780 m. It also tolerates wide variations in water conductivity (35 to 1112  $\mu\text{S}/\text{cm}$ ). *Habrophlebia dakkii* has a clear preference for poorly mineralized headwaters with moderate current velocity. The substrate is characterized by pebbles, gravel, sand and silt covered in some places with algae and submerged macrophytes, which provide excellent refuge for nymphs when the current is stronger.

The same remark was made by Gagneur and Thomas (1988) concerning *Habrophlebia hassainae* (sub. nom. *H. gr. fusca*) found in Algeria.

Since this species has been found in localities on the Oued Ouergha, which is the Rif tributary of the Oued Sebou, we believe that its presence in the Haut Sebou (Middle Atlas) and the coastal Meseta is highly probable.

Nymphs of *Habrophlebia dakkii* sp. nov. can be found all year long, but are most abundant in spring, when temperatures are optimal for their development.

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### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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# A review of the subgenus *Loxocera* Meigen, 1803 (Diptera, Brachycera, Psilidae) in China

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## Abstract

The subgenus *Loxocera* (*Loxocera* Meigen, 1803) (Diptera, Brachycera, Psilidae) in China is reviewed. Six species are recognized, including four new species: *L. (L.) chikuni* **sp. nov.**, *L. (L.) lonsdalei* **sp. nov.**, *L. (L.) maculithorax* **sp. nov.**, and *L. (L.) obscura* **sp. nov.** Two species originally placed in the subgenus *Loxocera*, *L. anulata* Wang & Yang, 1998 and *L. tianmuensis* Wang & Yang, 1998, are transferred to the subgenus *Loxocera* (*Imantimyia* Frey, 1925). A key to the species of the subgenus *Loxocera* occurring in China is provided.

**Key words:** Acalyptratae, Diopsoidea, *Imantimyia*, new species, Palaearctic Realm, Psilinae



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## Introduction

Psilidae is a group of small to medium-sized, yellow to black acalyptrate flies which can be diagnosed externally by their peculiar wing venation and reduced setation (Lonsdale 2020; Shatalkin 2021). With about 340 species described so far, the Psilidae is distributed in all zoogeographic regions, with the highest diversity occurring in the temperate areas of the northern hemisphere (Shatalkin and Merz 2010; Lonsdale 2020). The monophyly of Psilidae and its several subtaxa are well supported, whereas the generic classification within Psilinae needs further consideration (Buck and Marshall 2006a, 2006b; Lonsdale 2020; Zhou and Yang 2022).

The psiline genus *Loxocera* Meigen, 1803 is cosmopolitan and currently comprises about 50 species. This genus was traditionally divided into four subgenera: *Loxocera* s. str., *Asiopsila* Shatalkin, 1998, *Platystyla* Macquart, 1835, and *Tropeopsila* Shatalkin, 1983 (Shatalkin 1998). Based on a morphological phylogenetic analysis, Buck and Marshall (2006b) redefined the genus, recognized three subgenera (*Loxocera* s. str., *Imantimyia* and *Tropeopsila*), transferred *Asiopsila* to the genus *Psila* Meigen, 1803, and synonymized *Platystyla* with *Loxocera* s. str. The genus *Terarista* Yang & Wang, 2003 has also been confirmed as a junior synonym of the subgenus *Loxocera* recently (Zhou et al. 2022).

In the present study, we review and key the Chinese fauna of the subgenus *Loxocera* and document six species, four of which are described here as new. The other two Chinese species previously placed in the subgenus *Loxocera* are here transferred to *Imantimyia*.

Outside of China, an additional eight species of the subgenus *Loxocera* are known, with these occurring exclusively within the Palaearctic and northern Oriental realms (Iwasa 1992, 1993; Buck and Marshall 2006b; Zhou et al. 2022). A checklist for all described species of the subgenus *Loxocera* is also provided in this paper.

## Materials and methods

Specimens examined in this study are deposited in the Entomological Museum of China Agricultural University, Beijing, China (**CAU**) and the Smithsonian National Museum of Natural History, Washington, DC, USA (**USNM**).

Male terminalia were prepared by macerating the apical portion of the abdomen in heated 10% KOH solution for approximately 10 min, and then rinsing in distilled water. External structure and terminalia were examined using a Nikon SMZ745 stereoscopic microscope. After examination, the terminalia were transferred to fresh glycerol and stored in microvials pinned below the corresponding specimens.

Photographs were taken using a Canon 7D Mark II digital camera with a Canon macro lens EF 100 mm and MP-E 65 mm for habitus, and an Olympus BX51 microscope for terminalia. Figures were stacked using Helicon Focus v. 5.3 and assembled by Adobe Photoshop 2020. The distribution map was prepared using the online version of SimpleMappr (Shorthouse 2010). Terminology follows Buck and Marshall (2006b) and Lonsdale (2020). Measurements were obtained using a calibrated micrometer; body length is measured from apex of frons to apex of abdomen; interocular space is the width between eyes.

## Results

### Genus *Loxocera* Meigen, 1803

#### Subgenus *Loxocera* Meigen, 1803

*Loxocera* Meigen, 1803: 275. Type species: *Musca aristata* Panzer, 1801, by monotypy.

*Platystyla* Macquart, 1835: 374. Type species: *Loxocera hoffmannseggii* Meigen, 1826, by monotypy. Synonymized by Buck and Marshall (2006b: 199).

*Terarista* Wang, 1999: 268. Nomen nudum.

*Terarista* Yang & Wang in Wang & Yang, 2003: 563. Type species: *Terarista fuji-ana* Wang, 1999, by original designation. Synonymized by Zhou et al. (2022: 465).

**Diagnosis.** The subgenus *Loxocera* can be recognized by the following combination of character states: frontal vitta desclerotized, dull, velvety; lunule sclerotized, broadly exposed between antennal base and anterior margin of frons; fore wing with alula glabrous except margin; hind femur with a subapical patch of microtomentum on ventral surface; male sternite 8 broadly exposed and setulose, fused to tergite 6 and epandrium; female tergite 10 and cerci separated. For details on the character states used to define the subgenus, see Buck and Marshall (2006b).

### Key to species of subgenus *Loxocera* from China

- 1 Arista blackish brown, laterally compressed and very high, arising at apex of antennal first flagellomere (Figs 3, 4, 21); wing with broad, transverse, dark band at level of posterior crossvein (Figs 1, 19).....**2**
- Arista whitish yellow, thin, arising near midpoint of antennal first flagellomere (Figs 12, 29, 36); wing without transverse dark band (Figs 27, 35).....**4**
- 2 Frontal vitta uniformly black..... ***L. (L.) fujiana* (Wang)**
- Frontal vitta black with anterior part dark yellow or yellowish brown (Figs 2, 20).....**3**
- 3 Apex of antennal first flagellomere not produced beyond base of arista (Figs 3, 4); mesonotum blackish brown; hypandrial lobe small, short (Figs 5–8) ..... ***L. (L.) chikuni* sp. nov.**
- Apex of antennal first flagellomere clearly produced beyond base of arista (Fig. 21); mesonotum largely blackish brown, with irregular dark brown margin (Fig. 22); hypandrial lobe large, broad (Figs 23–26) ..... ***L. (L.) maculithorax* sp. nov.**
- 4 Antennal scape and pedicel subequal in length (Fig. 36)..... ***L. (L.) omei* Shatalkin**
- Antennal scape and pedicel unequal in length (Figs 12, 29).....**5**
- 5 Antennal scape distinctly shorter than pedicel (Fig. 12); antennal first flagellomere about 3 times as long as pedicel (Fig. 12); fore and mid femora blackish brown with apical half yellowish brown; pregonite short, simple in shape, apically blunt (Fig. 16); phallus shovel-like, abruptly widened apically with rounded posterior margin (Figs 14, 15).....***L. (L.) lonsdalei* sp. nov.**
- Antennal scape distinctly longer than pedicel (Fig. 29); antennal first flagellomere about 4.6 times as long as pedicel (Fig. 29); fore and mid femora dark yellow with base pale yellow, hind femur dark brown; pregonite rather long, slender, curved apically with sharp apex (Fig. 33); phallus droplet-like, abruptly narrowed apically (Figs 31, 32).....***L. (L.) obscura* sp. nov.**

#### ***Loxocera (Loxocera) chikuni* sp. nov.**

<https://zoobank.org/48776277-5971-456E-86BD-2EE0265B2A2D>

Figs 1–8

**Type material. Holotype** (♂): CHINA, Hubei, Shennongjia, Guanmenshan, 1560 m, 2019.viii.12, leg. Ding Yang (CAU).

**Diagnosis.** Generally blackish brown; face blackish; antennal scape and pedicel subequal in length; apex of antennal first flagellomere not produced beyond base of arista; arista laterally compressed and very high, arising at apex of first flagellomere and 1.8 times as long as the latter; wing with broad, transverse, dark band at level of posterior crossvein; hypandrial lobe small, short, covered with long setae; pregonite stout, lobate, apically blunt with rounded processes; phallus tongue-like, relatively short, with rounded posterior margin.

**Description. Male.** Body length 9.0 mm, wing length 7.2 mm, length of antenna 2.5 mm. Generally blackish brown, moderately shining (Fig. 1). Frontal vitta black, with anterior part yellowish brown (Fig. 2); parafacial, gena and posterior eye margin yellowish brown; proboscis and palpus pale brown. Wing slightly



1



2



3

4

Figures 1–4. *Loxocera (Loxocera) chikuni* sp. nov., holotype, male 1 habitus, lateral 2 head, dorsal 3 left antenna, dorsal 4 same, lateral. Scale bars: 3 mm (1); 0.5 mm (2); 1 mm (3, 4).

infumated, with broad, transverse, dark band at level of posterior crossvein, band more or less interrupted along center of cells  $r_{4+5}$  and dm (Fig. 1); wing veins yellowish brown to brown. Halter white with base slightly darkened. Legs with trochanters, apical half of fore and mid femora, fore and mid tibiae, basal half of hind tibia, and tarsomere I pale brown. Bristles on head and thorax black.

Head (Figs 1, 2) transverse oblong in dorsal view, largely glabrous; length along midline 0.8 times as long as width across eyes, width across eyes 1.9 times as broad as interocular space. Frons slightly protruding beyond level of anterior eye margin; frontal vitta with shallow depression at middle; frontal orbit with some short, scattered hairs. Ocellar triangle broad, smooth, with silvery tomentose stripes along lateral margins. Face strongly slanting, with weakly elevated median carina. Parafacial narrow, with a tomentose golden patch between anterior eye margin and lunule. Gena swollen; postgena covered with silvery tomentum. Occiput with a large silvery tomentose patch at middle above foramen. Head chaetotaxy: 1 ocellar seta, 1 postvertical seta, 1 inner vertical seta, 1 outer vertical seta. Antenna (Figs 3, 4) long and thick, with short dense setulae; scape nearly as long as pedicel, gradually widened towards apex of segment; first flagellomere about 2.1 times as long as pedicel, apex very weakly curving ventrally; arista laterally compressed and very high, arising at apex of first flagellomere, 1.8 times as long as first flagellomere, divided into small aristomere 1 and large aristomeres 2+3. Palpus elongate oval, with short, dense setulae and long, scattered setae.

Thorax (Fig. 1) robust, with short, dense, whitish-yellow setulae, except anepisternum (anterior half), anepimeron, katatergite, meron, scutellum and mediotergite (middle portion) glabrous; anatergite with fine tomentum; disc of scutellum with fine wrinkles. Scutum 1.4 times as long as wide. Scutellum subtriangular, slightly swollen, and wider than long. Thoracic chaetotaxy: 1 dorso-central seta, 1 notopleural seta, 2 posterior supra-alar setae, 1 apical scutellar seta. Wing (Fig. 1) with last sector of  $M_1$  strongly curved; apex of  $M_4$  nearly reaching wing margin. Legs with dense, whitish-yellow setulae, except ventral surface of fore and mid femora largely glabrous; femora subfusiform, slightly compressed laterally; tibiae gradually widened towards apex, fore and mid tibiae straight, hind tibia finely curved.

Abdomen elongate, with short, dense, whitish-yellow and black setae; syntergite 1+2 with several long, hair-like setae laterally.

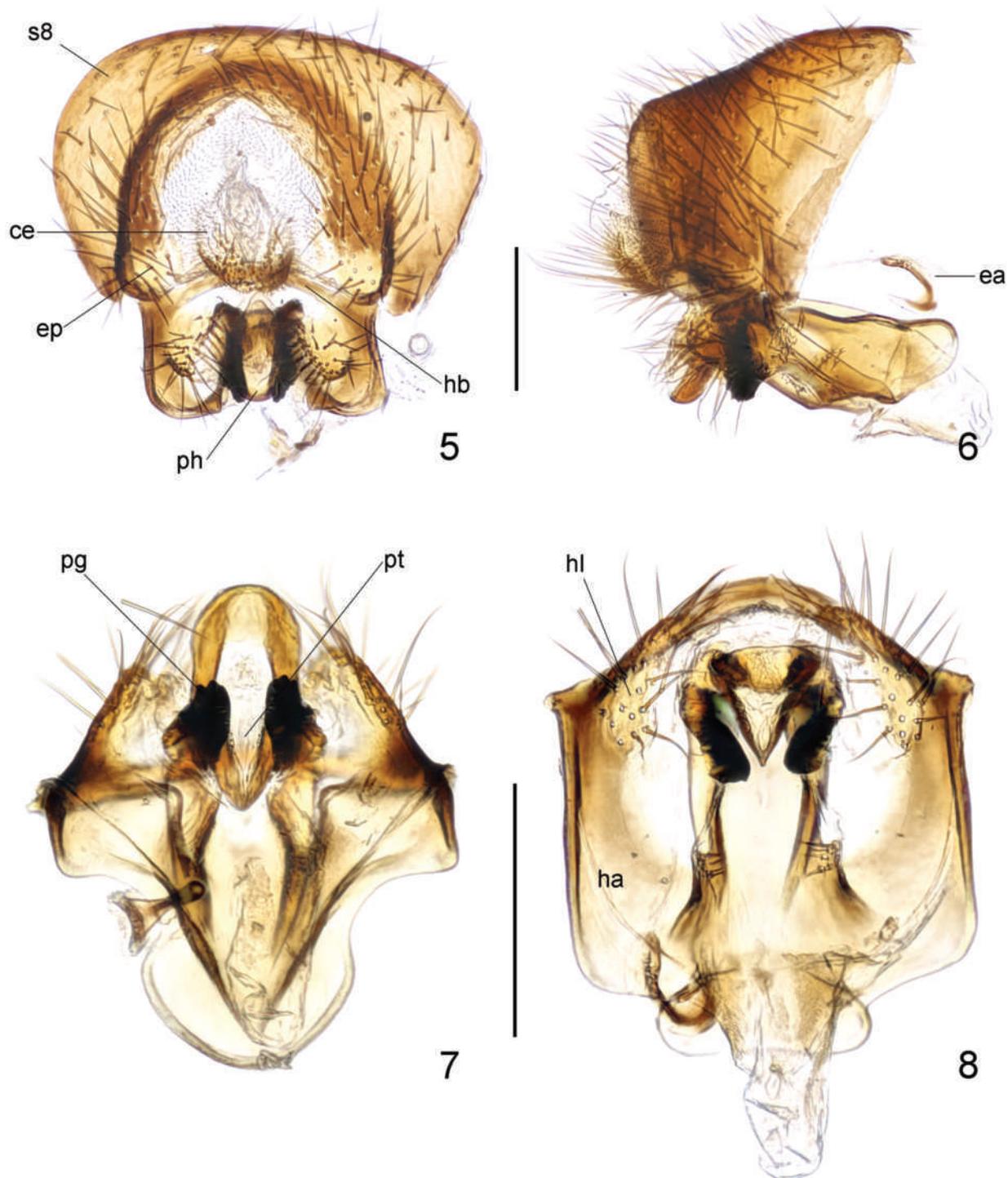
**Male genitalia:** Sternite 8 (Figs 5, 6) broad, relatively flattened, with long, dense setae. Cerci (Figs 5, 6) small, simple in shape, with short, dense setae. Hypandrium (Fig. 8) well developed; hypandrial arms fused posterodorsally, posteriorly produced into small, short, convex lobes covered with long setae (Figs 5–8). Hypandrial bridge present and robust. Pregonite (Figs 5–7) stout, lobate, apically blunt, with some rounded processes. Phallus (Figs 5–7) tongue-like, relatively short, with rounded posterior margin. Phallotrema (Fig. 7) large, flanked by some peculiar, tiny processes. Ejaculatory apodeme (Fig. 6) small, hook-like, strongly curved.

**Female.** Unknown.

**Etymology.** The specific epithet is dedicated to the Chinese entomologist Chikun Yang (1925–2006), in honor of his excellent contribution to Chinese insect taxonomy.

**Distribution.** China – Hubei: Shennongjia (Fig. 37).

**Comparative notes.** This new species is similar to *L. (L.) fujiana* (Wang, 1999) and *L. (L.) maculithorax* sp. nov. by sharing the laterally compressed and high arista which arises at the apex of the antennal first flagellomere, and the broad, transverse, dark band on the wing. The new species differs from *L. (L.) fujiana* by the following character states: head blackish brown with anterior



Figures 5–8. *Loxocera (Loxocera) chikuni* sp. nov., male genitalia 5 sternite 8 to genitalia, caudal 6 same, lateral 7 pregonite and phallus, ventral 8 hypandrium and associated structures, ventral. Abbreviations: ce = cercus, ea = ejaculatory apodeme, ep = epandrium, ha = hypandrial arm, hb = hypandrial bridge, hl = hypandrial lobe, pg = pregonite, s8 = sternite 8, ph = phallus, pt = phallotrema. Scale bar: 0.25 mm.

part of frontal vitta, parafacial, gena, and posterior eye margin yellowish brown [vs head blackish brown with gena slightly paler in *L. (L.) fujiana*]; arista 1.8 times as long as antennal first flagellomere [vs 2 times in *L. (L.) fujiana*]; hind tibia pale brown in basal half and blackish brown in apical half [vs uniformly

dark brown in *L. (L.) fujiana*]; transverse dark band on wing interrupted along cell  $r_{2+3}$  [vs not interrupted in cell  $r_{2+3}$  in *L. (L.) fujiana*]. The new species can be separated from *L. (L.) maculithorax* sp. nov. by the following character states: apex of antennal first flagellomere not produced beyond base of arista [vs produced beyond base of arista in *L. (L.) maculithorax* sp. nov.]; arista 1.8 times as long as antennal first flagellomere [vs 2.1 times in *L. (L.) maculithorax* sp. nov.]; mesonotum uniformly blackish brown [vs largely blackish with irregular brownish margin in *L. (L.) maculithorax* sp. nov.]; abdomen uniformly blackish brown [vs reddish brown with both ends darkened in *L. (L.) maculithorax* sp. nov.]; male sternite 8 flattened [vs with a small blunt protrusion in *L. (L.) maculithorax* sp. nov.]; hypandrial lobe of male small and short [vs large and broad in *L. (L.) maculithorax* sp. nov.].

### ***Loxocera (Loxocera) fujiana* (Wang, 1999)**

*Terarista fujiana* Wang, 1999: 268 (protologue); Wang and Yang (2003: 563, 565) (subsequent usage, redescription, lectotype designation, figure); Cui et al. (2009: 326) (listed); Liang (2016: 170) (listed); Tang et al. (2021: 236) (catalogue, distribution). Lectotype (♀): China, Fujian, Wuyishan, CAU.

*Loxocera (Loxocera) fujiana*: Zhou et al. (2022: 465) (new combination, redescription, distribution, photo).

**Type material examined.** **Lectotype** (♀): CHINA, Fujian, Wuyishan, Guadun, 1991.x.7, leg. Hong Wu (CAU).

**Diagnosis.** Generally blackish brown; face black; antennal scape and pedicel subequal in length; apex of antennal first flagellomere not produced beyond base of arista; arista laterally compressed and very high, arising at apex of antennal first flagellomere and 2 times as long as the latter; wing with broad, transverse, dark band at level of posterior crossvein; segment 8 of female with shallowly emarginated posterodorsal margin and deeply incised posteroventral margin.

**Distribution.** China – Fujian: Wuyishan (Fig. 37).

**Remarks.** For redescription and photographs of this species, see Zhou et al. (2022).

### ***Loxocera (Loxocera) lonsdalei* sp. nov.**

<https://zoobank.org/78375471-5312-46F1-89EB-59C7BDA7DAA2>

Figs 9–17

**Type materials.** **Holotype** (♂): CHINA, Shaanxi, Xi'an, Huiyuan, Zhuque Forest Park, 2606 m, 2020.vii.10, leg. Bing Zhang (CAU). **Paratypes:** CHINA, Qinghai, Haibei, Menyuan, Deqian vill., 2725 m, 2019.vii.18, leg. Jinlong Ren (1♀, CAU); CHINA, Qinghai, Haidong, Huzhu, Beishan Forest Farm, Zhalonggou, 2724 m, 2019.vii.2, leg. Qilemoge (1♂1♀, CAU); CHINA, Qinghai, Haidong, Huzhu, Yuanfugou, 2682 m, 2019.vii.3, leg. Qilemoge (1♀, CAU); same collection data as for holotype (5♂5♀, CAU); CHINA, Shaanxi, Xi'an, Zhouzhi, Wangjiahe, 1165 m, 2020.vii.6, leg. Bing Zhang (1♀, CAU).



9



10

Figures 9, 10. *Loxocera (Loxocera) lonsdalei* sp. nov., habitus, lateral **9** male, holotype **10** female, paratype. Scale bar: 3 mm.

**Diagnosis.** Generally brown to blackish brown; face yellowish brown; antennal pedicel distinctly longer than scape; antennal first flagellomere about 3 times as long as pedicel, gradually narrowed towards apex; arista whitish yellow, thin, arising near midpoint of antennal first flagellomere and 1.3 times as long as the latter; mesonotum blackish, with irregular brownish margin; wing without transverse dark band; hypandrial lobe large, broad, covered with short setae; pregonite short, simple in shape, apically blunt; phallus shovel-like, relatively long, abruptly widened apically with rounded posterior margin.

**Description. Male and female.** Body length 7.7–9.5 mm, wing length 6.0–6.5 mm, length of antenna 1.6–1.8 mm. Generally brown to blackish brown, moderately shining (Figs 9, 10). Frontal vitta black, with anterior part brown (Fig. 11); ocellar triangle and face yellowish brown; parafacial, gena and posterior eye margin dark yellow (Figs 11, 12). Antenna with arista whitish yellow (Fig. 12). Proboscis and palpus pale brown. Postpronotum brown to dark



Figures 11–13. *Loxocera (Loxocera) lonsdalei* sp. nov., paratype, female 11 head, dorsal 12 same, lateral 13 thorax, dorsal. Scale bar: 1 mm.

brown. Mesonotum largely blackish brown, with irregular brown to dark brown margin as shown in Fig. 13. Scutellum yellowish brown (Fig. 13). Mesopleuron dark yellow with variable brown to dark brown mottling (Fig. 10), or largely blackish brown (Fig. 9). Wing slightly infumated; wing veins yellowish brown to dark brown. Halter white with base slightly darkened. Legs dark brown to blackish brown; coxae brown to blackish brown; trochanters, apex of femora, tibiae, and tarsi yellowish brown; mid tibia with indistinct, narrow, dark ring subapically (Figs 9, 10); hind tibia with wide dark ring at middle (Figs 9, 10). Abdomen yellowish brown; anterior half and posterior margin of syntergite 1+2 and posterior portion of tergites 3–5 in male blackish brown (Fig. 9); anterior half and posterior margin of syntergite 1+2 and posterior portion of tergites 3–6 in female dark brown, posterior half of tergite 7 and anterior half of tergite 10 blackish brown (Fig. 10). Bristles on head and thorax black.

Head (Figs 11, 12) nearly rounded in dorsal view, largely glabrous; length along midline 0.9 times as long as width across eyes, width across eyes 2 times as broad as interocular space. Frons strongly protruding beyond level of anterior eye margin; frontal vitta with shallow depression at middle; frontal orbit with some short, scattered hairs. Ocellar triangle broad, smooth. Face strongly slanting, with weakly elevated median carina. Parafacial narrow, with a tomentose golden patch between anterior eye margin and lunule. Gena swollen; postgena covered with silvery tomentum. Occiput with a large silvery tomentose patch at middle above foramen. Head chaetotaxy: 1 ocellar seta, 1 postvertical seta, 1 inner vertical seta, 1 outer vertical seta. Antenna (Fig. 12) long and thick, with short dense setulae; pedicel distinctly longer than scape; first flagellomere about 3 times as long as pedicel, gradually narrowed towards apex; arista thin, arising near midpoint of first flagellomere, 1.3 times as long as first flagellomere, divided into small aristemere 1 and large aristemeres 2+3. Palpus elongate oval, with short dense golden setulae and long scattered black setae.

Thorax (Figs 9, 10, 13) robust, with short dense whitish yellow setulae, except anepisternum (anterior half), anepimeron, katatergite, katepisterum (middle portion), meron, scutellum and mediotergite (middle portion) glabrous; anatergite with fine tomentum. Scutum 1.2 times as long as wide. Scutellum (Fig. 13) subtriangular, slightly swollen and wider than long. Thoracic chaetotaxy: 1 dorsocentral seta, 1 notopleural seta, 2 posterior supra-alar setae, 1 apical scutellar seta. Wing with last sector of  $M_1$  strongly curved; apex of  $M_4$  nearly reaching wing margin. Legs with dense, whitish-yellow setulae, except ventral surface of fore and mid femora largely glabrous; femora subfusiform, slightly compressed laterally; tibiae gradually widened towards apex, nearly straight.

Abdomen elongate, with short, dense, whitish-yellow setae; syntergite 1+2 with several long hair-like setae laterally.

**Male genitalia:** Sternite 8 (Figs 14, 15) broad, relatively flattened, with long, dense setae. Cerci (Figs 14, 15) relatively slender, slightly curved, with short, dense setae. Hypandrium (Fig. 17) well developed; hypandrial arms posteriorly produced into very large, broad, convex lobes covered with short setae (Figs 14–17). Hypandrial bridge present and robust. Pregonite (Figs 14–16) short, simple in shape, apically blunt. Phallus (Figs 14, 15) shovel-like, relatively long, abruptly widened apically with rounded posterior margin. Phallostrema (Fig. 16) large, flanked by peculiar, short or long, simple or apically bifurcate processes. Ejaculatory apodeme (Fig. 15) small, hook-like, strongly curved.

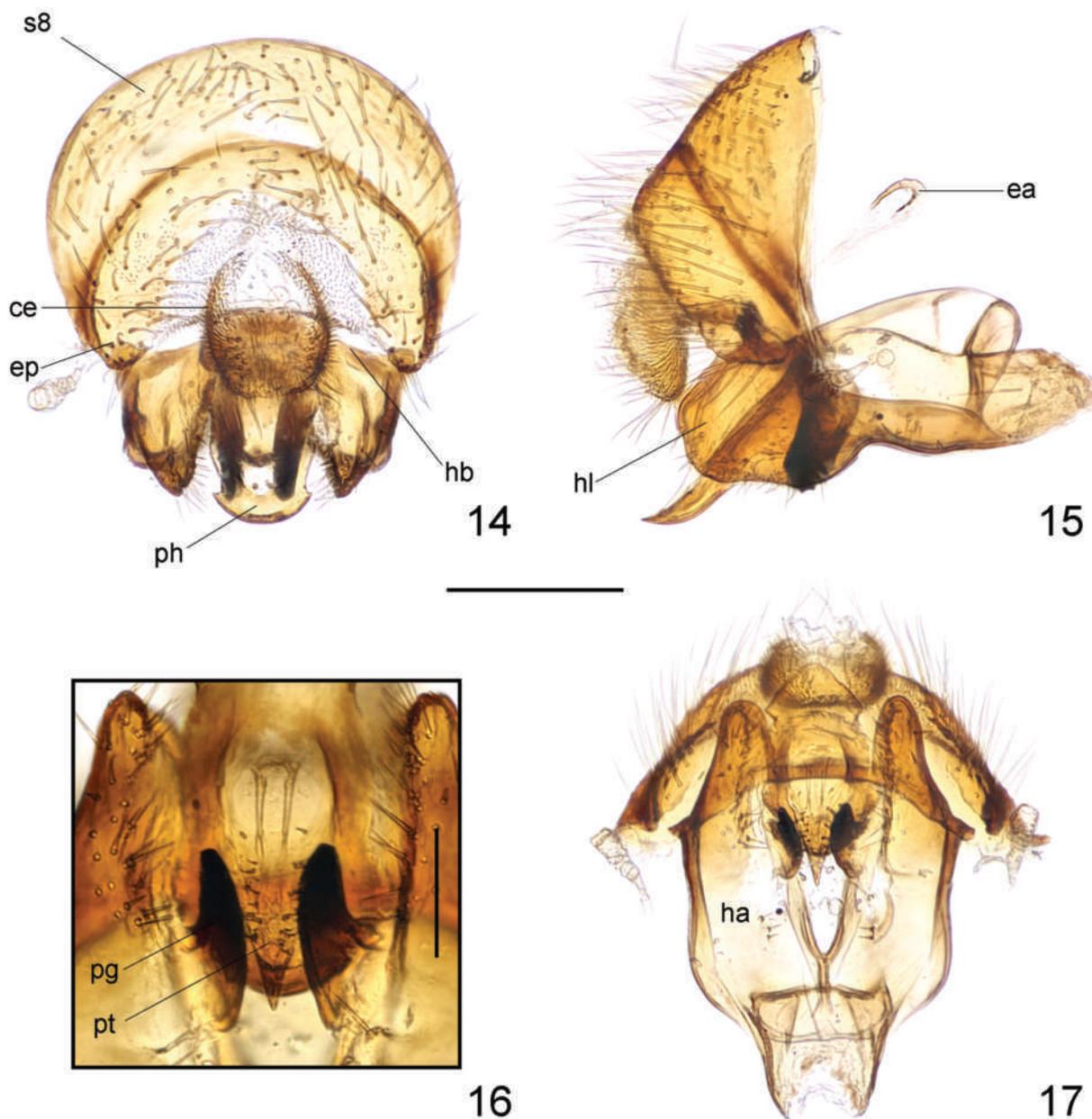
**Female terminalia:** Segment 7 laterally compressed; sternite 7 keeled along midline. Segment 8 coriaceous and longitudinally striate, posterodorsal margin shallowly emarginate, posterolateral margin forming blunt angular protrusion, posteroventral margin with deep linear incision. Tergite 10 relatively long and broad, with tiny, scattered setulae. Cerci relatively broad, separate from tergite 10, with rows of long setulae on posterior margin.

**Etymology.** The specific epithet is dedicated to the Canadian entomologist Owen Lonsdale (Agriculture and Agri-Food Canada, Ottawa, Canada), for his outstanding contribution to the systematics of Acalypratae, and his encouragement to the first author.

**Distribution.** China – Qinghai: Haibei, Haidong; Shaanxi: Xi'an (Fig. 37).

**Comparative notes.** This new species is most similar to *L. (L.) omei* Shatkin, 1998, but it can be easily distinguished from the latter by the following character states: antennal pedicel distinctly longer than scape [vs subequal in length in *L. (L.) omei*]; antennal first flagellomere distinctly narrowed towards apex [vs weakly narrowed in *L. (L.) omei*]; mesonotum largely blackish with irregular brownish margin [vs uniformly blackish in *L. (L.) omei*]; abdomen yellowish brown with distinct ring-like markings [vs blackish brown with apical segments reddish in *L. (L.) omei*].

Antennal morphology similar to that of this new species also presents in *L. (L.) hoffmannseggii* Meigen, 1826 from Central and West Europe and *L. (L.) matsumurai* Iwasa, 1992 from Japan. However, the coloration of head, thorax, and abdomen of the new species is very different from that of the latter two species. Additionally, the head of the new species is nearly rounded in dorsal view with the frons strongly protruding anteriorly, while in the latter two species, the head is transverse oblong in dorsal view and the frons weakly protrudes anteriorly.



Figures 14–17. *Loxocera (Loxocera) lonsdalei* sp. nov., male genitalia **14** sternite 8 to genitalia, caudal **15** same, lateral **16** pregonite and phallus, ventral **17** hypandrium and associated structures, ventral. Abbreviations: ce = cercus, ea = ejaculatory apodeme, ep = epandrium, ha = hypandrial arm, hb = hypandrial bridge, hl = hypandrial lobe, pg = pregonite, s8 = sternite 8, ph = phallus, pt = phallotrema. Scale bars: 0.25 mm (**14**, **15**, **17**); 0.1 mm (**16**).

***Loxocera (Loxocera) maculithorax* sp. nov.**

<https://zoobank.org/60A9A3DD-8E8A-4D3D-B507-9BB730CDC9F0>

Figs 18–26

**Type materials. Holotype** (♂): CHINA, Shaanxi, Baoji, Longxian, Guanshan Grassland, 2034 m, 2020.viii.13, leg. Bing Zhang (CAU). **Paratypes**: same collection data as for holotype (1♂1♀, CAU).

**Diagnosis.** Generally dark brown; face brown; antennal scape and pedicel subequal in length; apex of antennal first flagellomere produced beyond base



Figures 18, 19. *Loxocera (Loxocera) maculithorax* sp. nov., habitus, lateral **18** male, holotype **19** female, paratype. Scale bar: 3 mm.

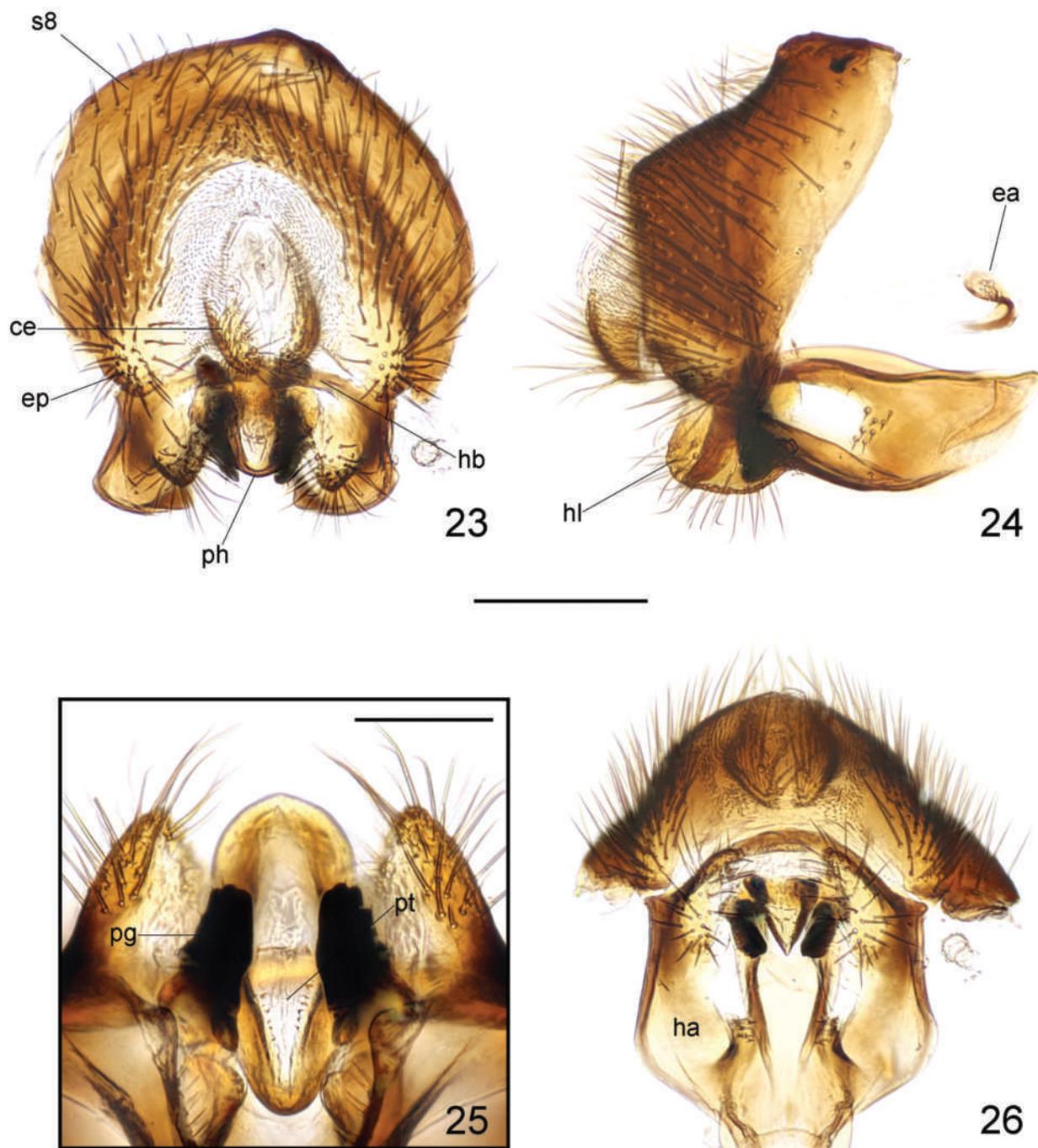
of arista; arista laterally compressed and very high, arising at apex of antennal first flagellomere and 2.1 times as long as the latter; mesonotum blackish, with irregular, brownish margin; wing with broad, transverse, dark band at level of posterior crossvein; hypandrial lobe large, short, covered with long setae; pregonite stout, lobate, apically blunt, with some rounded processes; phallus tongue-like, relatively short, with rounded posterior margin.



Figures 20–22. *Loxocera (Loxocera) maculithorax* sp. nov., holotype, male 20 head, dorsal 21 same, lateral 22 thorax, dorsal. Scale bar: 1 mm.

**Description. Male and female.** Body length 8.8–10.5 mm, wing length 6.2–7.0 mm, length of antenna 1.9–2.3 mm. Generally dark brown, moderately shining (Figs 18, 19). Frontal vitta black, with anterior and median parts dark yellow (Fig. 20); face brown; parafacial, gena, postgena, proboscis, and palpus pale brown (Fig. 21). Postpronotum brown. Mesonotum largely blackish brown, with irregular, dark brown margin as shown in Fig. 22. Wing slightly infumated, with broad, transverse, dark band at level of posterior crossvein, band more or less interrupted along center of cells  $r_{2+3}$ ,  $r_{4+5}$  and  $dm$ ; wing veins yellowish brown to brown. Halter white with base slightly darkened. Legs dark brown to blackish brown; trochanters, apex of femora, fore and mid tibiae, and tarsomere 1 yellowish brown; hind tibia in male dark brown except base and apex slightly paler (Fig. 18), in female yellowish brown, with wide, median, dark brown ring (Fig. 19). Abdomen reddish brown (♀, Fig. 19) to dark reddish brown (♂, Fig. 18); syntergite 1+2, tergite 6 and posterior portion of tergite 5 in male, and tergite 7 and posterior margin of tergite 6 in female blackish brown. Bristles on head and thorax black.

Head (Figs 20, 21) transverse oblong in dorsal view, largely glabrous; length along midline 0.7 times as long as width across eyes, width across eyes 2 times as broad as interocular space. Frons slightly protruding beyond level of anterior eye margin; frontal vitta with shallow depression at middle; frontal orbit with some short, scattered hairs. Ocellar triangle broad, smooth, with silvery tomentose stripes along lateral margins. Face strongly slanting, with moderately elevated median carina. Parafacial narrow, with a tomentose golden patch between anterior eye margin and lunule. Gena swollen; postgena covered with silvery tomentum. Occiput with a large silvery tomentose patch at middle above foramen. Head chaetotaxy: 1 ocellar seta, 1 postvertical seta, 1 inner vertical seta, 1 outer vertical seta. Antenna (Fig. 21) long and thick, with short, dense setulae; scape nearly as long as pedicel, gradually widened towards apex; first flagellomere about 2 times as long as pedicel, apex strongly curving ventrally,



Figures 23–26. *Loxocera (Loxocera) maculithorax* sp. nov., male genitalia **23** sternite 8 to genitalia, caudal **24** same, lateral **25** pregonite and phallus, ventral **26** hypandrium and associated structures, ventral. Abbreviations: ce = cercus, ea = ejaculatory apodeme, ep = epandrium, ha = hypandrial arm, hb = hypandrial bridge, hl = hypandrial lobe, pg = pregonite, s8 = sternite 8, ph = phallus, pt = phallotrema. Scale bars: 0.25 mm (**23**, **24**, **26**); 0.1 mm (**25**).

produced beyond base of arista; arista laterally compressed and very high, arising at apex of first flagellomere, 2.1 times as long as first flagellomere, divided into small aristomere 1 and large aristomeres 2+3. Palpus elongate oval, with short, dense setulae and long, scattered setae.

Thorax (Figs 18, 19, 22) robust, with short, dense, whitish-yellow setulae, except anepisternum (anterior half), anepimeron, katatergite, meron, scutellum, and mediotergite (middle portion) glabrous; anatergite with fine tomentum.

Scutum 1.4 times as long as wide. Scutellum (Fig. 22) subtriangular, slightly swollen, and wider than long. Thoracic chaetotaxy: 1 dorsocentral seta, 1 notopleural seta, 2 posterior supra-alar setae, 1 apical scutellar seta. Wing with last sector of  $M_1$  strongly curved; apex of  $M_4$  nearly reaching wing margin. Legs with dense, whitish-yellow setulae, except ventral surface of fore and mid femora largely glabrous; femora subfusiform, slightly compressed laterally; tibiae gradually widened towards apex, fore and mid tibiae straight, hind tibia finely curved.

Abdomen elongate, with short, dense, whitish-yellow setae; syntergite 1+2 with several long hair-like setae laterally.

**Male genitalia:** Sternite 8 (Figs 23, 24) broad, with long, dense setae, dorsally with a wide, blunt protrusion at middle. Cerci (Figs 23, 24) relatively broad, elongate, with short, dense setae. Hypandrium (Fig. 26) well developed; hypandrial arms fused posterodorsally, posteriorly produced into large, short, convex lobes covered with long setae (Figs 23–26). Hypandrial bridge present and robust. Pregonite (Figs 23–25) stout, lobate, apically blunt, with some rounded processes. Phallus (Figs 23–25) tongue-like, relatively short, with rounded posterior margin. Phallosome (Fig. 25) large, flanked by some peculiar, tiny processes. Ejaculatory apodeme (Fig. 24) small, hook-like, strongly curved.

**Female terminalia:** Segment 7 laterally compressed; sternite 7 keeled along midline. Segment 8 coriaceous and longitudinally striate, posterodorsal margin shallowly emarginate, posterolateral margin forming acute angular protrusion, posteroventral margin with deep linear incision. Tergite 10 relatively short and narrow, with scattered tiny setulae. Cerci relatively broad, separate from tergite 10, with rows of long setulae on posterior margin.

**Etymology.** The specific epithet is derived from Latin *macula* (meaning spotted) and Greek *thorax* (meaning thorax), referring to the mesonotum of the new species, which is blackish with an irregular brownish margin.

**Distribution.** China – Shaanxi: Baoji (Fig. 37).

**Comparative notes.** This new species is similar to *L. (L.) fujiana*, but it can be readily separated from the latter by the different coloration of head, mesonotum, hind tibia, and abdomen, the apically curved and produced first flagellomere, and the shortened scutum. It also resembles *L. (L.) chikuni* sp. nov., and their differences are discussed above [see under *L. (L.) chikuni* sp. nov.]. The Japanese species *L. (L.) monstrata* Iwasa, 1992 shares a similar antennal morphology with the new species, but its arista is less than 2 times as long as the first flagellomere, and it has different coloration on thorax, legs and abdomen.

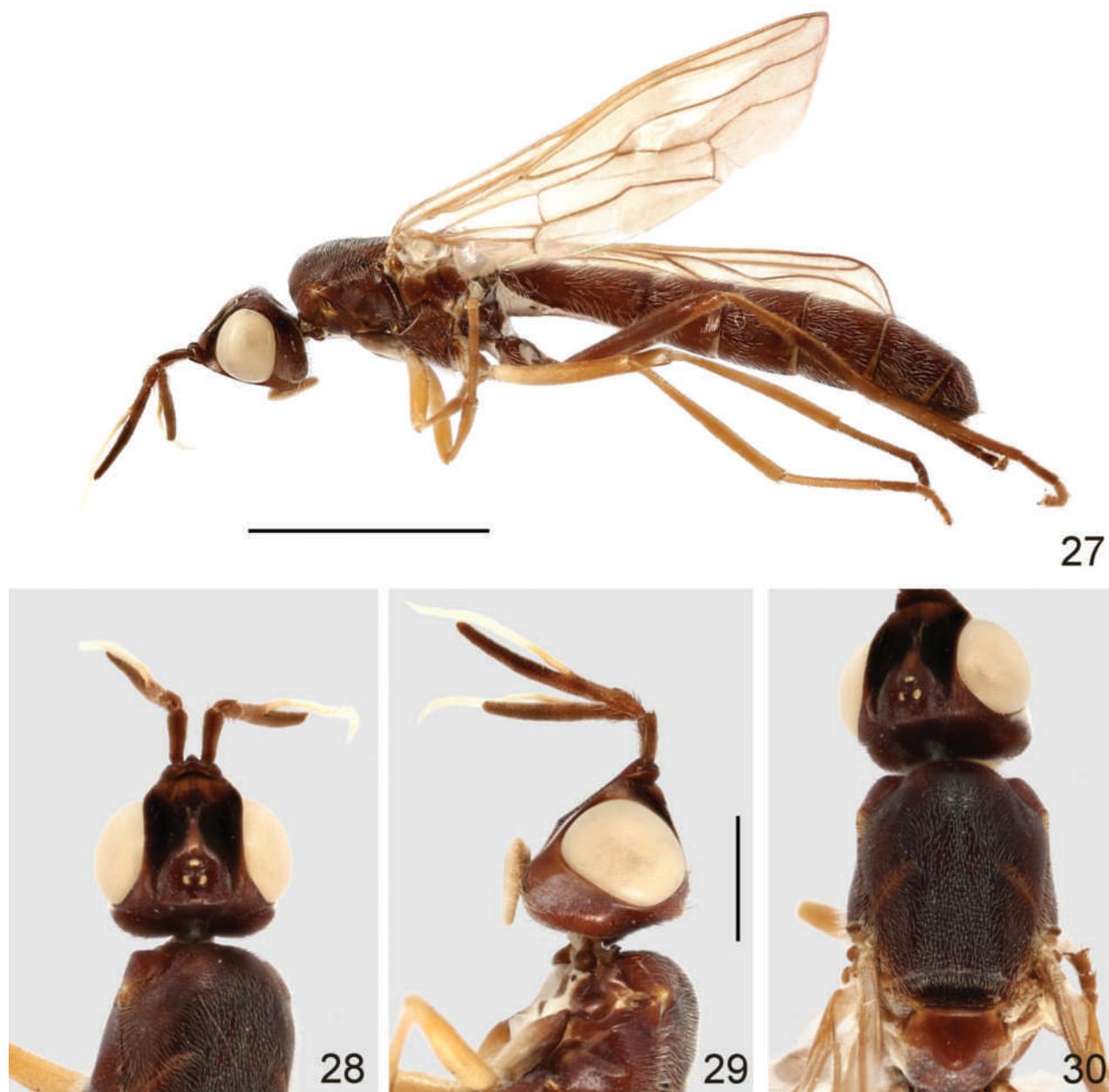
#### ***Loxocera (Loxocera) obscura* sp. nov.**

<https://zoobank.org/30ECC9F7-08E8-4782-90F9-F305B414E095>

Figs 27–34

**Type materials.** **Holotype** (♂): CHINA, Shaanxi, Xi'an, Zhouzhi, Houzhenzi, 2009. ix.29, leg. Maoling Sheng (CAU). **Paratypes:** same collection data as for holotype (4♂♂, CAU).

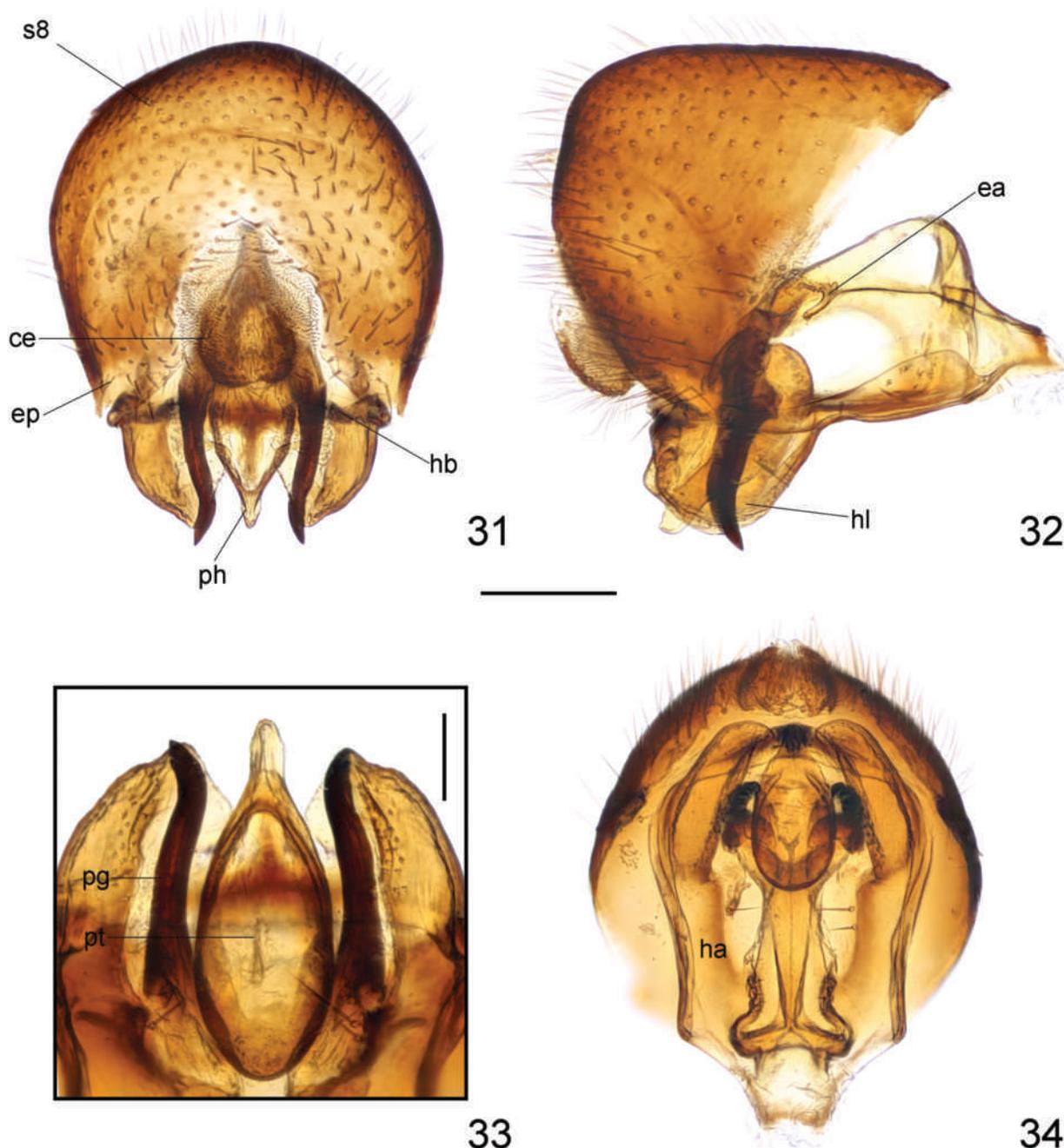
**Diagnosis.** Generally dark brown; face blackish; antennal scape distinctly longer than pedicel; antennal first flagellomere stick-like, weakly narrowed towards apex of segment; arista whitish yellow, thin, arising before midpoint of



Figures 27–30. *Loxocera (Loxocera) obscura* sp. nov., holotype, male 27 habitus, lateral 28 head, dorsal 29 same, lateral 30 thorax, dorsal. Scale bars: 3 mm (27); 1 mm (28–30).

antennal first flagellomere and 1.4 times as long as the latter; wing without transverse dark band; hypandrial lobe very large, broad, covered with short, sparse setae on inner surface; pregonite rather long, slender, curved apically with sharp apex; phallus droplet-like, elongate, abruptly narrowed apically.

**Description. Male.** Body length 8.9–10.2 mm, wing length 6.6–7.2 mm, length of antenna 2.2–2.5 mm. Generally dark brown, moderately shining (Fig. 27). Frontal vitta black except anterior and median parts brown (Fig. 28); face blackish brown; gena and postgena slightly paler (Fig. 29). Antenna with arista whitish yellow. Proboscis and palpus pale brown. Mesonotum blackish brown (Fig. 30). Scutellum brown (Fig. 30). Mesopleuron with anepimeron and katatergite slightly paler; portion above and below anterior spiracle yellow



**Figures 31–34.** *Loxocera (Loxocera) obscura* sp. nov., male genitalia **31** sternite 8 to genitalia, caudal **32** same, lateral **33** pregonite and phallus, ventral **34** hypandrium and associated structures, ventral. Abbreviations: ce = cercus, ea = ejaculatory apodeme, ep = epandrium, ha = hypandrial arm, hb = hypandrial bridge, hl = hypandrial lobe, pg = pregonite, s8 = sternite 8, ph = phallus, pt = phallosome. Scale bars: 0.25 mm (**31**, **32**, **34**); 0.1 mm (**33**).

(Fig. 29). Wing slightly infumated; wing veins yellowish brown to dark brown. Halter white with base slightly darkened. Fore and mid legs dark yellow, with coxae dark brown, base of femora pale yellow, and mid tarsomeres 2–5 slightly darkened. Hind leg brown, with coxa, femur (except apex), and wide median ring on tibia dark brown. Bristles on head and thorax black.

Head (Figs 28, 29) nearly rounded in dorsal view, largely glabrous; length along midline nearly as long as width across eyes, width across eyes 2 times

as broad as interocular space. Frons strongly protruding beyond level of anterior eye margin; frontal vitta with shallow depression at middle; frontal orbit with some short, scattered hairs. Ocellar triangle broad, smooth. Face strongly slanting, with moderately elevated median carina. Parafacial narrow, with a tomentose golden patch between anterior eye margin and lunule. Gena swollen; postgena covered with silvery tomentum. Occiput with a large silvery tomentose patch at middle above foramen. Head chaetotaxy: 1 ocellar seta, 1 postvertical seta, 1 inner vertical seta 1 outer vertical seta. Antenna (Fig. 29) long, relatively slender, with short, dense setulae; scape distinctly longer than pedicel; first flagellomere about 4.6 times as long as pedicel, stick-like, laterally compressed, weakly narrowed towards apex; arista thin, arising before midpoint of first flagellomere, 1.4 times as long as first flagellomere, divided into small aristomere 1 and large aristomeres 2+3. Palpus elongate oval, with short, dense, white setulae.

Thorax (Figs 27, 30) robust, with short, dense, white setulae, except anepisternum (anterior half), anepimeron, katatergite, meron, scutellum and mediotergite (middle portion) glabrous; anatergite with fine tomentum. Scutum 1.35 times as long as wide. Scutellum (Fig. 30) transverse, wider than long, with midportion distinctly swollen. Thoracic chaetotaxy: 1 dorsocentral seta, 1 notopleural seta, 2 posterior supra-alar setae, 1 apical scutellar seta. Wing with last sector of  $M_1$  strongly curved; apex of  $M_4$  nearly reaching wing margin. Legs with dense, whitish-yellow setulae, except ventral surface of fore and mid femora largely glabrous; femora subfusiform, slightly compressed laterally; tibiae gradually widened towards apex, hind tibia finely curved.

Abdomen elongate, with short, dense, white setae; syntergite 1+2 with several long, hair-like setae laterally.

**Male genitalia:** Sternite 8 (Figs 31, 32) broad, inflated, with long, dense setae. Cerci (Figs 31, 32) relatively broad, slightly curved, with short, dense setae. Hypandrium (Fig. 34) well developed; hypandrial arms posteriorly produced into very large, broad, convex lobes covered with sparse, short setae on inner surface (Figs 31–34). Hypandrial bridge present and robust. Pregonite (Figs 31–33) rather long, slender, curved apically with sharp apex. Phallus (Figs 31–33) droplet-like, elongate, abruptly narrowed apically. Phallotrema (Fig. 33) small, without processes. Ejaculatory apodeme (Fig. 32) small, V-like, strongly curved.

**Female.** Unknown.

**Etymology.** The specific epithet is derived from Latin *obscura* (meaning dark, indistinct), referring to the dark-brown body color of the new species.

**Distribution.** China – Shaanxi: Xi'an (Fig. 37).

**Comparative notes.** This new species is most similar to *L. (L.) malaisei* (Frey, 1955) (from Myanmar and Nepal) in having a relatively long antennal scape (longer than pedicel), a nearly parallel-sided and laterally compressed antennal first flagellomere, similar coloration of legs, and an enlarged and posteriorly inflated male sternite 8. It can be distinguished from the latter by the following character states: antennal scape about 1.5 times as long as pedicel [vs 2 times in *L. (L.) malaisei*]; antennal first flagellomere elongate, 4.6 times as long as pedicel [vs shorter, 4 times in *L. (L.) malaisei*]; arista slender [vs widened towards apex in *L. (L.) malaisei*]; phallus droplet-like with apex abruptly narrowed [vs elongate oval in *L. (L.) malaisei*].

***Loxocera (Loxocera) omei* Shatalkin, 1998**

Figs 35, 36

*Loxocera (Platystyla) omei* Shatalkin, 1998: 90, 97 (protologue). Holotype (♂): China, Sichuan, Emeishan, USNM.

*Loxocera (Loxocera) omei*: Buck and Marshall (2006b: 199) (subgeneric placement, distribution).

*Loxocera omei*: Tang et al. (2021: 234) (catalogue, distribution).

**Type material examined. Holotype** (♂): CHINA, Sichuan, Leshan, Emeishan, 1935.vii.21, leg. D.C. Graham (USNM).

**Diagnosis.** Generally blackish brown; face yellowish brown; antennal scape and pedicel subequal in length; antennal first flagellomere laterally compressed, about 3.6 times as long as pedicel, weakly narrowed towards apex; arista whitish yellow, thin, arising near midpoint of antennal first flagellomere and 1.3 times as long as latter; wing without transverse dark band.

**Distribution.** China – Sichuan: Leshan (Fig. 37).

**Remarks.** This species was described based on one male (the holotype) from Sichuan, China (Shatalkin 1998). The habitus photographs of the holotype (Figs 35, 36) are provided here for facilitating the identification of this species.

**Notes on species transferred to *Imantimyia* Frey, 1925**

***Loxocera (Imantimyia) anulata* Wang & Yang, 1998**

Figs 38, 39

*Loxocera (Loxocera) anulata* Wang and Yang 1998a: 440, 454 (protologue). Holotype (♂): China, Hubei, Shennongjia, CAU.

*Loxocera annulata*: Buck and Marshall (2006b: 199) (listed, distribution). Incorrect subsequent spelling.

*Loxocera anulata*: Tang et al. (2021: 234) (catalogue, distribution).

**Type material examined. Holotype** (♂): CHINA, Hubei, Shennongjia, Dajiuahu, 1977.vii.9, leg. Huanguang Zou (CAU).

**Distribution.** China – Hubei: Shennongjia.

**Remarks.** *Loxocera anulata* was originally described as a member of the subgenus *Loxocera* based on one male (the holotype) from Hubei, China (Wang and Yang 1998a). The specific epithet of this species was spelled as *anulata* in its original description, where the authors (Wang and Yang 1998a) intend to create the name from the Latin adjective *anulatus*, -a, -um (meaning ringed, referring to the annulations on the abdomen of this species), therefore it is considered as the correct original spelling. The subsequent usage of the specific epithet *annulata* (Buck and Marshall 2006b) is treated as an incorrect subsequent spelling. Buck and Marshall (2006b) failed to assign this species (as *L. annulata*) to a certain subgenus based on its brief original description. This species was catalogued by Tang et al. (2021) without subgeneric placement.

The holotype of *L. anulata* was examined during the present study. It satisfied the diagnosis of *Loxocera* s. lat. provided by Buck and Marshall (2006b). The sclerotized and sub-shining frontal vitta, the hiding lunule, the microtrichose



Figures 35, 36. *Loxocera (Loxocera) omei* Shatalkin, 1998, holotype, male, habitus with labels 35 lateral 36 dorsal. Scale bar: 3 mm.

on the alula, and the very reduced and bare male sternite 8 match the characters used to diagnose the subgenus *Imantimyia* Frey, 1925 (Buck and Marshall 2006b). Therefore, this species is herein transferred to *Imantimyia*.

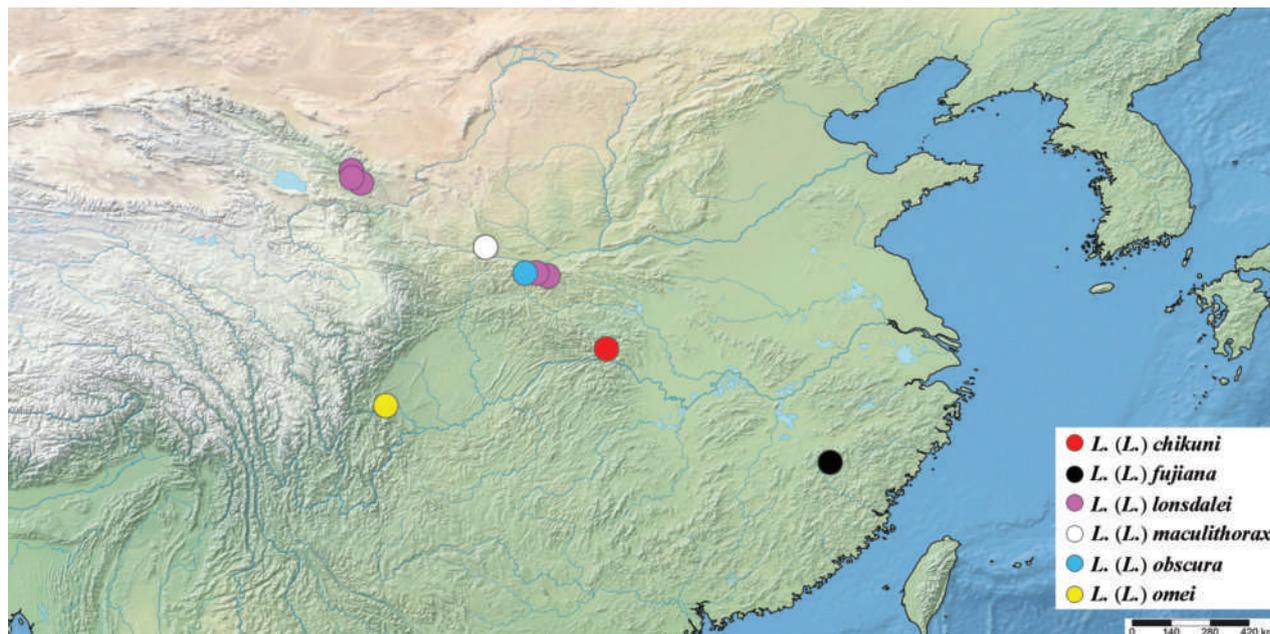


Figure 37. Known distribution of species of the subgenus *Loxocera* in China.

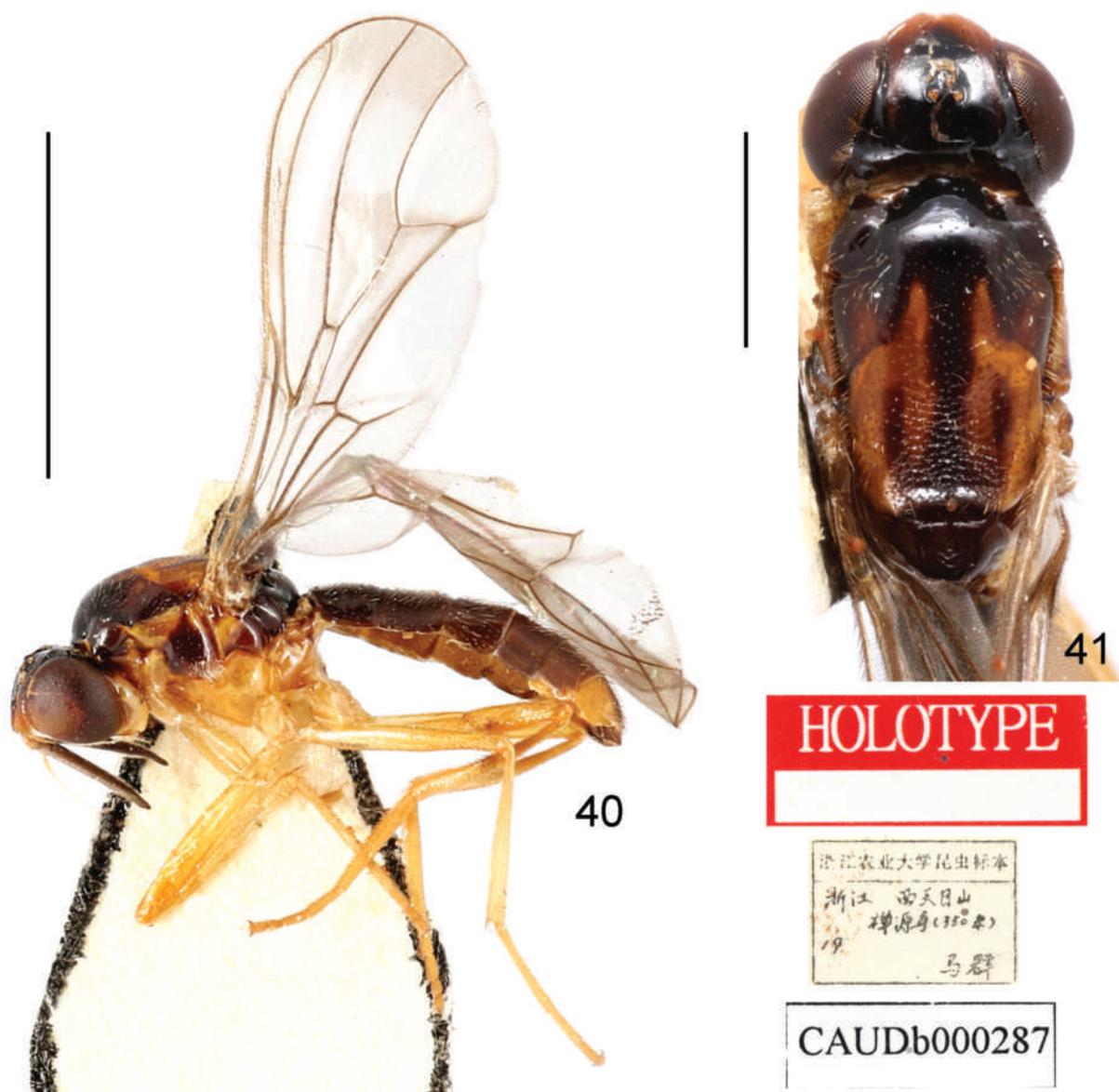


Figures 38, 39. *Loxocera (Imantimyia) anulata* Wang & Yang, 1998, holotype, male 38 lateral habitus with labels 39 head and thorax, dorsal. Scale bars: 3 mm (38); 1 mm (39).

***Loxocera (Imantimyia) tianmuensis* Wang & Yang, 1998**

Figs 40, 41

*Loxocera tianmuensis* Wang and Yang 1998b: 200, 201 (protologue); Buck and Marshall (2006b: 199) (listed, distribution). Holotype (♂): China, Zhejiang, Xitianmushan, CAU.



Figures 40, 41. *Loxocera (Imantimyia) tianmuensis* Wang & Yang, 1998, holotype, male **40** lateral habitus with labels **41** head and thorax, dorsal. Scale bars: 3 mm (**40**); 1 mm (**41**).

**Type material examined. Holotype** ( $\delta$ ): CHINA, Zhejiang, Lin'an, Xitianmushan, 350 m, 1987.ix.3, leg. Qun Ma (CAU).

**Distribution.** China – Zhejiang: Lin'an.

**Remarks.** *Loxocera tianmuensis* was originally described based on one male (the holotype) from Zhejiang, China (Wang and Yang 1998b), but the authors did not assign it to any subgenus. Buck and Marshall (2006b) mentioned that this species "cannot be confidently placed to subgenus" due to the inadequate original description. No new information has since been published on this species.

The holotype of *L. tianmuensis* was examined in the course of the present study. The date on the collection data label of the holotype does not match that in the original description. The authors (Wang and Yang 1998b) communicated personally with the specimen collector during the preparation of the manuscript, thus providing a precise date of specimen collection in the original

description. Based on the examination of the holotype, this species matches the current concept of *Imantimyia* (Buck and Marshall 2006b) and is herein placed under this subgenus.

## Discussion

The present study documents the Chinese fauna of the subgenus *Loxocera*, including two described species and four new species. The other two species originally assigned to the subgenus *Loxocera* are herein transferred to the subgenus *Imantimyia* based on examination of their holotypes. An identification key to the species of the subgenus *Loxocera* from China is also presented.

Antennal morphology of members of the subgenus *Loxocera* is diverse and useful in species identification (Zhou et al. 2022). The arista is particularly highly diagnostic, with half of the fauna having the arista dark colored, laterally compressed, very high and arising at the apex of the antennal first flagellomere, and the other half having it whitish yellow, slender, and arising near the midpoint of the antennal first flagellomere. The location and shape of the arista have been used by some authors to distinguish genus-level taxa within *Loxocera* s. lat. (e.g. Hennig 1941; Steyskal 1987; Iwasa 1992; Shatalkin 1998; Shatalkin and Merz 2010), while others consider it more appropriate for species-level identification (Buck and Marshall 2006b; Zhou et al. 2022). Whether these states characterize natural species groups remains to be proven.

Including the four species newly described in this study, the subgenus *Loxocera* currently comprises 14 species (Table 1). Many of these species are distributed in the Palaearctic Realm, while only three are reported from the northern part of the Oriental Realm. Among the six species currently recorded in China, only *L. (L.) fujiana* occurs in the Oriental Realm. Most of the Chinese species of the subgenus *Loxocera* are endemic and known only from their type locality, except for

**Table 1.** Described species of the subgenus *Loxocera* and their known distribution.

Species	Distribution	References
<i>L. (L.) aristata</i> (Panzer, 1801)	Europe, Iran, Israel	Panzer 1801; Freidberg and Shatalkin 2008; Khaghaninia and Gharajedaghi 2014; Whitters and Claude 2021
<i>L. (L.) atriceps</i> Bigot, 1886	Europe	Bigot 1886; Buck and Marshall 2006b
<i>L. (L.) chikuni</i> Zhou & Yang, sp. nov.	China	present study
<i>L. (L.) fujiana</i> (Wang, 1999)	China	Zhou et al. 2022; present study
<i>L. (L.) glandicula</i> Iwasa, 1993	Nepal	Iwasa 1993
<i>L. (L.) hoffmannseggii</i> Meigen, 1826	Europe	Whitters and Claude 2021
<i>L. (L.) lonsdalei</i> Zhou & Yang, sp. nov.	China	present study
<i>L. (L.) maculata</i> Rondani, 1876	Europe	Rondani 1876; Soós 1984; Buck and Marshall 2006b
<i>L. (L.) maculithorax</i> Zhou & Yang, sp. nov.	China	present study
<i>L. (L.) malaisei</i> Frey, 1955	Myanmar, Nepal	Frey 1955; Iwasa 1993
<i>L. (L.) matsumurai</i> Iwasa, 1992	Japan, Russia	Iwasa 1992
<i>L. (L.) monstrata</i> Iwasa, 1992	Japan	Iwasa 1992
<i>L. (L.) obscura</i> Zhou & Yang, sp. nov.	China	present study
<i>L. (L.) omei</i> Shatalkin, 1998	China	Shatalkin 1998; present study

*L. (L.) lonsdalei* sp. nov. which has been collected from four localities in central China. Considering the diversity presented from so few specimens, it is very likely that many more undescribed species will be found given additional collecting.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

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No ethical statement was reported.

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### Author contributions

Conceptualization, Jiale Zhou and Ding Yang; writing – original draft preparation, Jiale Zhou and Ding Yang; writing – review and editing, Jiale Zhou and Ding Yang; material, Jiale Zhou and Ding Yang; visualization, Jiale Zhou; funding, Jiale Zhou and Ding Yang.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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# A new bamboo-feeding species of the genus *Pseudosymplanella* Che, Zhang & Webb, 2009 (Hemiptera, Caliscelidae, Ommatidiotinae) from China

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## Abstract

A new planthopper species, *Pseudosymplanella maxima* **sp. nov.**, belonging to the genus *Pseudosymplanella* (Hemiptera: Fulgoromorpha: Caliscelidae: Augilini), is described and illustrated, from China. In common with other Chinese Augilini, the new species feeds exclusively on bamboo. Additionally, a key to the two species of *Pseudosymplanella* is provided.

**Key words:** Augilini, bamboo, identification key, morphology, Oriental region, planthopper, taxonomy



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## Introduction

The family Caliscelidae Amyot & Audinet-Serville, 1843, a worldwide distributed group, is divided into two subfamilies: Caliscelinae (including tribes Caliscelini and Peltonotellini) and Ommatidiotinae (including tribes Ommatidiotini, Augilini and Adenissini) (Gnezdilov and Wilson 2006; Emeljanov 2008; Gnezdilov 2008). The two subfamilies can be separated by nymphal characters (Gnezdilov and Wilson 2006), disparities in the first metatarsomere and the degree of aedeagus reduction (Gnezdilov and Bourgoin 2009).

Modern fauna of the tribe Augilini Baker, 1915 contains 16 genera and 41 species, including the new species described below (Zhang et al. 2020; Gong et al. 2021; Bourgoin 2023). A fossil genus and species was documented in Dominican amber in the New World (Bourgoin et al. 2015b). Now, seven genera and 21 species are recorded from southern China. It is worth noting that all these species have been documented to be bamboo-feeders (Chen et al. 2014; Gong et al. 2018, 2020, 2021; Zhang et al. 2020).

The planthopper genus *Pseudosymplanella* was established by Che, Zhang and Webb (2009) based on a single species, *Pseudosymplanella nigrifasciata*, from China and Thailand, and placed in the tribe Augilini of the subfamily Ommatidiotinae (Hemiptera: Fulgoroidea: Caliscelidae).

In the present paper, a new species, *Pseudosymplanella maxima* sp. nov. is described from Yunnan Province, China. Descriptions and illustrations are given, generic characteristics are redefined, and a key to species of *Pseudosymplanella* is provided.

## Material and methods

Terminology used for the external morphology and the male genitalia mainly follows the classifications proposed by Fennah (1987) and Chan and Yang (1994). The standard terminology for hind and forewing venation adheres to the principle outlined by Bourgoïn et al. (2015a). The methodology for describing the description of the female genitalia mainly follows Bourgoïn's (1993) approach. Dry specimens were used for the descriptions and illustrations. External morphology was observed under a stereoscopic microscope and characters were measured with an ocular micrometer. All dimensions were expressed in millimeters (mm); the body length was measured from the apex of the head to the apex of the forewing in repose. The genital segments of the examined specimens were subjected to maceration in a 10% NaOH and subsequently observed in glycerin jelly for illustration using a Leica MZ 12.5 stereomicroscope. Photographs were captured with a KEYENCE VHX-1000 system. Illustrations were scanned with CanoScan LiDE 200 and imported into Adobe Photoshop CS6 for labeling and plate composition. The dissected male genitalia were preserved in glycerine and then stored in small plastic tubes, which were pinned together with the specimens.

The type specimens and materials examined were deposited in the Institute of Entomology, Guizhou University, Guiyang, China (IEGU).

## Taxonomy

**Order Hemiptera Linnaeus, 1758**

**Suborder Fulgoromorpha Evans, 1946**

**Family Caliscelidae Amyot & Audinet-Serville, 1843**

**Ommatidiotinae Fieber, 1875**

**Tribe Augilini Baker, 1915**

***Pseudosymplanella* Che, Zhang & Webb, 2009**

Figs 1–21

*Pseudosymplanella* Che, Zhang & Webb, 2009: 49.

**Type species.** *Pseudosymplanella nigrifasciata* Che, Zhang & Webb, 2009, by original designation.

**Diagnosis.** Head with eyes as wide as or slightly narrower than pronotum; vertex with anterior margin a little convex or straight; second segment of antenna with a black transverse spot near apex. Mesonotum sometimes with pit along lateral margin, maximum width wider than medial length. Male with pygofer in lateral view, dorsal margin shorter than ventral margin, posterior margin with a rather slender and long process; genital style in lateral view elongate or

rather broad; aedeagus simple, tubular, slightly ventrally curved. Female genitalia with gonoplacs rounded or triangular in lateral view.

**Host plant.** Bamboo.

**Distribution.** Southern China (Yunnan Province) and Thailand (Fig. 22).

**Key to species of genus *Pseudosymplanella* Che, Zhang & Webb, 2009**

- 1 Body mainly brown, anal segment with anal pore located at mid-length, posterior margin of pygofer in profile with thick and short process near dorsal margin..... *P. nigrifasciata*
- Body grass green, anal segment with anal pore located in apical half, posterior margin of pygofer in profile with slender and long process near mid-length ..... *P. maxima* sp. nov.

***Pseudosymplanella maxima* sp. nov.**

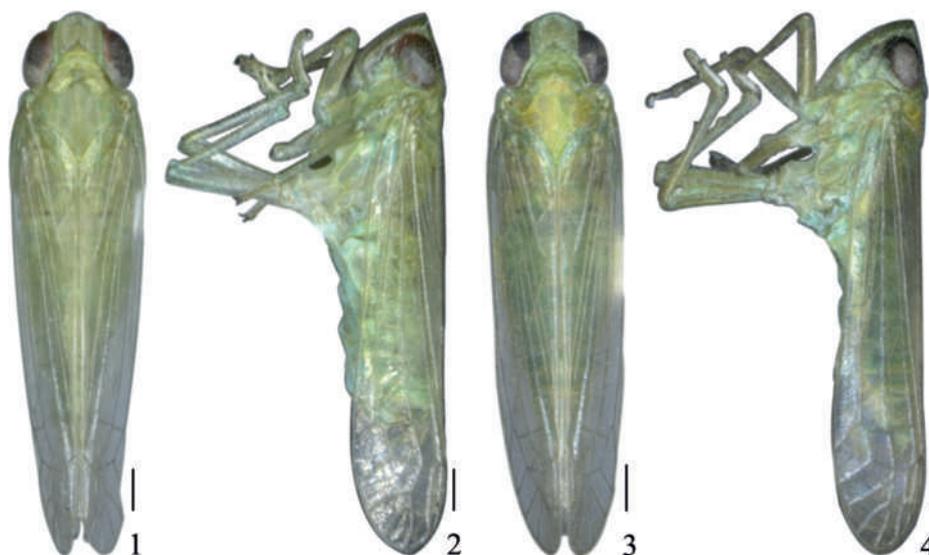
<https://zoobank.org/A2F51374-787E-4703-8C71-694657D3A9B2>

Figs 1–21

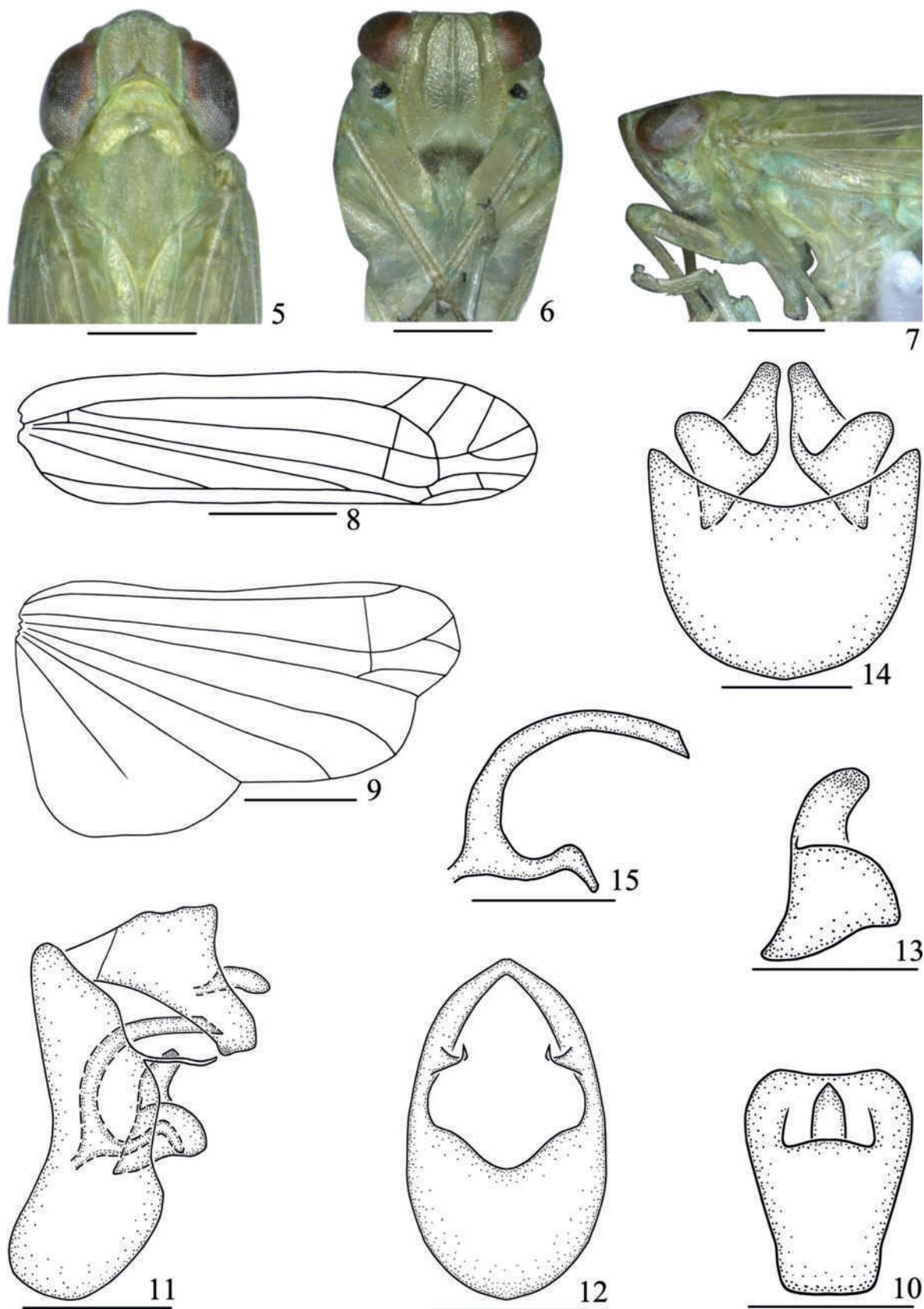
**Description. Measurements.** Body length including forewing: male 5.1 mm ( $N = 1$ ), female 6.0–6.2 mm ( $N = 3$ ); forewing length: male 4.2 mm ( $N = 1$ ), female 5.0–5.2 mm ( $N = 3$ ).

**Coloration.** Body (Figs 1–4) grass green. Eyes reddish brown, ocelli orange red. Second segment of antenna (Fig. 6) with a black transverse spot near apex. Clypeus (Fig. 6) with basal half brown.

**Head and thorax.** Head (Fig. 5) with eyes as wide as pronotum. Vertex (Fig. 5) with length in middle line 0.8 times than width at base. Frons (Fig. 6) with length in middle line 1.1 times than maximum width. Pronotum (Fig. 5) with length in middle line shorter than vertex (0.8:1). Mesonotum (Fig. 5) 1.2 times as long as



**Figures 1–4.** *Pseudosymplanella maxima* Gong, Yang & Chen, sp. nov. **1** male habitus, dorsal view **2** male habitus, lateral view **3** female habitus, dorsal view **4** female habitus, lateral view. Scale bars: 0.5 mm (1–4).

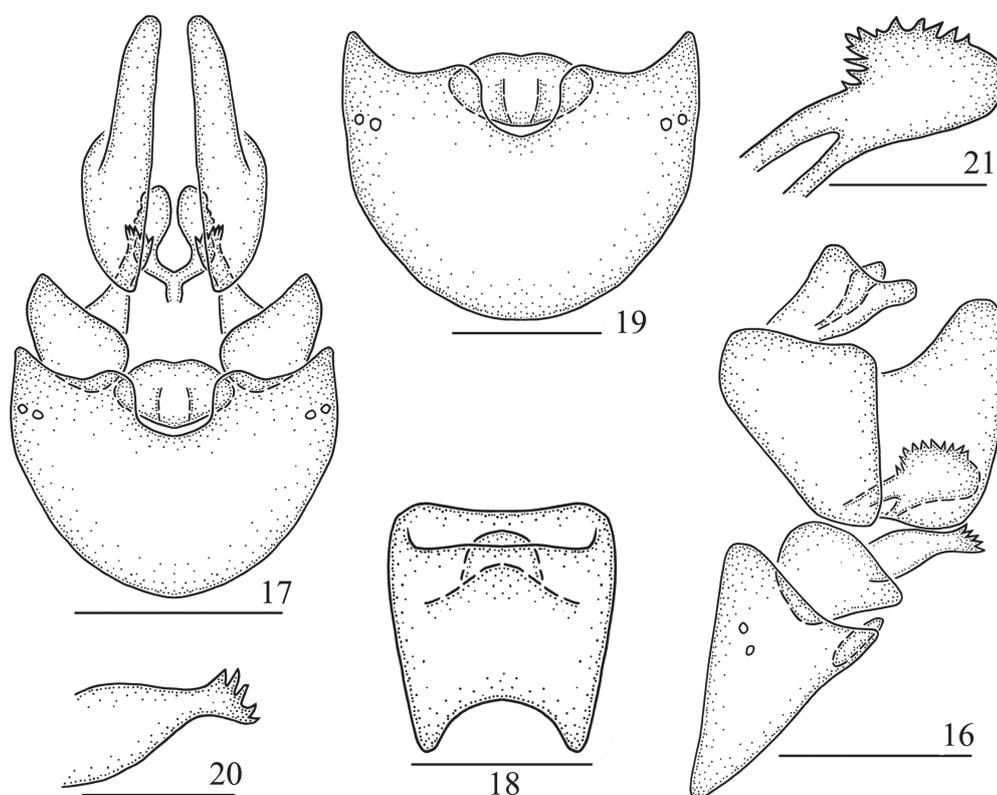


Figures 5–15. *Pseudosymplanella maxima* Gong, Yang & Chen, sp. nov., male 5 head and thorax, dorsal view 6 face 7 head and thorax, lateral view 8 forewing 9 hindwing 10 anal segment, dorsal view 11 male genitalia, lateral view 12 pygofer, posterior view 13 genital styles, lateral view 14 pygofer and genital styles, ventral view 15 aedeagus, lateral view. Scale bars: 1 mm (8, 9); 0.5 mm (5–7); 0.3 mm (11); 0.2 mm (10, 12–15).

vertex and pronotum together in middle line. Forewing (Fig. 8) longer in middle line than broad at widest part (3.8:1); veins distinct, without nodal line, R and MP with common stem; ScP, R and CuA single, MP with three branches, Pcu uniting A1 at basal half of clavus. Hindwing (Fig. 9) with length 1.7 times as long as broad at widest part, ScP and RP single, MP and CuA with two branches. Legs relatively long, hind tibia with a single lateral tooth; spinal formula of hind leg 6-0-0.

**Male genitalia.** Anal segment (Fig. 10) in dorsal view with length 1.3 times longer in mid-line than widest part, apical margin slightly concave; anal pore located at apical half; in lateral view (Fig. 11) dorsal margin sinuated, ventral margin slightly concave near apex with a small process, broadening distally and abruptly narrowed subapically. Pygofer in lateral view (Fig. 11) with dorsal margin distinctly shorter than ventral margin, posterior margin sinuated with a rather slender and long process near mid-length; in posterior view (Fig. 12), nearly oval, with length 1.7 times longer in mid-line than widest part; in ventral view (Fig. 14), posterior margin broadly concave. Genital style in lateral view (Fig. 13) rather broad, nearly triangle, apical margin roundly convex; a strong finger-like process apically arising from dorsal margin, slightly curved. Aedeagus (Fig. 15) simple, tubular, slightly ventrally curved.

**Female genitalia.** Anal segment small, short, in dorsal view (Fig. 18) nearly quadrangle, anal pore near apex. Abdominal sternite VII in ventral view (Fig. 19) rather large and broad, behind the posterior margin with a small oval ossification flake. Gonapophysis VIII (first valvula) (Fig. 20) elongate, with five spines



Figures 16–21. *Pseudosymplanella maxima* Gong, Yang & Chen, sp. nov., female 16 genitalia, lateral view 17 genitalia, ventral view 18 anal segment, dorsal view 19 abdominal sternite VII, ventral view 20 gonapophysis VIII, lateral view 21 gonapophysis IX, lateral view. Scale bars: 0.5 mm (16, 17); 0.3 mm (19); 0.2 mm (18, 20, 21).

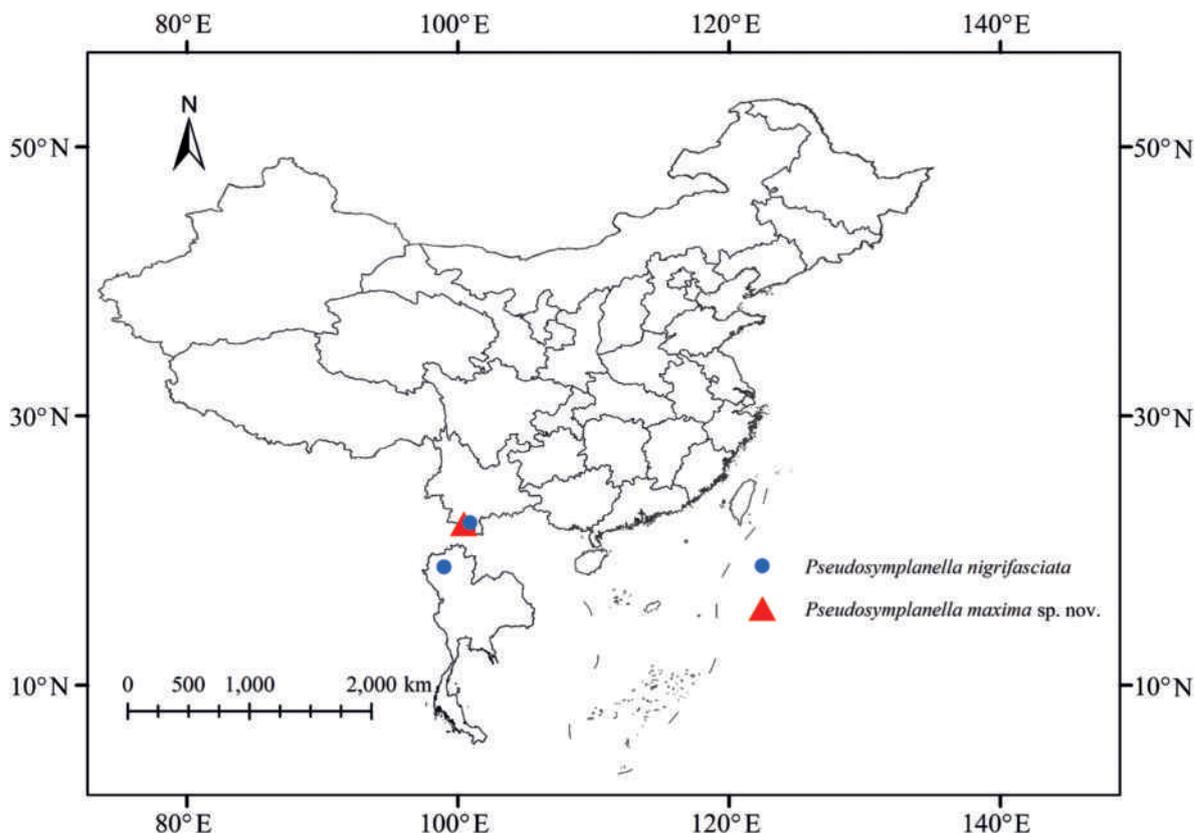


Figure 22. Geographic distributions of species of *Pseudosymplanella* Che, Zhang & Webb, 2009.

at apical margin. Gonapophysis IX (second valvula) (Fig. 21) with two symmetrical lobes, each lobe with many spines at dorsal margin. Gonoplac (third valvula) (Fig. 16) triangular, apical margin rounded.

**Host plant.** Bamboo.

**Distribution.** Southwestern China (Yunnan Province) (Fig. 22).

**Type material. Holotype:** ♂, CHINA: Yunnan Province, Menghai County, Mengzhe Reservoir (22°08'N, 100°26'E), 2019-X-4, Nian Gong. **Paratypes:** 1♂3♀, data same as holotype.

**Etymology.** The specific name is derived from the Latin word “maximus”, referring to the long process of the pygofer.

**Remarks.** This new species is closely related to *P. nigrifasciata* Che, Zhang & Webb, 2009, but differs in: 1) body mainly green, without stripe (body brown, with stripe in *P. nigrifasciata*); 2) anal segment with anal pore located in apical half (anal pore located at mid-length in *P. nigrifasciata*); and 3) posterior margin of pygofer in profile with slender and long process near the mid-length (posterior margin of pygofer in profile with thick and short process near dorsal margin in *P. nigrifasciata*).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Nian Gong conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures, authored or reviewed drafts of the paper, and approved the final draft. Xiang-Sheng Chen analyzed the data, prepared figures and tables, and approved the final draft. Lin Yang conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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# The tropiduchid planthopper genus *Connelicita* Wang & Bourgoïn, 2015: two new species from Central Vietnam and new records (Hemiptera, Fulgoromorpha, Tropiduchidae)

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## Abstract

Two new planthopper species of the tropiduchid genus *Connelicita* Wang & Bourgoïn, 2015, *C. bachmaensis* Constant & Pham, **sp. nov.**, and *C. phongdienensis* Constant & Pham, **sp. nov.** are described from Bach Ma National Park and from the Centre for Conservation of Vietnam Natural Resources and Rescue of Animals and plants, Phong Dien District in Thua Thien-Hue Province, respectively. These new records greatly extend the distribution of the genus, which was known from southern China (Guangxi) and North Vietnam, to the south, reaching the mid area of Central Vietnam. New records are provided for *C. haiphongensis* Wang & Zhang, 2015, extending the distribution of the species from Cat Ba Island to a large zone in North Vietnam. Illustrations of habitus, details, and male genitalia are given as well as a distribution map and photographs of the habitat. An identification key to the species of Vietnam is provided. The genus *Connelicita* now comprises five species.

**Key words:** Bach Ma National Park, biodiversity, Fulgoroidea, Indochina, Phong Dien District



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## Introduction

The family Tropiduchidae is distributed worldwide and contains 680 species in 197 genera, including 26 species in Vietnam (Bourgoïn 2023). Within the subfamily Elicinae Melichar, 1915, the tribe Elicini Melichar, 1915 counts 143 species in 37 genera distributed in most zoogeographical regions except the Palaearctic (Wang et al. 2015; Bourgoïn 2023).

The genus *Connelicita* Wang & Bourgoïn, 2015 was rather recently described to accommodate three species: one from southern China (Guangxi) and two from North Vietnam (Wang et al. 2015).

Study of the recent material of Tropiduchidae in the collections of Vietnam National Museum of Nature and Royal Belgian Institute of Natural Sciences revealed two undescribed species of *Connelicita* from Central Vietnam and several additional records of *C. haiphongensis* Wang & Zhang, 2015.

The present paper aims to describe the two new species as a new contribution to the Vietnamese tropiduchid fauna.

## Materials and methods

The genitalia were extracted after soaking the abdomen for some hours in a 10% solution of potassium hydroxide (KOH) at room temperature. The pygofer was separated from the abdomen, thoroughly rinsed in 70% ethanol, and the aedeagus dissected with a needle blade for examination. The whole was then placed in glycerin for preservation in a tube attached to the pin of the corresponding specimen. Photographs of collection specimens were taken with a Leica EZ4W stereomicroscope, stacked with CombineZ, and optimized with Adobe Photoshop; photographs from the field were taken with an Olympus Tough 6 camera. The map was produced with SimpleMappr (Shorthouse 2010) and includes records available from Wang et al. (2015). The external morphological terminology follows O'Brien and Wilson (1985), the wing venation terminology follows Bourgoin et al. (2015), and for the male genitalia, Bourgoin and Huang (1990). The classification used follows FLOW (Fulgoromorpha Lists on The Web – Bourgoin 2023). The metatiobiotarsal formula gives the number of spines on (side of metatibia) apex of metatibia / apex of first metatarsomere / apex of second metatarsomere.

The measurements were taken as in Constant (2004) and the following acronyms are used:

<b>BB</b>	maximum breadth of the body
<b>BF</b>	maximum breadth of the frons
<b>BTg</b>	maximum breadth of the tegmen
<b>BV</b>	maximum breadth of the vertex
<b>LF</b>	length of the frons at median line
<b>LT</b>	total length (apex of head to apex of tegmina)
<b>LTg</b>	length of the tegmen
<b>LV</b>	length of the vertex at median line

Acronyms used for the collections:

<b>RBINS</b>	Royal Belgian Institute of Natural Sciences, Brussels, Belgium
<b>VNMN</b>	Vietnam National Museum of Nature, Hanoi, Vietnam

Other abbreviations

<b>CCRR</b>	Centre for Conservation of Vietnam Natural Resources and Rescue of Animals and plants
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## Taxonomy

**Family Tropicuchidae Stål, 1866**

**Subfamily Elicinae Melichar, 1915**

**Tribe Elicini Melichar, 1915**

## Genus *Connelicita* Wang & Bourgoïn, 2015

**Type species.** *Connelicita backyensis* Stroiński & Bourgoïn, 2015 by original designation.

**Diagnosis.** Head capsule with frons widely developed below level of eyes; anterodorsal part of genae visible in dorsal view. Tegmina with costal area containing more than 16 cells, cells longer than wide; ScP regularly straight and presence of 2 or 3 veinlets pcu-cup.

**Distribution.** China: Southern China (Guangxi); Vietnam: North and Central.

### Species included.

*Connelicita bachmaensis* Constant & Pham sp. nov.

*Connelicita backyensis* Stroiński & Bourgoïn, 2015

*Connelicita haiphongensis* Wang & Zhang, 2015

*Connelicita lungchowensis* (Chou & Lu, 1977)

*Connelicita phongdienensis* Constant & Pham sp. nov.

## Key to the species of *Connelicita* Wang & Bourgoïn, 2015 from Vietnam

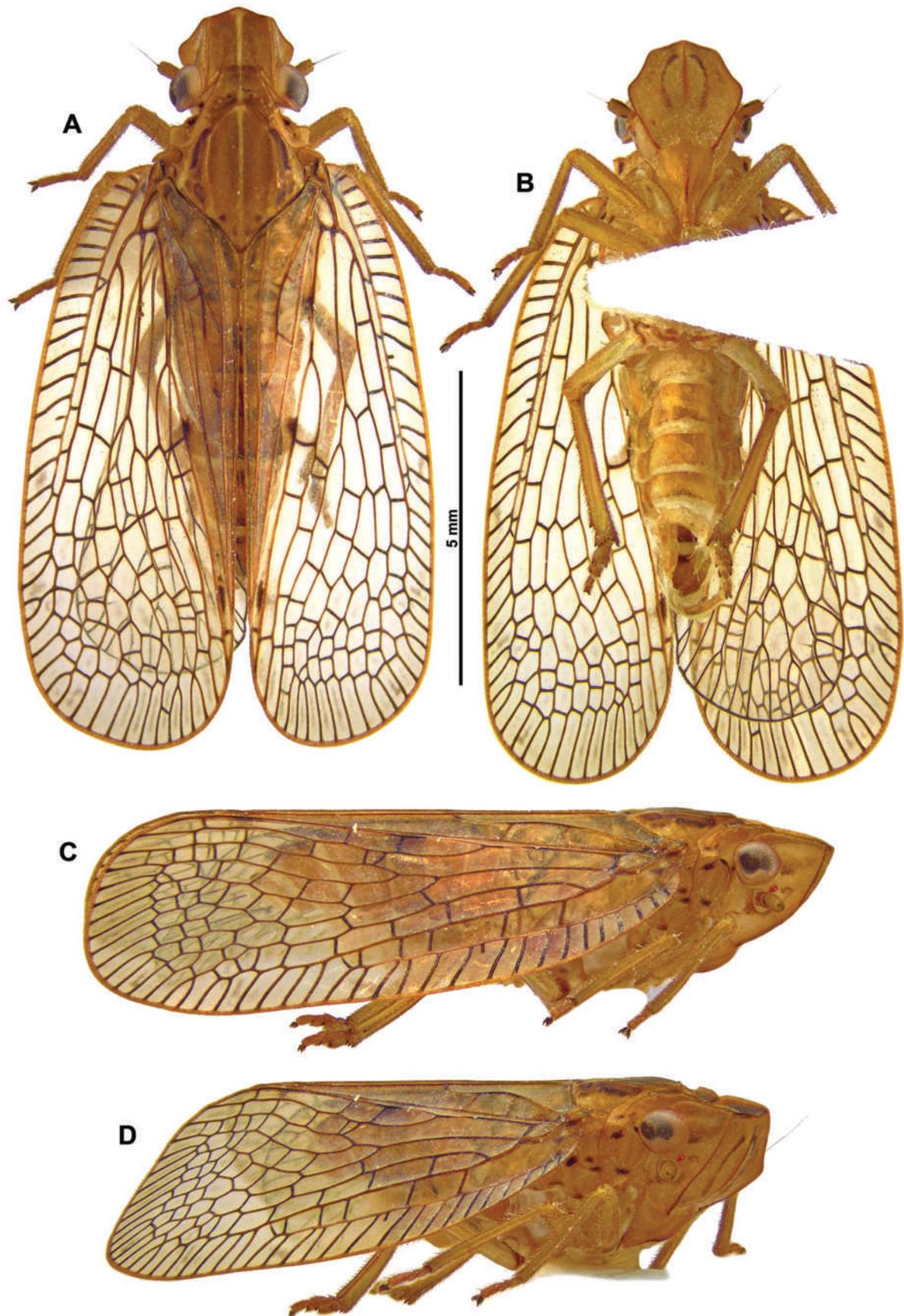
- 1 Anal tube in lateral view strongly narrowing in distal half (*An* – Fig. 9A); dorsal process of perianthrium spinose (*dpp* – Fig. 9E).....**2**
- Anal tube of male subcylindrical and elongate (*An* – Fig. 3A–C); dorsal process of the perianthrium forming a large lobe concave in distal portion (*dpp* – Fig. 3F, G) .....***C. bachmaensis* Constant & Pham, sp. nov.**
- 2 Capitulum of the gonostylus with two spines and without digitiform process (Wang et al. 2015: figs 12, 31); spinose dorsal process of the perianthrium moderately developed (Wang et al. 2015: figs 11, 30) .....**3**
- Capitulum of the gonostylus with one spine and a dorsal digitiform process (*ca* – Fig. 9A–D); spinose dorsal process of the perianthrium strongly developed (*dpp* – Fig. 9E) ..... ***C. phongdienensis* Constant & Pham, sp. nov.**
- 3 Capitulum of the gonostylus with two spines very unequal in size, proximal one much stronger than distal one (Wang et al. 2015: fig. 31); distal portion of the gonostylus in lateral view, rounded and developed dorsocaudad (Wang et al. 2015: fig. 31); in dorsal view, posterior and lateral margins of the anal tube concave in distal half (Wang et al. 2015: fig. 29) .....  
..... ***C. backyensis* Stroiński & Bourgoïn, 2015**
- Capitulum of the gonostylus with two spines more or less equal in size (Wang et al. 2015: fig. 12); distal portion of gonostylus in lateral view, rounded and developed caudad (Wang et al. 2015: fig. 12); in dorsal view, posterior and lateral margins of the anal tube rounded in distal half (Wang et al. 2015: fig. 10) ..... ***C. haiphongensis* Wang & Zhang, 2015**

### *Connelicita bachmaensis* Constant & Pham, sp. nov.

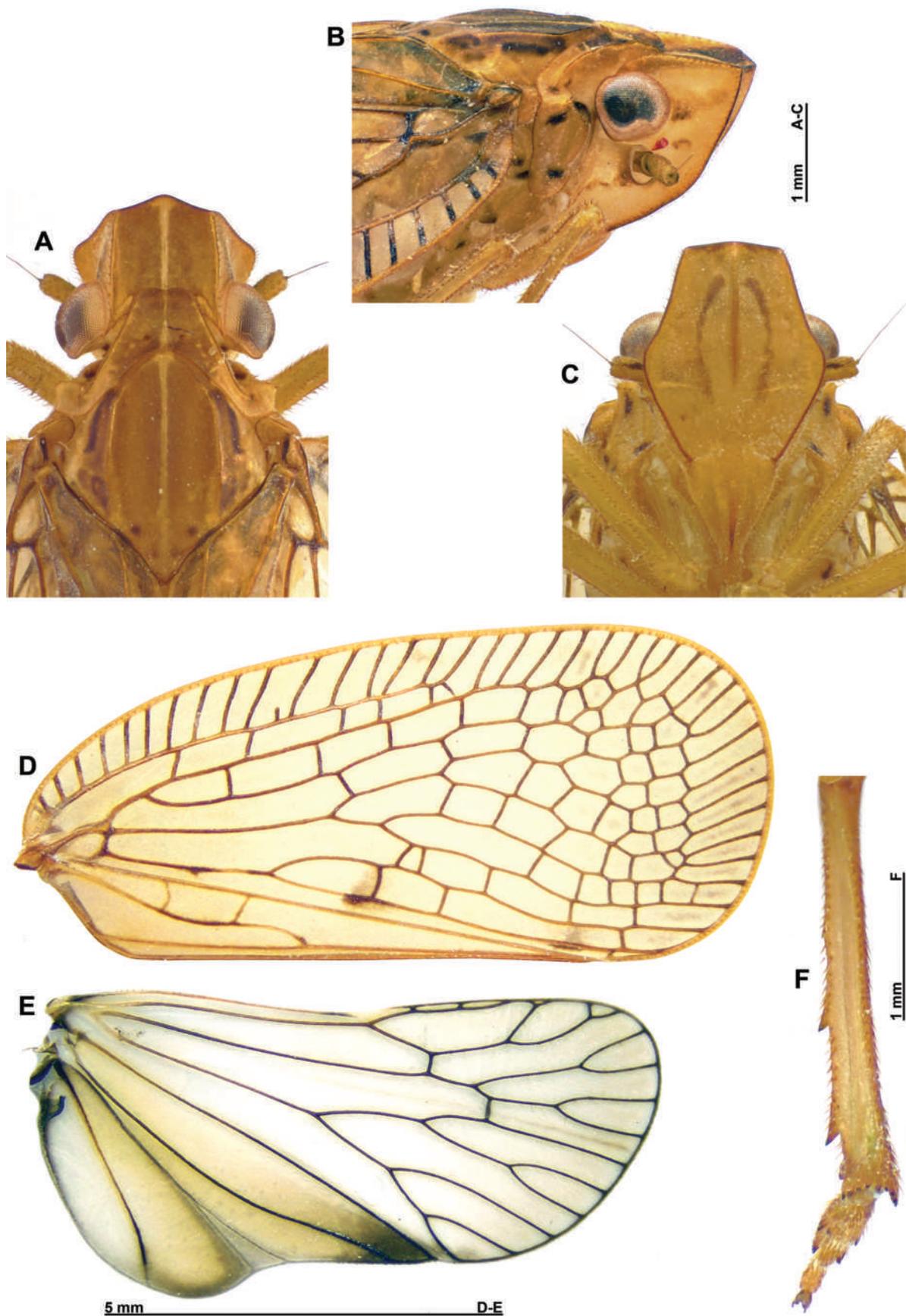
<https://zoobank.org/32D5F308-98C3-465F-85FE-C96DC1237A87>

Figs 1–5

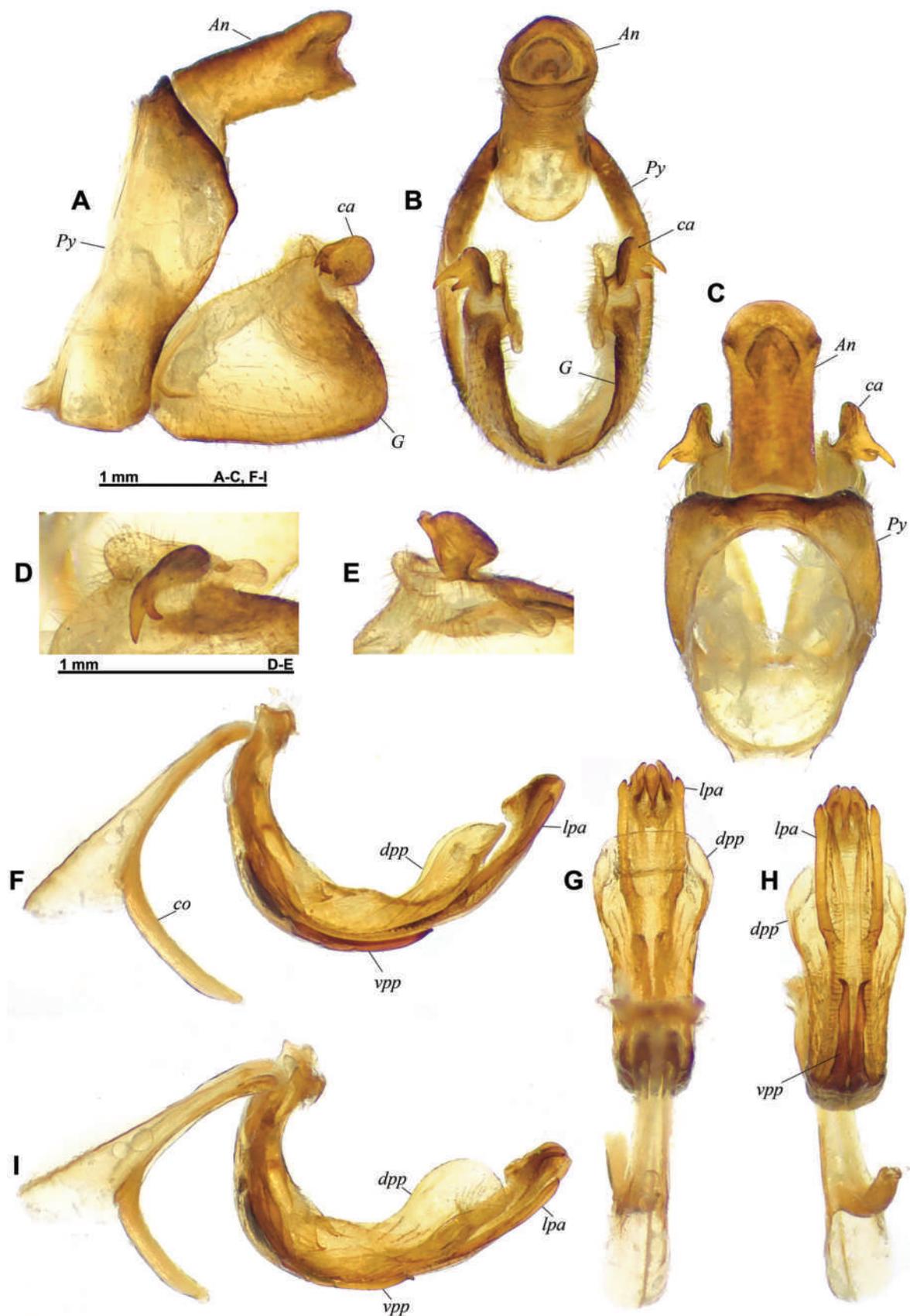
**Type materials.** *Holotype* ♂, VIETNAM – Thừa Thiên-Huế Province • Bach Ma National Park, Pheasant trail; 16°13'38"N, 107°51'20"E; 3 Mar. 2023; by net; Trung T. Vu leg.; VNMN.



**Figure 1.** *Connellicita bachmaensis* sp. nov., holotype ♂ **A** habitus, dorsal view **B** habitus, ventral view **C** habitus, lateral view **D** habitus, anterolateral view.



**Figure 2.** *Connellicita bachmaensis* sp. nov., holotype ♂ **A–C** detail of head and thorax **A** dorsal view **B** lateral view **C** perpendicular view of frons **D** right tegmen anterolateral view **E** right hind wing **F** right metatibia and metatarsus, ventral view.



**Figure 3.** *Connelicita bachmaensis* sp. nov., holotype ♂, terminalia **A–C** pygofer, gonostyli and anal tube **A** lateral view **B** caudal view **C** dorsal view **D–E** capitulum of left gonostylus **D** left laterodorsal view **E** right laterodorsal view **F–I** aedeagus, phallobase and connective **F** left lateral view **G** anterodorsal view **H** posteroventral view dorsal view **I** left lateroventral view.



**Figure 4.** *Connelicita bachmaensis* sp. nov., Vietnam, Bach Ma National Park, Pheasant Trail, 12 May 2023 **A** adult specimen sitting on leaf of unidentified plant **B** habitat.

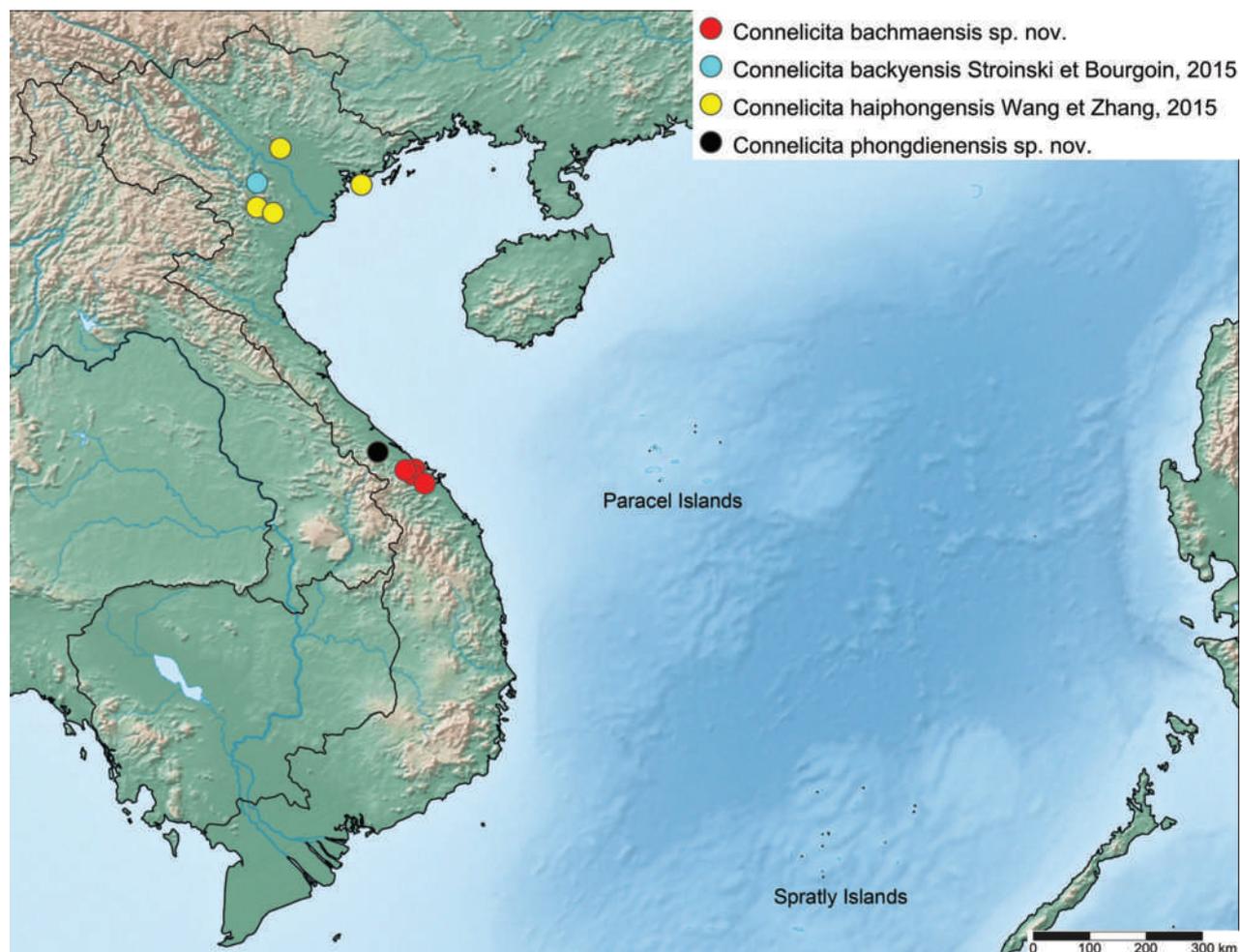


Figure 5. Distribution map of the species of *Connelicita* of Vietnam.

**Paratypes**, VIETNAM – **Thừa Thiên-Huế Province** • 3 ♀♀; same collection data as for holotype; VNMN • 2 ♂♂, 5 ♀♀; **Thừa Thiên-Huế Province** • Bach Ma National Park, Yes Hue Eco; 16°13'05"N, 107°42'27"E; 1 Jun. 2023; alt. 152 m; by net; Hoai T.T. Nguyen leg.; VNMN • 3 ♂♂, 1 ♀; Bach Ma National Park, Pheasant trail; 16°13'38"N, 107°51'20"E; 10–20 May 2023; alt. 500–600 m; J. Constant & L. Semeraro leg.; I.G.: 34.640; RBINS • 1 ♂, 1 ♀; Bach Ma National Park, near ranger station; 16°08'37"N, 107°49'36"E; 18 May 2023; alt. 300–600 m; J. Constant & L. Semeraro leg.; I.G.: 34.640; RBINS • 1 ♂; Bach Ma National Park, Yes Hue Eco; 16°13'05"N, 107°42'27"E; 17 May 2023; alt. 200–300 m; J. Constant & L. Semeraro leg.; I.G.: 34.640; RBINS – **Da Nang Province** • 1 ♂; Ba Na-Nui Chua; 16°00'N, 108°01'E; 16–19 Jul. 2017; GTI Project; J. Constant & J. Bresseel leg.; I.G.: 33.498; RBINS.

**Diagnosis.** The species can be separated from all other species of *Connelicita* by the following features of the male terminalia: anal tube subcylindrical and elongate (~1.9× as long as wide in dorsal view), with dorsal margin not emarginate in lateral view (Fig. 3A–C), capitulum of gonostylus placed at apicodorsal angle and bearing two strong lateral teeth (Fig. 3A–C), and dorsal process of perianthrium forming a large lobe concave in distal portion (Fig. 3F, G).

**Description. Measurements and ratios:** LT: ♂ ( $n = 6$ ): 12.56 mm (11.91–13.17); ♀ ( $n = 2$ ): 13.60 (13.50–13.70). LTg/BTg = 2.19; LW/BW = 2.02; LV/BV = 1.04; LF/BF = 1.15.

**Head** (Fig. 2A–C): narrower than thorax and elongate, with  $\sim 2/3$  of vertex length surpassing eyes and genae largely visible from above. Vertex brown, with median yellowish line, weakly concave,  $\sim 1.0\times$  as long in mid-line as broad basally, with lateral margins subparallel, anterior margin bisinuate, roundly produced anteriorly in middle portion, and posterior margin excavate. Frons pale yellowish brown, with two curved brown lines on disc; lateral margins finely lined in dark brown; convex in lateral view; smooth, with median carina reaching dorsal margin but not frontoclypeal suture. Genae yellowish, with brown markings between eye and anterior margin, between antennal insertion and posterior margin, and near red ocellus. Clypeus pale yellowish brown, triangular, with median carina in distal portion and with fronto-clypeal suture rounded. Labium yellowish, elongate, and narrow, reaching metacoxae, with apical segment elongate. Eyes globular (not emarginate) and protruding laterally; ocelli present. Antennae yellowish brown, with longitudinal black line along underside of pedicel; scape ring-shaped and pedicel cylindrical, longer than broad.

**Thorax** (Fig. 2A, B): pronotum yellowish brown, darker on middle portion, with median, yellowish carina and 3 or 4 small yellowish tubercles on sides of disc; paranotal fields with 3 black spots, one being behind eye. Mesonotum yellowish brown, with median and peridiscal carinae yellowish; blackish, slightly curved longitudinal line in lateral fields and 2 blackish points at base of scutellum. Tegulae yellowish brown.

**Tegmina** (Figs 1A–D, 2D): translucent, with brown spot near middle of vein CuP and vein CuA2 and apical cells weakly infusate in middle; costal and postclaval margins slightly diverging towards the posterior; distal margin widely rounded.

**Venation** (Fig. 2D): costal area well developed, with numerous cross-veins delimitating elongate cells, pterostigma absent; veins ScP+R, MP and CuA separated at base, first fork of vein ScP+R near base; first fork of vein MP after first fork of vein CuA; clavus long, reaching near  $4/5$  of tegmina length; veins Pcu and A1 fused at basal half of clavus.

**Hind wings** (Fig. 2E): translucent, with veins blackish; CuP-Pcu-A1 lobe weakly infusate, with apical angle blackish; A2 lobe weakly infusate and roundly protruding along postclaval margin.

**Venation:** main veins present; ScP+R, MP, and CuA running more or less parallel, slightly diverging towards posterior; Pcu curved around distal third of wing towards CuP but not reaching the latter; A2 complete, reaching margin.

**Legs** (Figs 1A, B, 2F): pale yellowish brown, with dark brown marking. Metabiotarsal formula: (2) 9 / 2 / 2.

**Abdomen** (Fig. 1B): Pale yellowish brown.

**Terminalia** ♂ (Fig. 3): pygofer (Py – Fig. 3A, B)  $\sim 1.6\times$  as high as wide in caudal view, in lateral view with posterior margin deeply inclined posteriorly at upper  $1/3$  and deeply inclined forward and slightly sinuate at lower  $2/3$ . Gonostyli (G – Fig. 3A–C) rather short in lateral view, with capitulum at apicodorsal angle, and with large laminate process behind capitulum; posteroventral angle rounded; capitulum (ca – Fig. 3A–E) laterally flattened, with posterior margin

rounded in lateral view and 2 strong lateral spines derived from anterior portion, projecting lateroventrad, the dorsal one about twice as long as the ventral one. Aedeagus (Fig. 3F–I) upcurved, with pair of lateral pointed processes (*lpa*) not reaching apex, and 3 small terminal processes, middle one with small, triangular lamina projecting anterodorsad; dorsal process of periandrium (*dpp*) large, foliaceous, somewhat shovel-shaped, and concave in distal half, and with apical margin widely rounded; paired ventral processes of periandrium (*vpp*) surpassing  $\frac{1}{2}$  length of aedeagus, elongate, and with pointed apex curved lateroposterad; connective (*co*) strongly curved. Anal tube (*An* – Fig. 3 A–C) subcylindrical, with basal ventral bulge,  $\sim 1.9\times$  as long as wide in dorsal view, with apical margin rounded in dorsal view, excavate in lateral view; epiproct short, located at distal third of anal tube.

**Female.** Similar to male.

**Etymology.** The species epithet *bachmaensis* refers to Bach Ma National Park where the new species was discovered.

**Biology.** The specimens were found sitting on leaves on the lower vegetation (Fig. 4A) in a subtropical evergreen forest (Fig. 4B) at the junction of the Northern Vietnam lowland rain forests, Southern Vietnam lowland rain forests, and Southern Annamites montane rain forests ecoregions, at rather low altitude (150–600 m).

**Distribution.** Vietnam, Thua Tinh-Hue Province, Bach Ma National Park and Da Nang Province, Ba Na-Nui Chua Nature Reserve (Fig. 5).

### ***Connellicita haiphongensis* Wang & Zhang, 2015**

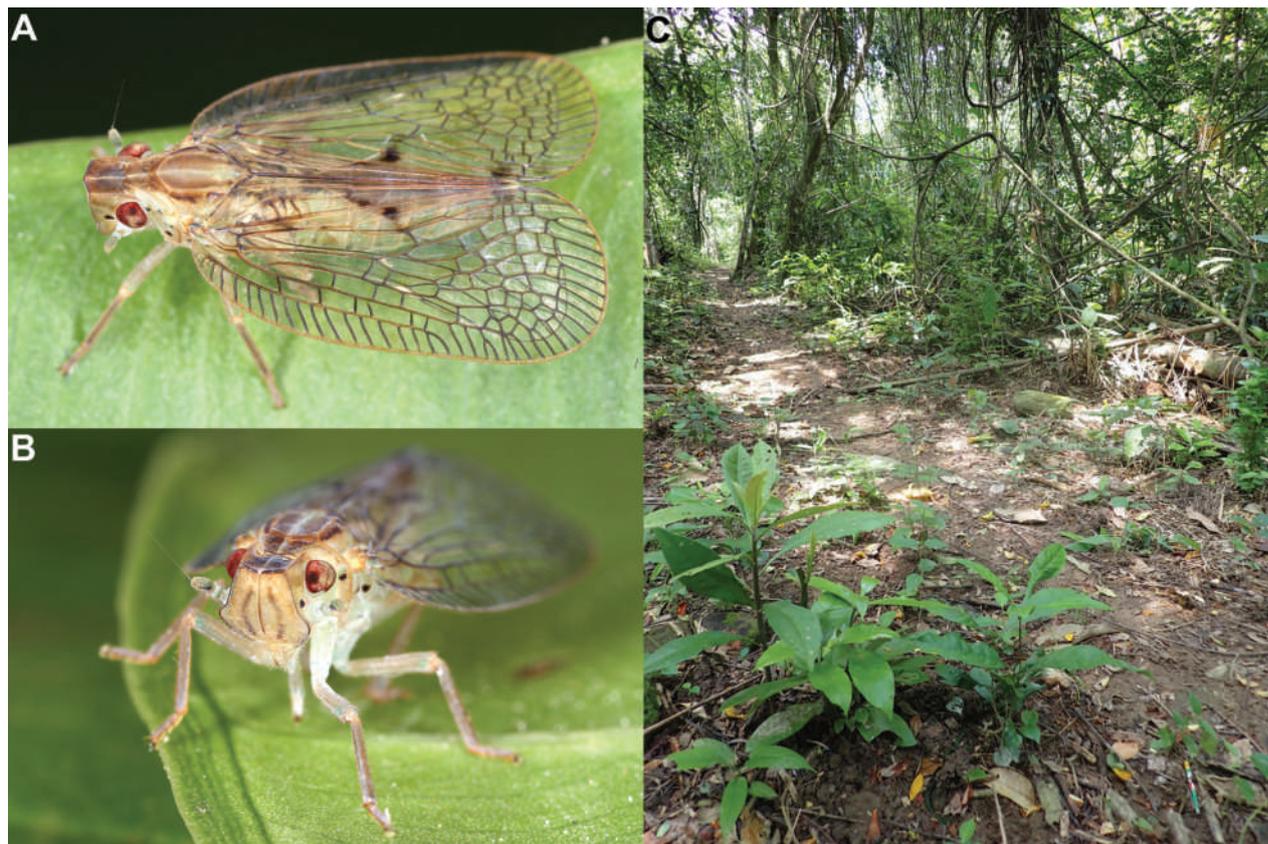
Figs 5, 6

*Connellicita haiphongensis* Wang & Zhang, 2015 in Wang et al. 2015: 569, figs 2–21.

**Materials examined.** VIETNAM – **Hai Phong Province** • 1 ♂; Cat Ba National Park; 20°48'00"N, 107°00'20"E; 12–16 Jul. 2013; J. Constant & J. Bresseel leg.; I.G.: 32.454; RBINS – **Vinh Phuc Province** • 5 ♂♂, 5 ♀♀; Me Linh Biodiversity Station; 21°23'04"N, 105°42'44"E; 29–30 Jun. 2023; J. Constant & L. Semeraro leg.; RBINS • 2 ♂♂, 1 ♀; same locality as preceding; 29 Jun. 2023; alt. 30 m; by net; Hoai T.T. Nguyen leg.; VNMN – **Hoa Binh Province** • 1 ♂, 1 ♀; Ngoc Son-Ngo Luong Nature Reserve; 20°26'16"N, 105°20'15"E; 25–30 Jul. 2016; GTI Project; J. Constant & J. Bresseel leg.; RBINS – **Ninh Binh Province** • 1 ♀; Cuc Phuong National Park; 20°20'53"N, 105°35'52"E; 31 Jul.–3 Aug. 2016; GTI Project; J. Constant & J. Bresseel leg.; RBINS.

**Note.** The species was previously only recorded from Cat Ba Island in Ha Long Bay (Wang et al. 2015). It is here recorded for the first time from the mainland, and its distribution is extended to the provinces of Vinh Phuc, Hoa Binh, and Ninh Binh (Fig. 5).

**Biology.** The specimens were found sitting on leaves on the lower vegetation (Fig. 6A, B) in subtropical evergreen forests (Fig. 6C) in the South China–Vietnam subtropical evergreen forests and Northern Indochina subtropical forests ecoregions.



**Figure 6.** *Connellicita haiphongensis* Wang & Zhang, 2015 **A, B** adult specimen on leaf of unidentified plant, Vietnam, Cuc Phuong National Park, 4 July 2019 Gernot Kunz, with permission **A** laterodorsal view **B** anterodorsal view **C** habitat in Vietnam, Me Linh Biodiversity Station, 30 June 2023.

***Connellicita phongdienensis* Constant & Pham, sp. nov.**

<https://zoobank.org/556D7EFE-52A9-4D76-869B-7C59FDDC754C>

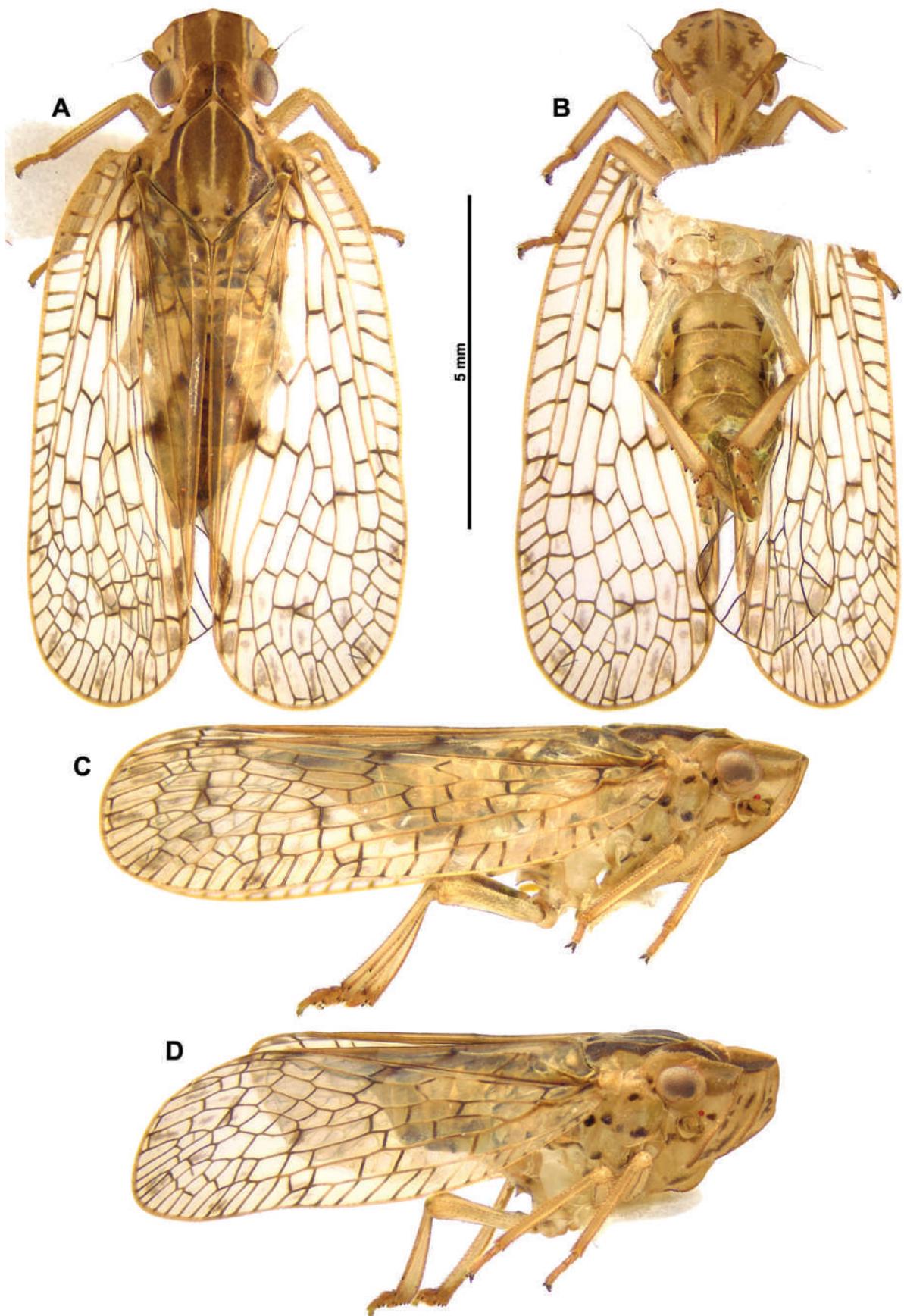
Figs 5, 7–10

**Type materials.** *Holotype* ♂, VIETNAM – Thừa Thiên-Huế Province • Phong Dien Nature Reserve, CCRR; 16°30'27"N, 107°16'05"E; 23 May 2023; alt. 350–400 m; J. Constant & L. Semeraro leg.; I.G.: 34.640; VNMN.

**Paratypes**, VIETNAM – Thừa Thiên-Huế Province • 1 ♂; same collection data as for holotype; RBINS • 1 ♀; Phong Dien Nature Reserve, CCRR; 16°30'27"N, 107°16'05"E; 23 May 2023; alt. 350–400 m; Trung T. Vu leg.; VNMN • 1 ♀; same collection data as for preceding; RBINS.

**Diagnosis.** The species can be separated from the other species of *Connellicita* by the following characters of the male terminalia: anal tube in lateral view abruptly narrowing in distal half (Fig. 9A), posterior margin of pygofer rounded in lateral view (Fig. 9A), capitulum of gonostylus with a lateral spine in ventral portion, and a dorsal curved digitiform process (Fig. 9A, D), dorsal process of periandrium tooth-shaped, strong, and projecting dorsocephalad (*dpp* – Fig. 9E) and absence of a strong digitiform process directed dorsally at apex of aedeagus (Fig. 9E–G).

The closest species are *C. backyensis* and *C. haiphongensis*, from which *C. phongdienensis* Constant & Pham, sp. nov. can be separated by the dorsal



**Figure 7.** *Connelicita bachmaensis* sp. nov., holotype ♂ **A** habitus, dorsal view **B** habitus, ventral view **C** habitus, lateral view **D** habitus, anterolateral view.

digitiform process of the capitulum of the gonostylus, which is absent in both other species (compare with Wang et al. 2015: figs 12, 31).

**Description. Measurements and ratios:** LT: ♂ ( $n = 2$ ): 10.15–10.77 mm. LTg/BTg = 2.26; LW/BW = 1.89; LV/BV = 0.85; LF/BF = 1.04.

**Head** (Fig. 8A–C): narrower than thorax and elongate, with  $\sim 2/3$  of vertex length surpassing eyes and genae largely visible from above. Vertex brown, with median yellowish line, weakly concave,  $\sim 1.0\times$  as long in mid-line as broad basally, concave on each side, with lateral margins subparallel, anterior margin roundly produced anteriorly in middle portion and posterior margin excavate. Frons yellowish brown, with irregular brown markings; convex in lateral view; smooth, with median carina reaching dorsal margin but not frontoclypeal suture. Genae yellowish, with brown marking between eye and anterior margin, brown line between antennal insertion and posterior margin, and a dark brown spot near red ocellus. Clypeus pale yellowish brown, with median carina and one curved line on each side, brown, triangular, with median carina in distal portion and with fronto-clypeal suture rounded. Labium yellowish, elongate, and narrow, reaching metacoxae, with apical segment elongate. Eyes globular (not emarginate) protruding laterally. Antennae yellowish brown, with longitudinal black line along underside of pedicel; scape ring-shaped and pedicel cylindrical, longer than broad.

**Thorax** (Fig. 8A, B): Pronotum brown, with median carina, posterolateral angles and 3 or 4 small yellowish tubercles on sides of disc, yellowish; paranotal fields yellowish, with 3 black spots, one being behind eye. Mesonotum brown, with median and peridiscal carinae, anterior portion of lateral angles, scutellum, and area before latter yellowish; lateral fields darker and 2 blackish points at base of scutellum. Tegulae yellowish brown.

**Tegmina** (Figs 7A–D, 8D): translucent, with brown spot near middle of vein CuP and vein CuA2, extending into clavus and irregular, greyish markings in cells in distal half of tegmen; costal and postclaval margins slightly diverging towards posterior; distal margin widely rounded.

**Venation** (Fig. 8D): costal area well developed, with numerous cross-veins delimitating elongate cells, pterostigma absent; veins ScP+R, MP, and CuA separated at base, first fork of vein ScP+R near base; first fork of vein MP after first fork of vein CuA; oblique, transverse cross-vein between MP and CuA1 in basal portion of latter; clavus long, reaching near  $4/5$  of tegmina length; veins Pcu and A1 fused at basal half of clavus.

**Hind wings** (Fig. 8E): translucent, with veins blackish; CuP-Pcu-A1 lobe infuscate in distal portion, with apical angle largely blackish; A2 lobe weakly infuscate and roundly protruding along postclaval margin.

**Venation:** main veins present; ScP+R, MP, and CuA running more or less parallel, slightly diverging towards posterior; Pcu curved around distal third of wing towards CuP but not reaching the latter; A2 complete, reaching margin.

**Legs** (Figs 7A, B, 8F–G): pale yellowish brown, with dark brown marking along dorsal portion of metafemora and basiventral portion of metatibiae. Metatibiotarsal formula: (2) 9 / 2 / 2.

**Abdomen** (Fig. 7B): pale yellowish brown, with narrow brown line interrupted in middle, along posterior margin of sternites.

**Terminalia** ♂ (Fig. 9): pygofer (Py – Fig. 9A, B)  $\sim 1.9\times$  as high as wide in caudal view, in lateral view with posterior margin deeply inclined posteriorly

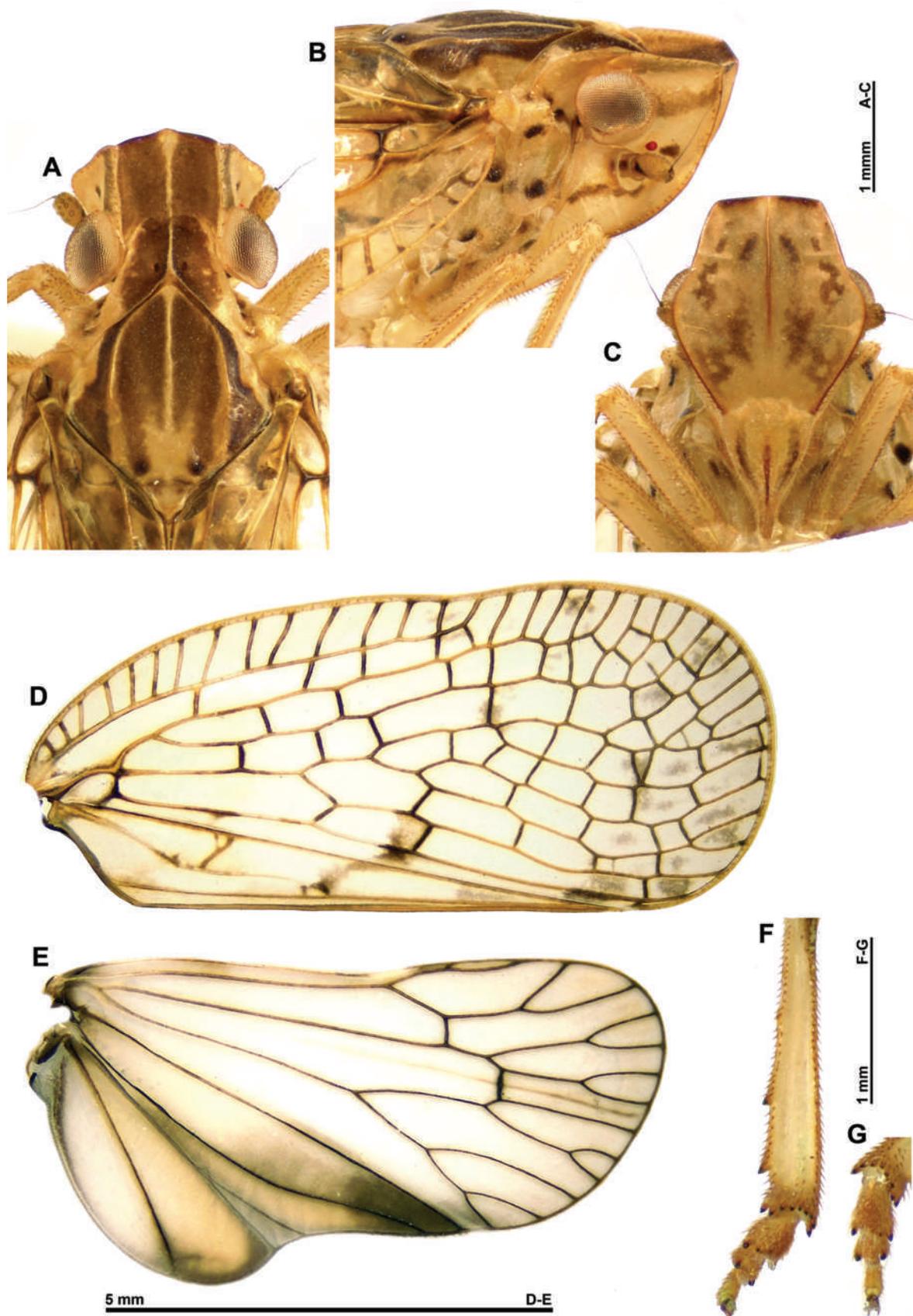
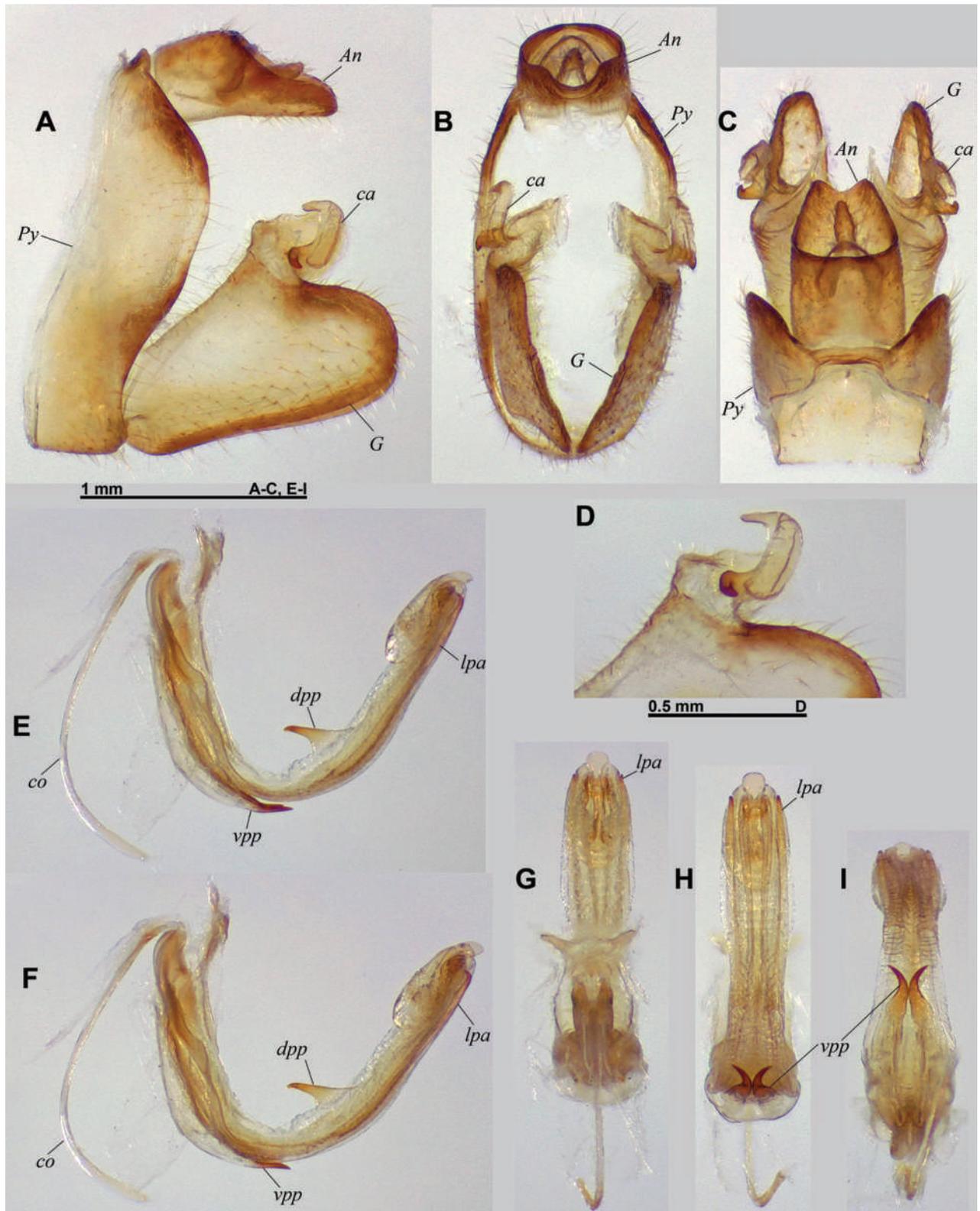


Figure 8. *Connelicita phongdienensis* sp. nov., holotype ♂ **A–C** detail of head and thorax **A** dorsal view **B** lateral view **C** perpendicular view of frons **D** right tegmen **E** right hind wing **F** right metatibia and metatarsus, ventral view **G** left metatarsus, ventral view.



**Figure 9.** *Connelicita phongdienensis* sp. nov., holotype ♂, terminalia **A–C** pygofer, gonostyli and anal tube **A** lateral view **B** caudal view **C** dorsal view **D–E** capitulum of left gonostylus **D** left laterodorsal view **E** right laterodorsal view **F–I** aedeagus, phallobase and connective **F** left lateral view **G** anterodorsal view **H** posteroventral view dorsal view **I** left lateroventral view.



Figure 10. Habitat of *Connellicita phongdienensis* sp. nov., Vietnam, Phong Dien, CCRR, 23 May 2023.

at upper 1/3 then broadly rounded, and sinuate at lower 2/3. Gonostyli (*G* – Fig. 9A–C) rather elongate in lateral view, with capitulum at about 2/3 of gonostylus length, and with large laminate process projecting medially behind capitulum; posterior lobe rounded; capitulum (*ca* – Fig. 9A–D) laterally flattened, with posterior margin rounded in lateral view and bearing a complex lateral process, with upper digitiform process curved cephalodorsad and sinuate ventral spine directed posteroventrad. Aedeagus (Fig. 9E–I) strongly upcurved at mid-length, with pair of slender lateral pointed processes (*lpa*) not reaching apex of aedeagus, and with apical point directed anterodorsad; 3 small terminal processes, middle one shortly projecting posterad; dorsal process of perianthrium (*dpp*) tooth-shaped, strong and projecting dorsocephalad, placed slightly after mid-length of aedeagus; paired ventral processes of perianthrium (*vpp*) not reaching 1/2 length of aedeagus, elongate, sinuate in distal portion, and with pointed apex directed lateroposterad; connective (*co*) moderately curved. Anal tube (*An* – Fig. 9A–C) ~1.26× as long in median line, as wide, in dorsal view; subcylindrical in proximal half, then with dorsal margin excavate and sinuate in lateral view; in dorsal view, lateral margins subparallel in proximal half, then regularly converging in distal half; posterior margin deeply concave in dorsal view, with basal ventral bulge; epiproct rather large, located at 1/2 length of anal tube.

**Female.** Similar to male.

**Etymology.** The species epithet *phongdienensis* refers to Phong Dien District, the locality where the new species was discovered, at the Centre for Conservation of Vietnam Natural Resources and Rescue of Animals and plants.

**Biology.** The specimens were found sitting on leaves on the lower vegetation in a subtropical evergreen forest (Fig. 10) in the Northern Vietnam lowland rain forests ecoregion at rather low altitude (150–600 m).

**Distribution.** Vietnam, Thua Tinh-Hue Province, Phong Dien District, CCRR (Fig. 5).

## Discussion

The present work adds two species of *Connellicita* to the fauna of Vietnam, leading to a total of four species for the country, while one additional species is known from China. The new records also greatly extend the distribution of the genus to the south but leaves a gap of more than 500 km without any record of *Connellicita* in the northern half of Central Vietnam where new species will certainly be discovered in the future. Although the species are easily identified from male terminalia characters, their external morphology is extremely similar, and the species should not be identified based only on photographs. Citizen-science platforms like iNaturalist should refrain from their members identifying such insects to species level, unless good photographs of the genitalia of the corresponding specimen are provided to support the identification.

In Phong Dien District, VNMN is conducting an ambitious project of forest restoration at the Centre for Conservation of Vietnam Natural Resources and Rescue of Animals and plants, not far from the forest where *C. phongdienensis* Constant & Pham sp. nov. was discovered. The return of such planthopper species in this area in the future would be a great indicator of a successful project.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization, J.C. and T.-H.P.; Funding acquisition, T.-H.P. and J.C.; Investigation, J.C.; Methodology, J.C. and T.-H.P.; Project administration, T.-H.P., C.V.C.L. and J.C.; Field work, J.C., T.-H.P., C.V.C.L., T.T.V., H.T.T.N.; Field logistics, T.-H.P., C.V.C.L., T.T.V., H.T.T.N.; Supervision, J.C. and T.-H.P.; Validation, J.C. and T.-H.P.; Writing – original draft, J.C.; Writing – review and editing, J.C., T.-H.P., C.V.C.L., T.T.V., H.T.T.N. All authors have read and agreed to the published version of the manuscript.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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# Description of the first stygobiotic species of the atyid shrimp genus *Sinodina* (Decapoda, Caridea, Atyidae) from Yunnan Province, China

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## Abstract

*Sinodina* Liang & Cai, 1999, a genus of atyid shrimp, is endemic to China and distributed only in the Yunnan-Guizhou Plateau. We describe here the thirteen species of *Sinodina*, and the first cave-dweller of the genus, *Sinodina ashima* **sp. nov.**, collected from a limestone cave in Shilin County, Yunnan Province. This species can be distinguished from its congeners by the completely degraded pigment and eyes, the extremely long rostrum, the rostral formula and the absence of sexual dimorphism of the third and fourth pereopods. A phylogenetic analysis based on four genes (COI, 16S, 18S, H3) shows that the new species strongly clustered with the type species of this genus, *Sinodina gregoriana* (Kemp, 1923), supporting the generic status of this new species.

**Key words:** Diversity, morphology, new species, phylogeny, stygobiont, taxonomy

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## Introduction

The genus *Sinodina* Liang & Cai, 1999 belongs to the order Decapoda and the family Atyidae. It was established by Liang and Cai (1999) on the type species *Caridina gregoriana* Kemp, 1923. In the paper, the authors also transferred *Caridina yui* Liang & Yan, 1985, *Caridina acutipoda* Liang, 1989 and *Caridina bispinosa* Liang & Yan, 1990 (in Liang 1990) to *Sinodina*, and published three new species, *Sinodina dianica* Liang & Cai, 1999, *S. wangtai* Liang & Cai, 1999 and *S. lijiang* Liang & Cai, 1999. Chen and Liang (2002) described a new species *Sinodina yongshengica* Chen & Liang, 2002 from Yongsheng, Yunnan, China. Liang (2002) based on the specimens from Jiangchuan, Yunnan, described *Sinodina angulata* Liang, 2002. Liang (2004) reviewed the genus and placed *Caridina leptopropoda* Liang, 1990, *Caridina heterodactyla* Liang & Yan, 1985 and *Caridina banna* Cai & Dai, 1999 into *Sinodina*. Ultimately, a total of 12 species have been recorded (Liang 2004; De Grave and Fransen 2011), making it the third largest genus of Atyidae in the Chinese fauna, after *Caridina* and *Neocaridina*. All of these species are endemic to a narrow range

and only distributed in Yunnan Province, southwest China, except *Sinodina gregori-ana* (Kemp, 1923), which has a relatively larger distribution range, not only in some lakes in Yunnan but also in Caohai Lake, Guizhou Province (Liang 2004).

The morphology of *Sinodina* is similar to that of the genera *Caridina* and *Neocaridina*, and they share the same branchial formula. *Sinodina* can be identified by the simple and lamellar podobranch of the second maxilliped and the obvious sexual dimorphism, that is, the male possesses more spines and distinctive dilation on the propodus of the third and fourth pereopod (Liang and Cai 1999). According to Liang (2004), both the simple podobranch and the dilated pereopod with a large number of spines of the male are plesiomorphic. Thus, *Sinodina* probably is a more basal group than *Caridina* and *Neocaridina*.

There are numerous caves in the karst areas of south China, which provided refuge for organisms in this area during the Neogene when the climate and habitat had been changing, especially after the Oligocene-Miocene boundary (Li et al. 2022). Many species have adapted to the cave environment and have undergone morphological changes, such as degeneration of eye and body coloration and elongation of the limbs. Some of them have completely adapted to the subterranean surroundings and live exclusively in the cave, becoming stygobionts/troglobionts. Cave shrimp is an interesting group among the stygofauna of this region. At present, four genera (*Caridina*, *Mancicaris*, *Neocaridina* and *Typhlocaridina*) and 27 species of Atyidae have been discovered and described from Chinese caves, distributed in Guangxi (12 species), Guizhou (8 species), Yunnan (3 species), Hunan (3 species) and Hubei (1 species) provinces (Cai and Ng 2018; Xu et al. 2020; Feng et al. 2021; Guo et al. 2022), and the number has continued to increase.

We surveyed Xiangshuiqing Cave in Shilin County, Yunnan Province twice in April and June 2023 and collected a total of 14 atyid shrimp specimens with strong cave morphological features. They were identified as a new species of *Sinodina* through morphological observations and molecular analysis. This species is the first stygobiont in the genus and the fourth cave atyid species in Yunnan Province, after three *Caridina* species, *Caridina feixiana* Cai & Liang, 1999, *Caridina alu* Cai & Ng, 2018 and *Caridina* aff. *heterodactyla* Liang & Yan, 1985 (Cai and Ng 2018).

## Materials and methods

### Specimen collecting and preservation

Specimens were collected by cage nets from a limestone cave in Shilin, Yunnan, southern China. Live animals were observed and photographed with a Sony A7R4A camera with a Sony FE 90 mm macro lens. Most of the specimens were preserved in 75% ethanol for morphological studies, and the remainder were preserved in absolute ethanol and stored at  $-40^{\circ}\text{C}$  for molecular research. All specimens are deposited at the Institute of Biology, Guizhou Academy of Sciences, Guiyang, China (IBGAS).

### Morphological study

Specimens were examined, photographed and measured using a Leica M205A stereomicroscope equipped with a Leica DFC450 camera and LAS X software (v. 5.1, Leica, Germany). All images were edited with PHOTOSHOP CC 2019 software (v. 20.0.0, Adobe, USA).

The following abbreviations are used in the text: **alt** (altitude), **cl** (carapace length, measured from the postorbital margin to the posterior margin of the carapace), **rl** (rostral length, measured from the rostral tip to the postorbital margin) and **tl** (total length, measured from the rostral tip to the posterior margin of the telson). All measurements are in millimeters.

## Molecular analyses

To verify the classification of the new species, a multi-genes phylogenetic analysis was conducted. Four specimens of *Sinodina ashima* sp. nov. were sampled. The ingroup of the matrix was composed of *Sinodina gregoriana* (Kemp, 1923), two cave-dweller species of *Caridina*, *Caridina cavernicola* Liang & Zhou, 1993 and *Caridina sinanensis* Xu et al., 2020, two species of *Neocaridina*, *Neocaridina palmata* (Shen, 1948) and *Neocaridina hofendopoda* (Shen, 1948), and *Paracaridina guizhouensis* (Liang & Yan, 1986). *Macrobrachium nipponense* (De Haan, 1849) of Palaemonidae was selected as the outgroup. Detailed geographical information and sequence metadata are listed in Table 1.

Four loci, including two mitochondrial genes (cytochrome c oxidase subunit I and 16S rDNA) and two nuclear genes (18S rDNA and histone H3 gene) were used to conduct the analysis. Primer sequences are in Table 2. Except for that of *Caridina cavernicola* and *Caridina sinanensis*, all sequences of this matrix were obtained in this research.

Raw sequences were edited and assembled using SEQMAN PRO software (Lasergene v. 7.1; DNA Star, Inc., Madison, Wis., USA). Protein-coding gene sequences (COI and H3) were aligned based on amino acid translation using CLUSTALW in MEGA 7.0 (Kumar et al. 2016). The more variable sequences (16S and 18S) were aligned using the online version of MAFFT v. 7.0 (Kato and Standley 2013) under the algorithm, Q-INS-i. All other settings were left as default. After manual trimming, the resulting sequences were concatenated using MESQUITE v. 3.6 (Maddison and Maddison 2015).

PARTITIONFINDER 2 (Lanfear et al. 2016) was used to determine the optimum partitioning scheme and the best-fitting model for each partition, using the corrected Akaike Information Criterion (AICc). We input the partition file that contained six partitions, in which the protein-coding genes (COI and H3) was divided into codon positions for each fragment.

Maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted to infer the phylogeny. ML was performed in RAXML v. 8.2.0 (Stamatakis 2014) under a GTRGAMMA model, and the six partitioning schemes, using 1000 rapid bootstrap replicates and a random seed value set to 12345. BI was implemented in MRBAYES v. 3.2.5 (Ronquist et al. 2012) following the parameters obtained from PARTITIONFINDER and with two simultaneous Monte Carlo Markov (MCMC) runs for 1 million generations, and tree samples were output every 1000 generations with a burn-in of 25%. Trees were visualized and edited with FIGTREE v. 1.44 (Rambaut 2016).

In addition, the pairwise p-distances between COI and 16S genes of all specimens of *Sinodina ashima* sp. nov. and *Sinodina gregoriana* were calculated with MEGA 7.0. One 16S sequence of *Sinodina* sp. derived from Genbank (Table 1) was also calculated for their interspecific distances.

**Table 1.** Details of the specimens used for the molecular analyses.

Taxon	Voucher number	Collection data	GenBank number				Reference
			COI	16S	18S	H3	
<i>Sinodina ashima</i> sp. nov.	GBZD-676	Xiaoliao Cave, Shilin, Yunnan, China, 4. VI. 2023, X.K. Jiang leg.	–	OR537884	OR539523	–	This study
	GBZD-677		OR536642	OR537885	OR539524	–	This study
	GBZD-678		OR536643	OR537886	OR539525	–	This study
	GBZD-679		OR536644	OR537887	OR539526	–	This study
<i>Sinodina gregoriana</i>	GBZD-238	Yangwanqiao Reservoir, Weining, Guizhou, China, 17. X. 2020, X.K. Jiang & H.M. Chen leg.	–	OR537881	OR539518	OR540202	This study
	GBZD-239		–	–	OR539519	OR540203	This study
	GBZD-240		–	–	OR539520	OR540204	This study
	GBZD-241		–	OR537882	OR539521	OR540205	This study
<i>Sinodina</i> sp.	ZMB DNA-651	Yunnan, China	–	FN995388	–	–	von Rintelen et al. 2012
<i>Caridina cavernicola</i>	–	Hechi, Guangxi.	MZ753498	MZ753801	–	–	Guo et al. 2022
<i>Caridina sinanensis</i>	–	Sinan, Guizhou, 25. I. 2019	MT433963	MT434874	–	–	Xu et al. 2020
<i>Neocaridina palmata</i>	GBZD-098	Lisong, Hezhou, Guangxi, China, 25. IV. 2021, X.K. Jiang leg.	OR536639	–	OR539516	OR540200	This study
<i>Neocaridina hofendopoda</i>	GBZD-141	Sijia River, Yacai, Sanjiang, Guangxi, China, 15. III. 2021, X.K. Jiang, H.M. Chen & J.C. Lv leg.	OR536640	–	OR539517	OR540201	This study
<i>Paracaridina guizhouensis</i>	GBZD-562	Longquan, Maopo, Yuping, Guizhou, China, 29. IV. 2022, X.K. Jiang, H.M. Chen & L.P. Ye leg.	OR536641	OR537883	OR539522	OR540206	This study
<i>Macrobrachium nipponense</i>	GBZD-001	Guangzhao Reservoir, Qinglong, Guizhou, China, 14. I. 2021, H.M. Chen leg.	OR536638	OR537880	OR539515	OR540199	This study

**Table 2.** Primers used for PCR and sequencing.

Genes	Primer	Sequence (from 5' to 3')	Reference
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAATCA	
16S	16sA	ACTTGATATATAATTAAGGGCCG	Wowor et al. 2009
	16sB	CTGGCGCCGGTCTGAACTCAAATC	
18S	18s ai	CCTGAGAAACGGCTACCACATC	DeSalle et al. 1992
	18s bi	GAGTCTCGTTCTATCGGA	
H3	H3 AF	ATGGCTCGTACCAAGCAGAC(AGC)GC	Colgan et al. 1998
	H3 AR	ATATCCTTRGGCATRARTGTGAC	

## Results

### Taxonomy

#### *Sinodina ashima* sp. nov.

<https://zoobank.org/B4D759DE-18AD-4F4E-A47D-16DE20D99B13>

Figs 1–5

**Type material. Holotype:** male (rl 5.1 mm, cl 5.8 mm, tl 26.7 mm), China, Yunnan Province, Kunming City, Shilin County, Xiangshuiqing Cave, 24°45'27.53"N, 103°19'54.88"E, alt. 1790 m, 4. VI. 2023, Jiang X.K. leg. **Paratypes.** 2 males (rl 5.0–5.9 mm, cl 6.0–6.6 mm, tl 27.5–29.5 mm) and 8 females (rl 4.6–6.5 mm, cl 5.4–6.6 mm, tl 23.5–28.7 mm), collected with holotype; 3 females (rl 5.7–9.0 mm, cl 6.7–8.2 mm, tl 28.9–40.0 mm), same locality, III. 2023, Zhou J.J. leg.

**Diagnosis.** Body color and eyes strongly degenerated. Rostrum extremely elongated and upturned, obviously beyond end of scaphocerite, rostral formula: 7–11 + 14–15/8–14. Male propodus of third and fourth pereopod normal without dilation. Dactylus of third pereopod with 4–6 spinules. Telson with 6–7 pairs of dorsal spines.

**Description.** *Body slender* (Fig. 1). Rostrum long, slightly to strongly upturned (Fig. 2), reaching obviously beyond end of scaphocerite, 0.85–1.1 times of cl, armed dorsally with 22–26 (holotype 23) teeth, including 7–11 (holotype 8) situated posterior to orbital margin, ventrally with 8–14 (holotype 11) teeth, rostral formula: 7–11 + 14–15/8–14 (Figs 1–3A).

**Eyes** small, highly reduced, without ocular peduncle, only centre of cornea slightly pigmented (Figs 1–3A).

**Carapace** smooth, glabrous, antennal spine acute, pterygostomial margin subrectangular, pterygostomial spine absent (Figs 1–3A).

**Antennule** (Fig. 3C) peduncle three-segmented, c. 0.6 times as long as carapace. Basal segment about 1.5 times as long as second and 2.0 times as long as third. All segments with submarginal setae. Stylocerite almost reaching end of basal segment. Anterolateral angle reaching one third of 2<sup>nd</sup> segment. Flagella long and simple.

**Antennal** (Fig. 3D) peduncle about 0.4 length of scaphocerite. Scaphocerite about 3.0 times as long as wide, outer margin straight, aetose, ending in a strong sub-apical spine, inner and anterior margins with long plumose setae.

**Mandible** incisor process with six irregular and blunt teeth. Molar process truncated (Fig. 3B).

**Maxillula** (Fig. 3E) lower lacinia broadly rounded, with several rows of plumose setae. Upper lacinia elongate, with numerous small teeth and short setae on inner margin. Palp digitiform, slightly expanded distally, with few long setae.

**Maxilla** (Fig. 3F) with palp slender and slightly curved. Upper endites subdivided. Scaphognathite tapering posteriorly with some long, curved setae.

**First maxilliped** (Fig. 3G) epipod small. Palp rounded, with several terminal plumose setae. Exopod flagellum distinct, well developed and with plumose marginal setae. Caridean lobe narrow, with dense plumose marginal setae.

**Second maxilliped** (Fig. 3H) slender. Ultimate and penultimate segments of endopod fused. Inner margin of ultimate, penultimate and basal segments with long straight setae. Exopod long and slender, with several plumose setae distally. Podobranch simple.

**Third maxilliped** (Fig. 4A) endopod three-segmented, basal segment about 7 times as long as broad, second segment about 10 times as long as broad and 0.95 times as long as basal segment, distal segment as long as second segment, ending in small claw-like apical spine surrounded by simple setae, preceded by 7 spines along distal third of posterior margin, a clump of long and simple setae proximally. Exopod reaching beyond end of basal segment of endopod, with long plumose setae distally.

**First pereopod** (Fig. 4B) stout, chela about 1.8 times as long as wide, 0.9 times length of carpus, movable finger about 2.8 times as long as wide, and 1.2 times length of palm, fingertips rounded, with numerous long setae. Carpus excavated anterodorsally, 2.3 times as long as wide and as long as merus. Merus slightly narrower than carpus. Ischium about 0.5 length of merus and about 2 times as long as basis.

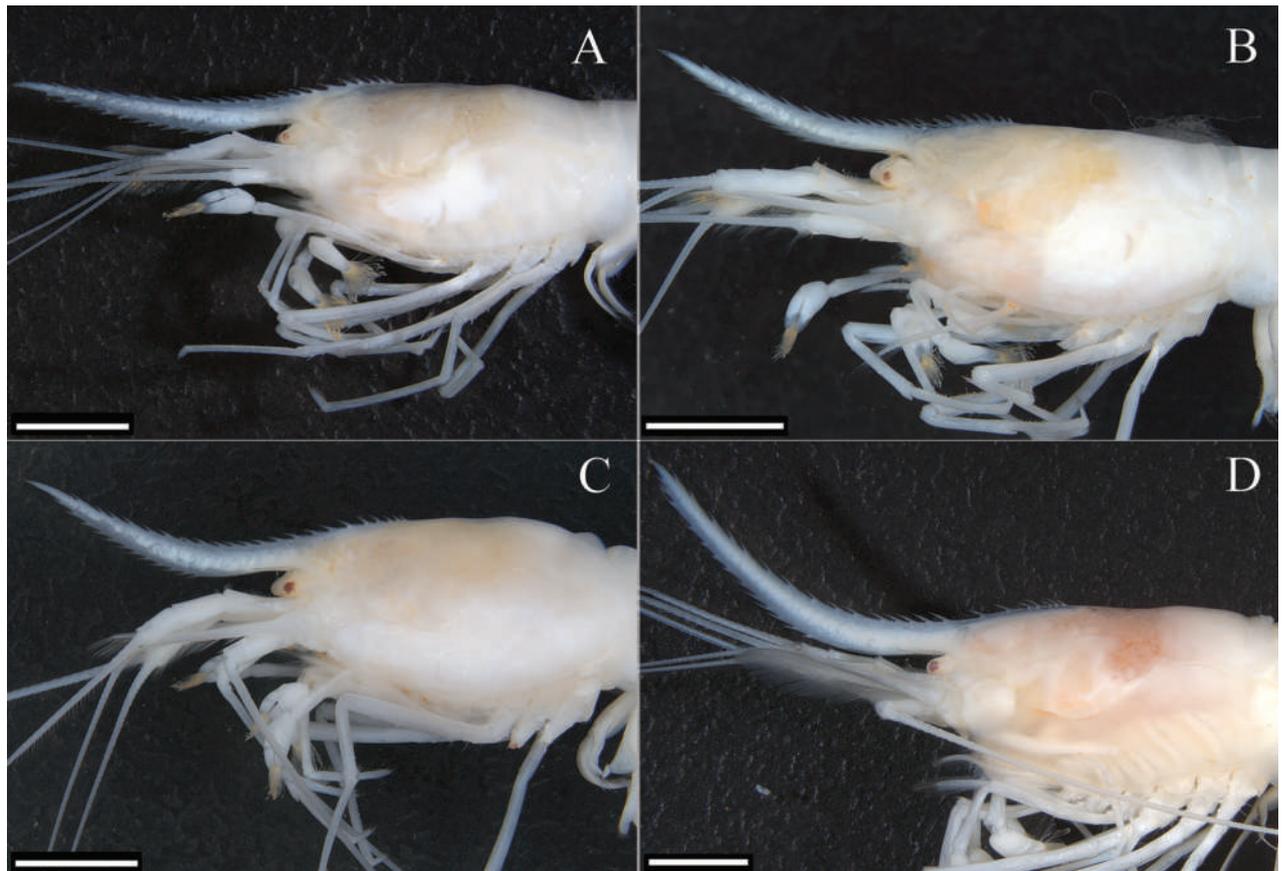


Figure 1. Live specimens of *Sinodina ashima* sp. nov.

**Second pereiopod** (Fig. 4C) slender and longer than first pereiopod. Chela 2.2 times as long as wide, 0.72 times length of carpus. Movable finger 3.5 times as long as wide and 1.5 times as long as palm, setal brushes well developed. Carpus 5.2 times as long as wide, distal part normal, about 0.7 times length of merus.

**Third pereiopod** (Fig. 4D) slender. Dactylus 2.8 times as long as wide (Fig. 5A) (female 2.4, Fig. 5B), ending in prominent claw-like spine surrounded by simple setae and 4–6 spines. Propodus 5.5 times as long as dactylus, bearing about 20 thin spinules evenly and loosely distributed on ventral margin, 13.5 times as long as wide. Carpus 0.71 times length of propodus. Merus 1.8 times length of carpus, with about 3–4 strong spines on the posterior margin. Ischium with a spine on the posterior margin.

**Fifth pereiopod** (Fig. 4E) dactylus 3.6 times as long as wide, ending in prominent claw-like spine surrounded by simple setae, inner margin with about 30 and comb-like spines. Propodus 5.7 times length of dactylus, bearing about 15 spinules in two rows on ventral margin, 19.4 times as long as wide. Carpus 0.51 times length of propodus. Merus 1.5 times length of carpus, with about 3 strong spines on the posterior margin. Ischium about 0.3 times length of merus and 2.1 times length of basis.



**Figure 2.** Cephalothorax of *Sinodina ashima* sp. nov., lateral view, showing the variation of the rostrum **A** female paratype, tl 23.5 mm **B** holotype **C** male paratype, tl 27.5 mm **D** female paratype, tl 40 mm. Scale bars: 2.5 mm.

**First pleopod** (Fig. 4F) endopod tongue-like, about 2.0 times as long as wide, 0.4 times length of exopod, both inner and outer margin with spine setae, appendix interna well developed, arising from distal 1/5 of endopod, overreaching end of endopod, with cincinnuli distally. Exopod 5.3 times as long as wide.

**Second pleopod** endopod slender. Appendix masculina (Fig. 4G) strong, about 3/5 length of endopod, bearing about 25 long, spine-like setae distally as well as on distal part of inner margin. Appendix interna of endopod reaching 1/2 of appendix masculina, with cincinnuli distally (Fig. 4G).

**Telson** (Fig. 4H) about 0.5 times the postorbital carapace length and as long as sixth abdominal somite, tapering posteriorly and ending in a small median projection, dorsal surface with about 6–7 pairs of submarginal spines. Posterior margin with a pair of outermost spines and 5 pairs of intermediate spines that are slightly shorter than the lateral pair. Exopod of uropod longer and wider than endopod, both with plumose marginal setae. Diaeresis bearing 8–11 (holotype 11) spines.

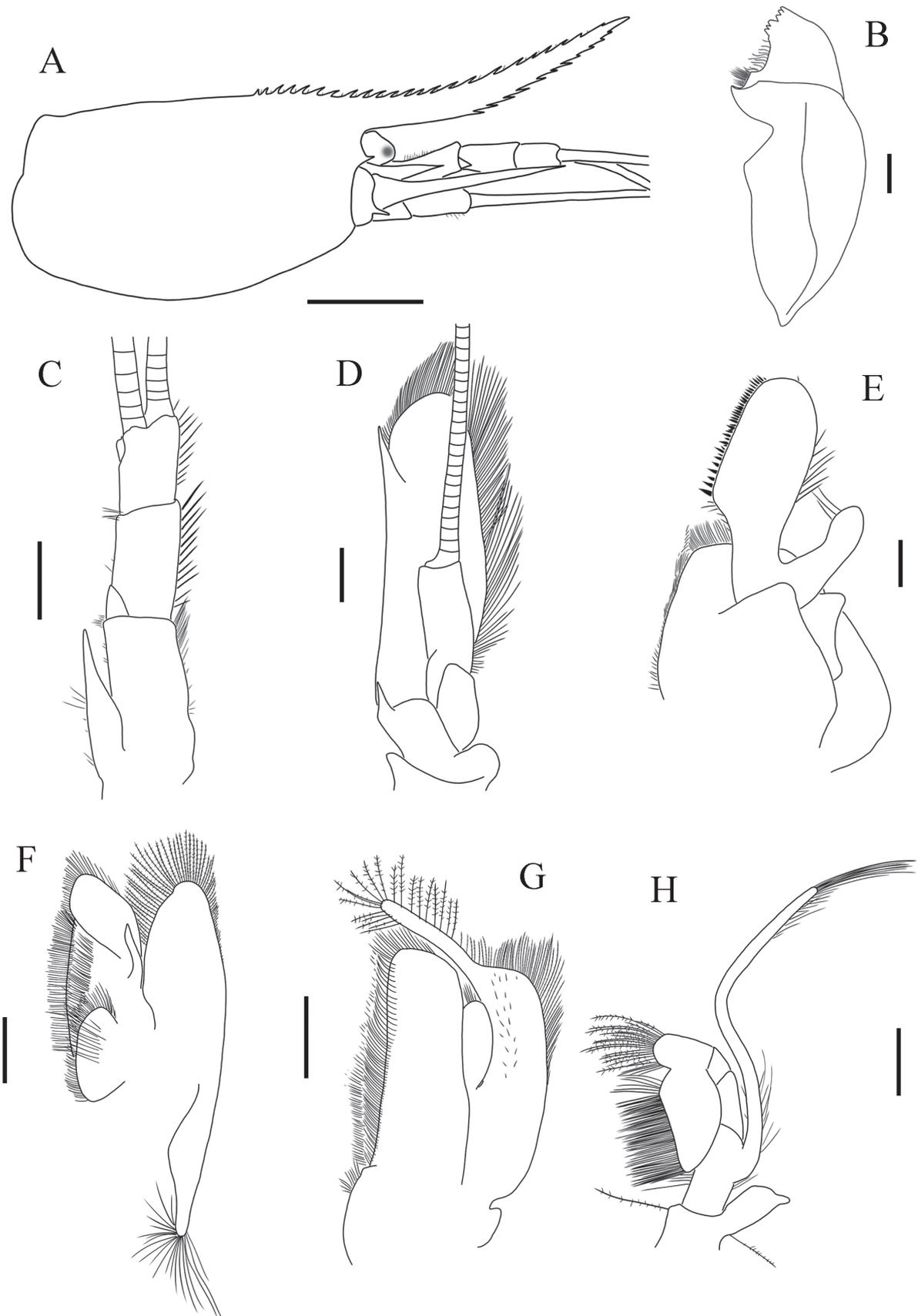
**Eggs** 0.85–0.91 × 1.20–1.27 in diameter.

**Color** strongly degenerated, translucent to flavescent (Fig. 1).

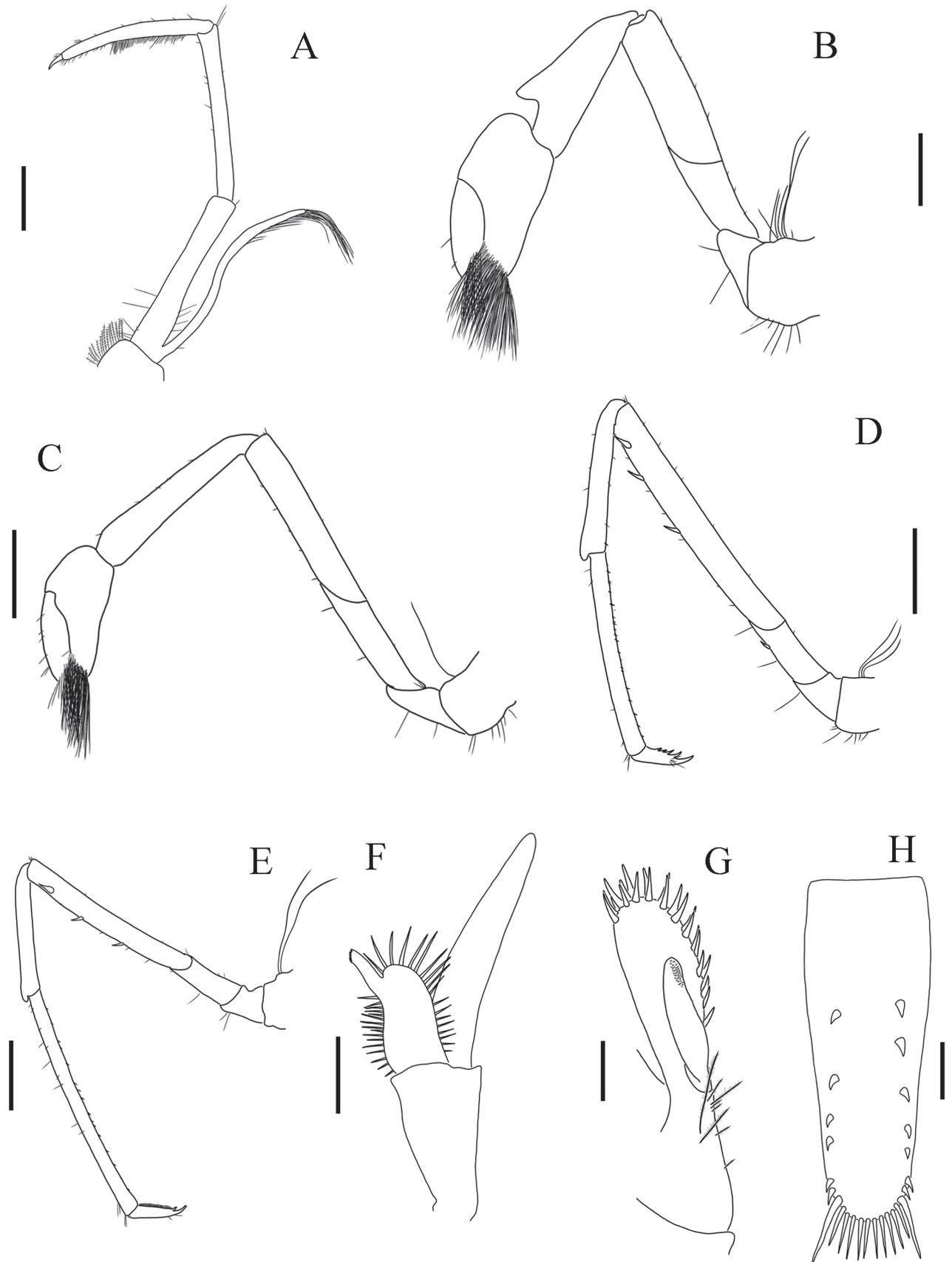
**Etymology.** The specific name is in honor of Ashima, who is a famous female character of the local legend spreading among the Yi nationality and is a symbol of love and bravery.

**Distribution.** Yunnan Province (Xiangshuiqing Cave), China.

**Habitat.** Subterranean river in a karst cave.



**Figure 3.** Holotype of *Sinodina ashima* sp. nov. **A** cephalothorax and cephalic appendages, lateral view **B** mandible **C** antennule **D** antenna **E** maxillula **F** maxilla **G** first maxilliped **H** second maxilliped. Scale bars: 2.5 mm (**A**); 0.25 mm (**B**, **E**); 0.75 mm (**C**, **D**); 0.5 mm (**F**–**H**).



**Figure 4.** Holotype of *Sinodina ashima* sp. nov. **A** third maxilliped **B** first pereopod **C** second pereopod **D** third pereopod **E** fifth pereopod **F** first pleopod **G** appendix masculina and appendix interna of second pleopod **H** telson. Scale bars: 0.75 mm (**A**, **C**, **F**); 0.5 mm (**B**, **H**); 1 mm (**D**, **E**); 0.25 mm (**G**).

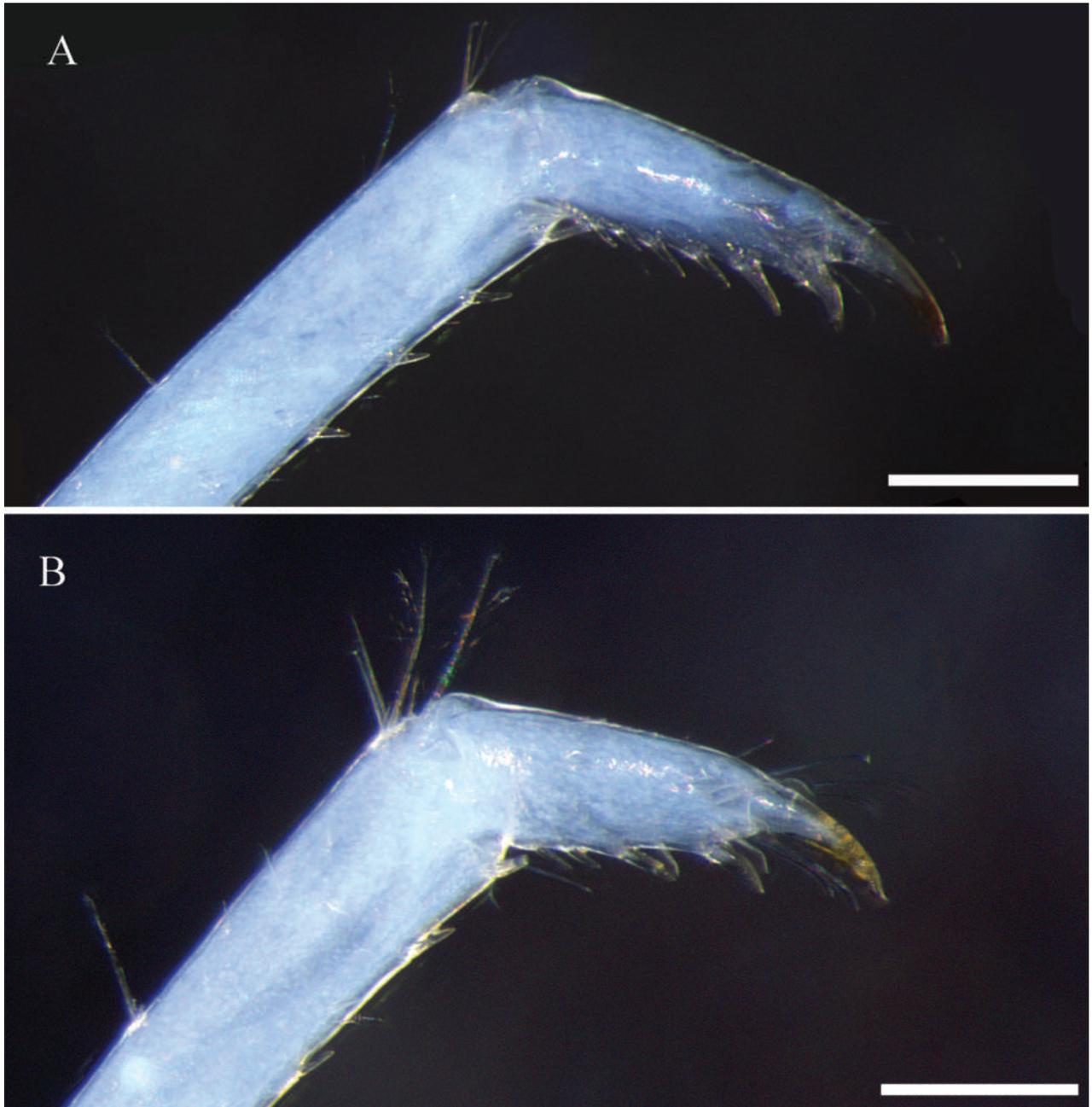
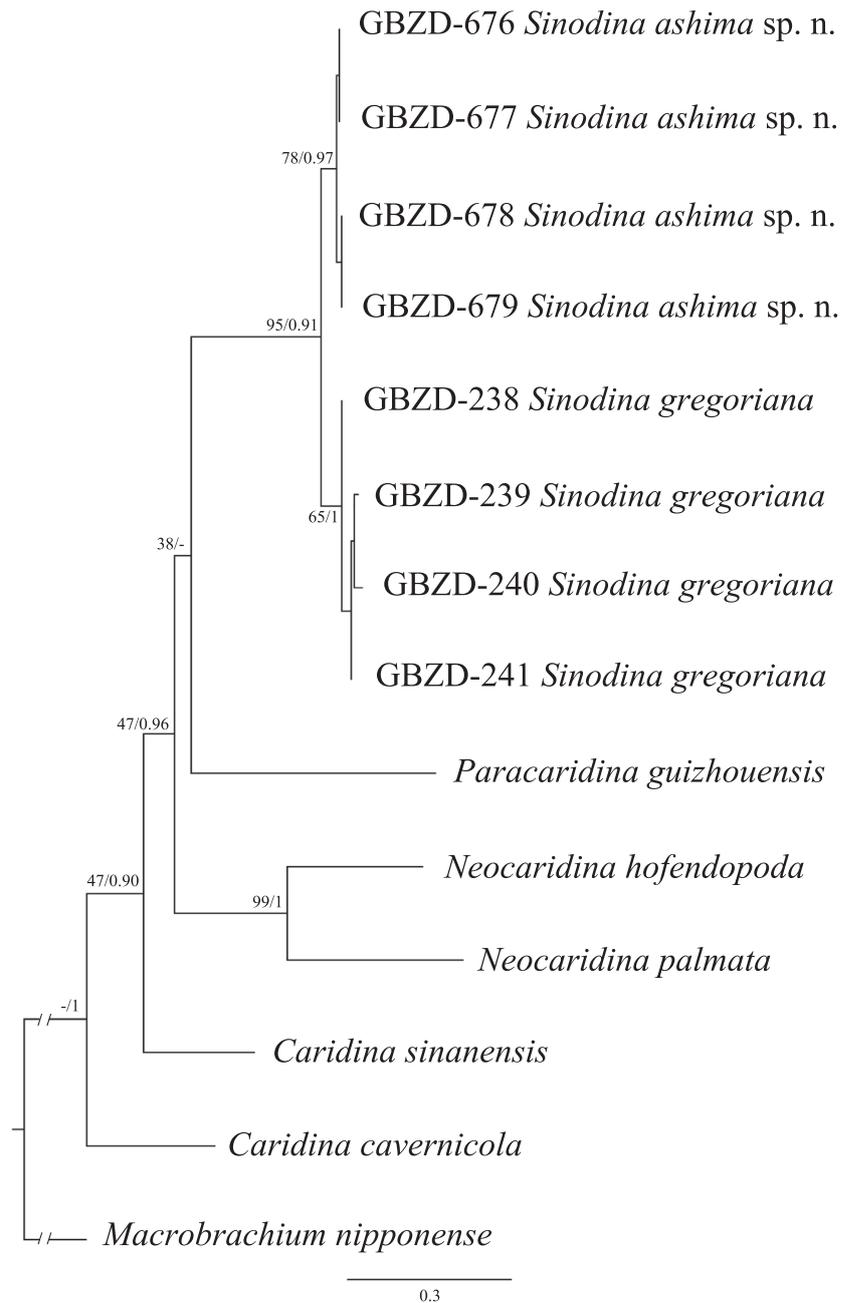


Figure 5. Dactylus of third pereopod **A** holotype **B** female paratype. Scale bars: 0.25 mm.

### Molecular analyses results

The phylogenetic matrix included 14 terminals with 2262 nucleotides (COI: 647 bp; 16S: 453 bp; 18S: 867 bp; H3: 295 bp). The best-fitting evolutionary model for the first codon of COI, 18S and the first and second codons of H3 was TRNEF+I+G. The best model for the second codon of COI was HKY+G. TRNEF+I was the optimal model for the third codon of COI. HKY+I+G and TVM+G suited the 16S and the third codon of H3 respectively.

The only difference between the topologies derived from the ML and BI analyses was the position of *Paracaridina guizhouensis*. It was either sister to *Sinodina* spp. (ML) (Fig. 6) or clustered with the clade of *Sinodina* spp. and *Neocaridina* spp. (BI). Two pairs of sister species received strong support. One



**Figure 6.** ML tree based on the concatenated dataset (COI + 16S + 18S + H3). Numbers at nodes are maximum likelihood percent bootstrap values (left) and Bayesian posterior probabilities (right).

clade showed that the new species, *Sinodina ashima* sp. nov., was clustered with *Sinodina gregoriana* (bootstrap value and posterior probability = 95% and 0.91). Another branch contained the two *Neocaridina* spp. (bootstrap value and posterior probability = 99% and 1).

The COI sequences were successfully obtained from three specimens of *Sinodina ashima* sp. nov., but failed in all specimens of *Sinodina gregoriana*. The intraspecific p-distances of COI of the new species were 0% and 1.85%. Nevertheless, the 16S sequences of all specimens of *Sinodina* spp., but two ones of *Sinodina gregoriana*, have been obtained. No intraspecific variation in 16S of *Sinodina ashima* sp. nov. was detected, and the intraspecific p-distance

of *Sinodina gregoriana* was 0.45%. The interspecific p-distances between *Sinodina ashima* sp. nov. and *Sinodina gregoriana* were 4.55% and 4.32%, between *Sinodina ashima* sp. nov. and *Sinodina* sp. were 2.05% and 2.27%, and between *Sinodina gregoriana* and *Sinodina* sp. was 4.09%.

## Discussion

Some morphological characteristics of *Sinodina* seem to be plesiomorphic. Its simple lamellar podobranch is the same as that of *Caridina* during the metamorphosis from the zoea to the first post-larval stage, without further development (Liang 2004). The distention and spininess on the distal ventral margin of the propodus of the male third and fourth pereopod also appear in the basal genus *Paratya* whose pereopods still possess exopods (Liang and Cai 1999; Liang 2004). Therefore, *Sinodina* is considered to be a relatively basal genus. In a previous phylogeny, *Sinodina* was detected as a sister group to all sampled taxa from China and Japan by three genes, including 16S, 18S and H3 (von Rintelen et al. 2012). However, our preliminary molecular analysis with low support values for the higher-level phylogenetic relationships does not reflect this relationship. To better clarify the taxonomic status and phylogenetic position of this genus, future studies should include more taxa and additional molecular data.

The new species with the simple and lamellar podobranch and its distribution is certainly a member of the genus *Sinodina*. This result is also supported by the phylogenetic analysis, in which *Sinodina ashima* sp. nov. is firmly clustered with the type species *Sinodina gregoriana*. As the first cave-dweller described in the genus, it can be easily distinguished from other species by its degraded body color and eyes. Besides, *Sinodina ashima* sp. nov. is similar to *S. heterodactyla* (Liang & Yan, 1985) and *S. banna* (Cai & Dai, 1999). They all show nearly no sexual dimorphism on the third and fourth pereopod. The new species differs from the two species not only in the stygomorphic traits, but also in the extremely elongated and upturned rostrum, obviously beyond the end of scaphocerite (vs. *S. heterodactyla* reaching the end of scaphocerite and *S. banna* only reaching the end of the first segment of the antennular peduncle); the rostrum with 8–14 teeth ventrally (vs. 5–9 in *S. heterodactyla* and no ventral tooth in *S. banna*); the dactylus of male third pereopod with 4–6 spines (vs. 7 in *S. heterodactyla* and 8–10 in *S. banna*); the body length 23–40 mm (vs. 23–32 mm in *S. heterodactyla* and 14–17 mm in *S. banna*).

Cave organisms in the karst region of south China have a long evolutionary history and high diversity (Li et al. 2022). In the past two decades, the knowledge of the subterranean fauna of China has rapidly increased, making this region a newly emerged world-class diversity hotspot (Deharveng and Bedos 2018). Our research adds a new generic-level taxon to the stygofauna of China. The first subterranean shrimp species from China was described in 1981, *Typhlocaridina lanceifrons* Liang & Yan, 1981, and 13 species have been published in the last century. Entering the 21<sup>st</sup> century, there has been a significant increase in the rate of the discovery of subterranean atyids, with 15 species reported, 11 of which have been published in the last five years. It is believed that as the investigation goes further, more new species and new high-level taxa will be discovered.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

Formal analysis: XJ. Investigation: JZ, XJ. Methodology: HC. Resources: WC. Writing - original draft: XJ. Writing - review and editing: JW, JZ, WC, HC.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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# Revisiting *Szeptyckitheca* Betsch & Weiner (Collembola, Symphypleona, Sminthuridae): new species, updated diagnoses, and a key

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## Abstract

*Szeptyckitheca* Betsch & Weiner is a genus of Sphyrothecinae (Sminthuridae) similar to *Sphyrotheca* Börner, with 13 nominal species. Most descriptions of *Szeptyckitheca* taxa lack valuable data in face of the current taxonomy of Symphypleona. In this study the previously described species of the genus were surveyed, aiming to provide updated diagnoses for them. Three species were also transferred to *Szeptyckitheca*: *Sphyrotheca karlarum* Palacios-Vargas, Vázquez & Cuéllar, 2003, *S. peteri* Palacios-Vargas, Vázquez & Cuéllar, 2003, and *S. koreana* Betsch & Weiner, 2009, based on trochanteral and/or dental chaetotaxy. Two new Brazilian species of the genus are described and illustrated, *S. andrzejki* Medeiros, Bellini & Weiner, **sp. nov.**, with a remarkable reduced ventral dental chaetotaxy not seen in other Neotropical species, and *S. cyanea* Oliveira, Medeiros & Bellini, **sp. nov.** with a distinctive large set of head vertex spines (18). Finally, an updated key to all the valid species of the genus is presented.

**Key words:** Chaetotaxy, Neotropical fauna, *Sphyrotheca* Börner, Sphyrothecinae, taxonomy



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## Introduction

*Szeptyckitheca* Betsch & Weiner, 2009 is the second largest genus of the small subfamily Sphyrothecinae (Sminthuridae) with 13 described species, after *Sphyrotheca* Börner, 1906 with 21 (Bellinger et al. 1996–2023). The genus was erected to gather *S. kesongensis* Betsch & Weiner, 2009 and taxa previously assigned to *Sphyrotheca*, but with a trochanteral spine in the first pair of legs (usually in the trochanter II as well), anterior (ventral) dental chaetotaxy with three or four transversal lines of chaetae (one distal, one or two subdistal and one basal), cephalic chaeta **A1** often absent, and lacking clearly curved rough macrochaetae on frontal area of the head and dorsal large abdomen (Betsch 1980; Betsch and Weiner 2009). Further characteristics are presented ahead in the updated diagnosis of the genus.

*Szeptyckitheca* has a mainly Holotropical distribution, with few species occurring from other regions but under subtropical climates (Bellinger et al. 1996–2023; Betsch and Weiner 2009; Zeppelini et al. 2018). Such distribution is remarkably similar to that of *Sphyrotheca*; however, this last genus also holds a few taxa recorded from more temperate regions of the Holarctic (Bellinger et al. 1996–2023; Bretfeld 1999). As most Symphypleona lineages, the genus and its diagnostic features were never tested under a modern phylogenetic approach (Medeiros et al. 2022; Bellini et al. 2023), so the genus validity is solely based on its morphological description and its listed differences from other Sphyrothecinae, specially *Sphyrotheca*. Even so, as noted by Zeppelini et al. (2018), most species of *Szeptyckitheca* need to be redescribed or their diagnoses should be expanded, since many taxa were poorly described and many features used in the modern taxonomy of Symphypleona are not available for them.

Here we described in detail two new species of *Szeptyckitheca* from Brazil. We surveyed previously described taxa of the genus to present the current state of the knowledge of the group. We also transferred *Sphyrotheca karlarum* Palacios-Vargas, Vázquez & Cuéllar, 2003, *S. peteri* Palacios-Vargas, Vázquez & Cuéllar, 2003, and *S. koreana* Betsch & Weiner, 2009 to *Szeptyckitheca*. With this study we were able to present update diagnoses of *Szeptyckitheca* and its species, provide some taxonomic notes, and an updated identification key for them.

## Materials and methods

The studied specimens of *Szeptyckitheca* were preserved in 70% ethanol at 6 °C, clarified in Nesbitt's solution, washed in Arlé's liquid, and mounted on glass slides in Hoyer's medium, combining procedures outlined by Arlé and Mendonça (1982) and Jordana et al. (1997). Habitus of the new species were photographed using a Leica EC4 camera attached to a Leica S8APO stereomicroscope, under LAS v. 4.12 software. Drawings were firstly made under a Leica DM750 microscope with a drawing tube, and photographs of the structures were taken with a Leica MC170 HD camera under LAS v. 4.12 software. Final figures were prepared with CorelDraw 2022 software.

For our survey of the previously described *Szeptyckitheca* species, we consulted the original descriptions as well as the redescription of *S. santiagoi* (Yosii, 1959) by Lawrence (1968). For the species distributions, we also checked Bellinger et al. (1996–2023), Mari-Mutt and Bellinger (1990, 1996), Mari-Mutt et al. (1997–2021), and Greenslade (2023).

The terminology used in the diagnoses and descriptions follows Cipola et al. (2014) for the labral chaetotaxy; Fjellberg (1999) for the labial palp; Betsch and Waller (1994) for the head and anterior large abdomen chaetotaxy; Vargovitsh (2009, 2012, 2013) for the posterior large abdomen chaetotaxy, with adaptations; and Betsch (1997) for the small abdomen chaetotaxy. Drawings and observations of the new species were made based on the entire type series.

Abbreviations used in the descriptions and figures are: **Abd.** –abdominal segment(s); **Ant.** –antennal segment(s); **mac** –macrochaeta(e); **Th.** –thoracic segment(s).

On the figures, chaetae present or absent are marked with white arrows; unpaired chaetae on head and trunk are marked with an asterisk, \*. Ant. IV subsegments are counted from the base to the apex. The head vertex was

considered as the frontal and interantennal areas sensu Betsch and Waller (1994) combined. Dens dorsal chaetae were considered as the dorsal, internal, and external chaetae rows combined. We considered as spines the strong and mostly stiff, pointed, blunt, or capitate/knobbed modified chaetae seen in the head vertex, trochanters, and femur I (the curved chaeta), following Bretfeld (1999) and Betsch and Weiner (2009).

Head, trunk (thorax + abdomen), and furca chaetotaxy are given by half body, with the exception of head vertex chaetae which are listed as their total number. Chaetae labels are marked in bold.

The type series of the new species are deposited at the Collembola Collection of the Biosciences Center of the Federal University of Rio Grande do Norte (CC/UFRN), Brazil.

## Results

### Taxonomic account

**Order Symphypleona Börner, 1901 sensu Bretfeld, 1986**

**Suborder Appendiciphora Bretfeld, 1986**

**Superfamily Sminthuroidea Bretfeld, 1994**

**Family Sminthuridae Lubbock, 1862**

**Subfamily Sphyrothecinae Betsch 1980**

***Szeptyckitheca* Betsch & Weiner, 2009**

**Genus diagnosis.** Cuticle granulation rough. Specimens pigmented, eyepatches dark. Ant. IV with 8–12 subsegments. Eyes 8+8, head without vesicles or tubercles, eyepatches with 0–2 interocular chaetae, head vertex with a total of 4–18 strong erect large spines, cephalic chaeta **A1** present or absent. Large abdomen without mesothoracic vesicles, dorsally with spine-like, blunt, or curved mac, adults with bothriotricha **A–D**, parafurcal area with 1+1 neosminthuroid chaetae. Small abdomen without bothriotrichum **E**, female subanal appendage short (not reaching the apex of the ventral anal valves), long (surpassing the ventral anal valves) or very long (surpassing the dorsal anal valve). Trochanteral spines present on legs I and III, sometimes on leg II as well, ungues usually with the internal tooth, pseudonychia and tunica. Manubrium with six or seven dorsal chaetae. Ventral (anterior) dental chaetotaxy with two to four transversal rows of chaetae, with the following resumed chaetotaxy formula from the apex to the base: 4–2,2–0,2–0...1, dorsal dens with 13–24 chaetae. Mucro without chaeta, with an apical notch (data based on Denis 1948; Yossii 1959, 1965, 1966; Delamare-Deboutteville and Massoud 1964a, b; Betsch 1965; Hüther 1967; Lawrence 1968; Snider 1978; Bretfeld 2005; Betsch and Weiner 2009; Zeppelini et al. 2018).

**Type species.** *Szeptyckitheca kesongensis* Betsch & Weiner, 2009.

**Remarks.** The closest genus to *Szeptyckitheca* is *Sphyrotheca*, and many features in their diagnoses overlap (Betsch and Weiner 2009). They share a subsegmented Ant. IV, Th. II without vesicles, the presence of bothriotrichum **D** in adult specimens, and the trochanteral spine in leg III (Bretfeld 1999; Betsch and Weiner 2009). Here we considered as the main unequivocal diagnostic feature to separate both genera the presence of at least one trochanteral spine

in leg I of *Szeptyckitheca* (absent in *Sphyrotheca*) (Betsch and Weiner 2009). Other features like the reduction of the ventral dens chaetotaxy and presence of **A1** cephalic chaeta vary within *Szeptyckitheca*, as well as the presence of curved mac on dorsal head and large abdomen of *Sphyrotheca*, and cannot be used alone to separate the genera (Tables 1, 2; see also the Discussion).

**Table 1.** Main features of *Szeptyckitheca* species from the Americas.

Species / features	<i>S. andrzeji</i> sp. nov.	<i>S. cyanea</i> sp. nov.	<i>S. bellingeri</i>	<i>S. kac</i>	<i>S. karlarum</i>	<i>S. mucroserrata</i>	<i>S. peteri</i>	<i>S. vanderdrifti</i>
Trunk color pattern	pinkish, with purple dorsal spots	dark bluish	dorsally dark brownish with lateral sides bluish	with dark spots	♂ pale bluish with purple spots and stripes, ♀ yellowish with brown spots and stripes	with lateral weak purple bands, posteriorly purple	♂ pale bluish with purple spots, ♀ yellowish with brown spots	yellowish with indistinct areas of pigment, or brownish
Ant. IV subsegments	11	11	8	9	9–10	9–10	9–10	~10
Ant. III chaetae	19	21**	18	14	19	?	17?	19
Ant. II chaetae	13	15	14?	13	15	?	12	14
Ant. II long chaetae	3	4	3	3	2	?	2	2–3
Ant. I chaetae	6	6	5?	7	6	?	4?	6
Interocular chaetae	-	2	2	-	2	2	1	2
Frontal head <b>A1</b>	-	+	-	-	-	-	-	-
Head vertex large spines (total)	14	18	14	16	16	16	16	16
Head vertex rough spines	-	-	-?	-?	+	+	+	-
Head frons sec. reduced chaetae	+	-	+	+	+	-?	+	+
Head interantennal bifid chaetae (♀)	-	-	-	-	-	-	-	-
Head clypeal f line mac	-	-	-	4	-	-	-	-
Tr. I n° of spines	2	2	2	1	1	?	1	1
Tr. I spine(s) apex shape	capitate	capitate	acuminate	capitate	blunt	?	?	acuminate
Tr. II spine apex shape	blunt	capitate	blunt	knobbed	-	?	knobbed	acuminate?
Tr. III spine apex shape	blunt	blunt	blunt	blunt	blunt	blunt	blunt	capitate
Tr. III regular chaetae	5	5	5	3	5	5	5	4
Tr. III oval organs	1	1	-?	-	1	+	1	-?
Femur I spine	+(1)	+(1)	+(1)	+(1)	+(1)	?	+(1)	?
Ungues inner tooth	+	+	+	+	+	+	+	+
Ungual tunica	+	+	+	+	+	+	+	+
Ungual pseudonychia	weak	weak	strong	weak	+	-	+	strong
Unguiculus I internal tooth	-	+	-	+	+	-	+	+
Unguiculus III apical filament	thin	thin	thick	thin	thin	thin	thin	thin
Unguiculus III filament length compared to unguis III	=	<	>	=	<	=	>	<
Large abdomen capitate mac	+	+	-	+	+	-	+	-
Female anal valve <b>m<sub>ps1</sub></b> winged	-	-	-	-	-	-	-	-
Female subanal appendage shape	short, spoon-like, apically serrated	long, slightly curved at the apex, unilaterally serrated	long, slightly curved at the apex, smooth or unilaterally serrated	long, blunt, apically serrated	long, spatulated, serrated at the tip	long, spatulated or acuminate, serrated at the tip	long, spatulated, serrated at the tip	long, acuminate, apically or almost entirely serrated
Manubrial chaetae	7	7	6	?	?	7	?	?
Dens ventral chaetotaxy	3...1	3,2...1	2,2...1	4,1...1	3,2...1	3,2...1 <sup>§</sup>	3,2...1	2,2...1
Dens dorsal chaetae	17	16	17	15	12	14 <sup>§</sup>	17	16
Prominent mucronal notch	-	+	+	+	-	-	-	-

Data based on the original descriptions. Legends: 'sec.' secondarily, 'Tr.' trochanter; '+' present; '-' absent; '=' subequal; '>' surpassing the tip of unguis; '<' not reaching the tip of unguis; '?' unknown, unclear data; '\*' described as mesochaetae; '\*\*' = including the peculiar small sensilla on cavities outside the apical organ; '§' = the proximal chaeta portrayed in the ventral dens (Snider 1978: 235, fig. 207) is lateral and large, and does not correspond to the typical reduced proximal ventral chaeta seen in other species of the genus. Due to position and size, we believe this chaeta was represented in the dorsal dens (Snider 1978: 235, fig. 208).

**Table 2.** Main features of *Szeptyckitheca* species from the Old World.

Species / features	<i>S. boneti</i>	<i>S. coerulea</i>	<i>S. formosana</i>	<i>S. implicata</i>	<i>S. kesongensis</i>	<i>S. koreana</i>	<i>S. machadoi</i>	<i>S. nepalica</i>	<i>S. santiagoi</i>	<i>S. spinimicronata</i>
Trunk color pattern	dorsally black or purplish-red	bluish with white spots	dorsally yellowish with lateral sides purplish	dark yellowish with purple stripes and spots	pale	lateral large abdomen violet	with transversal stripes and spots of dark pigment	pale, with diffused pigment and violet patches	variable, usually laterally dark and dorsally pale	whitish, with diffused fields of purple pigment
Ant. IV subsegments	10–12	9–10	9–10	11	10	9	10	10	10	~10
Ant. III chaetae	24*	?	?	?	23	23	21*	?	15?	?
Ant. II chaetae	?	15–16	?	>8	16	16	15*	?	15?	?
Ant. II long chaetae	?	-?	?	-	-	-	4	-?	1	?
Ant. I chaetae	?	7	4?	5	7	7	5?	?	5	?
Interocular chaetae	2	2	-?	1	2	2	2?	-?	1	-?
Frontal head A1	+	+	+	+	-	+	+	+	-	+
Head vertex large spines (total)	10	6	15	16	16	4****	16	11	14 or 16	16
Head vertex rough spines	-?	-	+	+	+/-	+/-	-?	-?	+?	+
Head frons sec. reduced chaetae	+	-	-?	-	-	+	-	-?	+?	-
Head interantennal bifid chaetae (♀)	-	-	-	-	+	-	-	-	-	-
Head clypeal f line mac	-	-	-	-	-	-	-	-	-	-
Tr. I n° of spines	?	1	?	1	1	1	1	1	1	?
Tr. I spine apex shape	?	blunt	?	blunt	capitate	capitate	blunt	blunt	blunt	?
Tr. II spine apex shape	?	blunt	?	blunt	capitate	capitate	-	blunt	blunt	?
Tr. III spine apex shape	?	blunt	?	blunt	capitate	capitate	blunt	blunt	blunt	blunt
Tr. III regular chaetae	?	5	?	5	5	4	4	?	5	5
Tr. III oval organs	?	-?	?	-?	2	2	-	?	?	-?
Femur I spine	?	+(1)	?	?	+(1)	+(1)	+(1)	?	+(2)	?
Ungues inner tooth	+	+	+	+	+	+	+	+	-	+
Ungual tunica	-**	+	+	+	+	+	+	+	+	+
Ungual pseudonychia	weak	strong	-	weak	strong	strong	strong	strong	weak	-
Unguiculus I internal tooth	+	-	+	+	-	+	+	+/-	+/-	-
Unguiculus III apical filament	thin	thin	thin	thin	thin	thin	thin	thin?	thin	thin
Unguiculus III filament compared to unguis III	<	<	<	<	<	=	>	<	=	<
Large abdomen capitate mac	?	+	-	+	-	-	?	-	-	-
Female anal valve mps1 winged	-	-	-	-	+	-	-	-	-	-
Female subanal appendage shape	very long, acuminate, serrated at the middle	long, acuminate, unilaterally serrated	long, blunt, apically serrated	long, acuminate, apically serrated	long, acuminate, apically serrated	long, blunt, apically unilaterally serrated	long, spatulate, apically serrated	long, acuminate, apically serrated	long, with a bidentate apex, smooth or apically serrated	long, acuminate, apically serrated
Manubrial chaetae	?	7	7	7	?	?	?	6	?	7
Dens ventral chaetotaxy	3,2,2...1 or 3,2...1	4,2...1	3,1...1	2,2...1	3,2...1	3...1 or 2...1	2,2...1	3,2...1	2,2...1***	3,2...1
Dens dorsal chaetae	17–18	16	15	19	20	17	24	13	13***	17
Prominent mucronal notch	+	+	+	+	+	+	+	+	+	+

Data based on the original descriptions, with exception of *S. santiagoi*, which was based on Yosii (1959) and Lawrence (1968). Legends: 'sec.' secondarily, 'Tr.' trochanter; '+' present; '-' absent; '~' about; '=' subequal; '>' surpassing the tip of unguis; '<' not reaching the tip of unguis; '?' unknown, unclear data; '\*' = including the peculiar small sensilla on cavities outside the apical organ; '\*\*' = unguis I and II without tunica, unguis III possibly with a small distal rudiment; '\*\*\*' = we considered dental chaetae as described by Yosii (1959), since Lawrence's (1968) redescription is imprecise regarding this feature and may be mistaken; '\*\*\*\*' = the large head vertex spines of *S. koreana*, comb. nov. are mixed with large curved rough mac.

In our survey and the descriptions of the new species we observed some species hold reduced spines on the frontal head. We considered such reduction as secondary, since other taxa of the genus present all frontal head chaetae well developed, a feature also seen in other genera and families of Symphypleona (Betsch 1980; Bretfeld 1999).

## Species diagnoses

### ***Szeptyckitheca bellingeri* (Betsch, 1965)**

*Sphyrotheca bellingeri* Betsch, 1965: 444.

**Diagnosis.** Yellowish ground, head with dark blue bands dorsally in a V-shape, dorsal trunk dark brownish with bronze, bluish or yellowish effects, other regions including appendages bluish. Ant. IV with 8 subsegments; Ant. III with 18 chaetae other than the sensory clubs; Ant. II with three chaetae clearly longer than the others. Eyepatches with two interocular chaetae modified into strong spines each. Head vertex with a total of 14 large spines, two of them unpaired; unpaired chaeta **A1** absent; secondarily reduced chaeta near the spines present. Trochanters I–III with 2,1,1 spines, respectively, trochanter I spines acuminate, trochanters II and III spines blunt; trochanter III with five regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I without the internal tooth; unguiculus III filament thick and surpassing the tip of the unguis III. Large abdomen lacking capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), slightly curved at the apex (hook-like), smooth or with the external border serrated. Manubrium with 6+6 dorsal chaetae; dens ventral chaetotaxy formula from the apex to the base as: 2,2...1, dorsal chaetotaxy with 17 chaetae; mucronal notch prominent (adapted from Betsch 1965).

**Remarks.** *Szeptyckitheca bellingeri* is the sole species of the genus with eight subsegments of the Ant. IV, while all others have nine or more. It also shares with the two new described species a pair of trochanteral spines on leg 1, a feature not reported in any other taxon of the genus (see Tables 1, 2).

**Habitat.** Specimens were found on mosses and liverworts growing on logs and stony ground, over the vegetation, litter layer, dead branches and directly upon the soil (Betsch 1965).

**Known distribution.** Jamaica (Betsch 1965).

### ***Szeptyckitheca boneti* (Denis, 1948)**

*Sminthurus Boneti* [sic] Denis, 1948: 298.

**Diagnosis.** Dorso-posterior head and dorsal trunk blackish to purplish-red, legs and antennae weakly pigmented, ventral side pale. Ant. IV with 10–12 subsegments; Ant. III with 24 chaetae other than the sensory clubs, including two peculiar small sensilla within cavities. Eyepatches with two regular interocular chaetae each. Head vertex with a total of ten large spines, two of them unpaired; unpaired chaeta **A1** present; secondarily reduced chaetae near the spines present. Ungues with a single inner tooth, without tunica (unguis III

possibly with a small distal rudiment of tunica) and with a weakly developed pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Female with a very long subanal appendage (surpassing the dorsal anal valve), acuminate, feathered (serrated) at the middle region on its both edges. Dens ventral chaetotaxy formula from the apex to the base as: 3,2...1 or 3,2,2...1, dorsal chaetotaxy with 17 or 18 chaetae; mucronal notch prominent (adapted from Denis 1948).

**Remarks.** *Szeptyckitheca boneti* is unique compared to all its congeners by the very long subanal appendages of the females, surpassing the dorsal anal valves. However, we could not find any data on the species legs chaetotaxy, especially the presence and shape of the trochanteral spines. Apparently the species was revised by Betsch (Betsch 1980; Betsch and Weiner 2009) before its inclusion in the genus, but it is in need of redescription, since many important data on its morphology are unknown (see Table 2).

**Habitat.** Specimens were found in bushes (Denis 1948).

**Known distribution.** Vietnam (Denis 1948).

### ***Szeptyckitheca coerulea* (Bretfeld 2005)**

*Sphyrotheca coerulea* Bretfeld, 2005: 32.

**Diagnosis.** Head and body almost completely blue, with pale spots. Ant. IV with nine or ten subsegments; Ant. II with 15 or 16 chaetae; Ant. I with seven chaetae. Eyepatches with two regular interocular chaetae each. Head vertex with a total of six large smooth spines, none of them unpaired; unpaired chaeta **A1** present; secondarily reduced chaetae near the spines absent. Trochanters I–III with 1,1,1 spines, respectively, all blunt; trochanter III with five regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I without the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen dorsally with several capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), acuminate, apically serrated on its internal face. Manubrium with 7+7 dorsal chaetae; dens ventral chaetotaxy formula from the apex to the base as: 4,2...1, dorsal chaetotaxy with 16 chaetae; mucronal notch prominent (adapted from Bretfeld 2005).

**Remarks.** *Szeptyckitheca coerulea* is the only species of the genus with six spines on the head vertex. Further data on the species are presented in Table 2.

**Habitat.** Specimens were found on shrubs and grasses near a stream of water (Bretfeld 2005).

**Known distribution.** Yemen (Socotra Island) (Bretfeld 2005).

### ***Szeptyckitheca formosana* (Yosii, 1965)**

*Sphyrotheca formosana* Yosii, 1965: 49.

**Diagnosis.** Yellowish ground, antennae diffusely pigmented, head almost pale, large abdomen laterally purplish, dorsally pale. Ant. IV with nine or ten subsegments. Head vertex with a total of 15 large spines, three of them

unpaired; unpaired chaeta **A1** present. Ungues with a single inner tooth, with tunica and without pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen without capitate chaetae. Female's subanal appendage long (surpassing the ventral anal valves), blunt, apically serrated on its external face. Dens ventral chaetotaxy formula from the apex to the base as: 3,1...1, dorsal chaetotaxy with 15 chaetae; mucronal notch prominent (adapted from Yosii 1965).

**Remarks.** Like *S. boneti*, *S. formosana* was apparently revised by Betsch (Betsch 1980; Betsch and Weiner 2009). Nevertheless, many important data on its morphology are still lacking (see Table 2), and it is in need of redescription.

**Habitat.** Unknown.

**Known distribution.** Taiwan (Yosii 1965).

### ***Szeptyckitheca implicata* (Hüther, 1967)**

*Sphyrotheca implicata* Hüther, 1967: 252.

**Diagnosis.** Dark yellowish ground, with dark purple spots and two longitudinal stripes on the dorsal large abdomen, appendages pale purple. Ant. IV with 11 subsegments; Ant. II with more than eight chaetae, none of them clearly longer than the others; Ant. I with five chaetae. Eyepatches with one regular interocular chaeta each. Head vertex with a total of 16 large spines, two of them unpaired; unpaired chaeta **A1** present; secondarily reduced chaetae near the spines absent. Trochanters I–III with 1,1,1 spines, respectively, all blunt; trochanter III with five regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and weak pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen dorsally with several capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), acuminate, apically serrated. Manubrium with 7+7 chaetae, dens ventral chaetotaxy formula from the apex to the base as: 2,2...1, dorsal chaetotaxy with 19 chaetae; mucronal notch prominent (adapted from Hüther 1967).

**Habitat.** Specimens were found associated to bushes (Hüther 1967).

**Known distribution.** South Sudan (Hüther 1967).

### ***Szeptyckitheca kac* Zeppelini, Lopes & Lima, 2018**

*Szeptyckitheca kac* Zeppelini, Lopes & Lima, 2018: 3.

**Diagnosis.** Trunk with dark spots of pigment, mucro and legs less pigmented. Ant. IV with nine subsegments; Ant. III with 14 chaetae other than the sensory clubs; Ant. II with 13 chaetae, three of them clearly longer than the others; Ant. I with seven chaetae; some chaetae of Ant. II–IV capitate. Eyepatches lacking interocular chaetae. Head vertex with a total of 16 large spines, two of them unpaired; unpaired chaeta **A1** absent; secondarily reduced chaetae near the spines present. Clypeal area with 4+4 mac near the antennae. Trochanters I–III with 1,1,1 spines, respectively, trochanter I spine capitate, II knobbed and

III blunt; trochanter III with three regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and weak pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and reaching the tip of the unguis III. Large abdomen dorsally with several capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), blunt, apically serrated on its internal face. Dens ventral chaetotaxy formula from the apex to the base as: 4,1...1, dorsal chaetotaxy with 15 chaetae; mucronal notch prominent (adapted from Zeppelini et al. 2018).

**Remarks.** *Szeptyckitheca kac* is unique within the genus due the presence of 4+4 modified mac on the upper clypeus (**f** line) and the dens ventral chaetotaxy formula of 4,1...1 chaetae. Further data on the species are presented in Table I.

**Habitat.** Specimens were found in the canopy of rainforest (Zeppelini et al. 2018).

**Known distribution.** Brazil (Zeppelini et al. 2018).

***Szeptyckitheca karlarum* (Palacios-Vargas, Vázquez & Cuéllar, 2003), comb. nov.**

*Sphyrotheca karlarum* Palacios-Vargas, Vázquez & Cuéllar, 2003: 303–306, 308, figs 4–6, Mexico, Quintana Roo, Reserva de la Biosfera de Sian Ka’na (orig. descr.).

**Diagnosis.** Males pale bluish, with purple or blue spots on antennae, dorsal head and furca, dorsal trunk striped; females yellowish, with brown spots and stripes with the same distribution of males. Ant. IV with nine or ten subsegments, with some proximal chaetae capitate; Ant. III with 19 chaetae other than the sensory clubs, 4–6 of them longer than the others and capitate; Ant. II with 15 chaetae, two of them clearly longer than the others, two of them modified into spines; Ant I with six chaetae. Eyepatches with two interocular chaetae each, one of them modified into a spine. Head vertex with a total of 16 large and rough spines, two of them unpaired; unpaired chaeta **A1** absent; secondarily reduced chaetae near the spines present. Trochanters I–III with 1,0,1 spines, respectively, trochanters I and III spines blunt; trochanter III with five regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen with capitate chaetae. Female with a long subanal appendage (slightly surpassing the ventral anal valves), spatulated, apically serrated on both faces. Dens ventral chaetotaxy formula from the apex to the base as: 3,2...1, dorsal chaetotaxy with 12 chaetae; mucronal notch discrete (adapted from Palacios-Vargas et al. 2003).

**Remarks.** *Sphyrotheca karlarum* is herein transferred to *Szeptyckitheca* due to the presence of robust and mostly erect spines on the head vertex and dorsal large abdomen, presence of three transversal rows of dental ventral chaetae and presence of spines on trochanters I and III, features used by Betsch and Weiner (2009) to separate *Szeptyckitheca* from *Sphyrotheca*.

**Habitat.** Specimens were found in low flooded jungle (Palacios-Vargas et al. 2003).

**Known distribution.** Mexico (Palacios-Vargas et al. 2003).

### ***Szeptyckitheca kesongensis* Betsch & Weiner, 2009**

*Szeptyckitheca kesongensis* Betsch & Weiner, 2009: 40.

**Diagnosis.** Specimens pale or very clear. Ant. IV with ten subsegments; Ant. III with 23 chaetae other than the sensory clubs; Ant. II with 16 chaetae, none of them clearly longer than the others; Ant. I with seven chaetae. Eyepatches with two regular interocular chaeta each. Head vertex with a total of 16 large spines, two of them unpaired; unpaired chaeta **A1** absent; secondarily reduced chaetae near the spines absent. Head interantennal area with 1+1 bifid chaetae. Trochanters I–III with 1,1,1 spines, respectively, all capitate; trochanter III with five regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I without the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen without capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), acuminate, apically serrated on both edges. Dens ventral chaetotaxy formula from the apex to the base as: 3,2...1, dorsal chaetotaxy with 20 chaetae; mucronal notch prominent (adapted from Betsch and Weiner 2009).

**Remarks.** *Szeptyckitheca kesongensis*, the type species of the genus, shows a very peculiar head morphology, with the female bearing bifid chaetae in the interantennal area, while the male lack such morphology (Betsch and Weiner 2009). Although only one couple of specimens was analyzed for this description, no other *Szeptyckitheca* species has such a sexual dimorphism, including the species herein described.

**Habitat.** Specimens were found associated to *Robinia* sp., shrubs, pines, and plant debris (Betsch and Weiner 2009).

**Known distribution.** North Korea (Betsch and Weiner 2009).

### ***Szeptyckitheca koreana* (Betsch & Weiner, 2009), comb. nov.**

*Sphyrotheca koreana* Betsch & Weiner, 2009: 36–39, figs 1–13, North Korea, Kaesong-si province, Chonma-sun Mountains (orig. descr.).

**Diagnosis.** Pale background, with antennae, frontal area (interocular field) and lateral sides of large abdomen violet. Ant. IV with nine subsegments; Ant. III with 23 chaetae other than the sensory clubs; Ant. II with 16 chaetae, none of them clearly longer than the others; Ant. I with seven chaetae. Eyepatches with two regular interocular chaetae each. Head vertex with a total of four large erect spines, plus four short spines and nine rough curved mac, three of them unpaired; unpaired chaeta **A1** present; secondarily reduced chaetae near the spines present. Trochanters I–III with 1,1,1 spines, respectively, all capitate; trochanter III with four regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and reaching the tip of the unguis III. Large abdomen without capitate mac, but with rough curved mac. Female with a long subanal appendage (surpassing the ventral anal valves), blunt, apically serrated on its internal edge. Dens ventral chaetotaxy formula from the apex to the base as: 3...1 or 2...1, dorsal chaetotaxy with 17 chaetae; mucronal notch prominent (adapted from Betsch and Weiner 2009).

**Remarks.** *Sphyrotheca koreana* is herein transferred to *Szeptyckitheca* due to the presence of one spine on trochanters I–III. This feature, which was listed as diagnostic of the later genus by Betsch and Weiner (2009), was overlooked in the original paper possibly due to the overall morphology of the species, which, disregarding the trochanteral chaetotaxy, better matches *Sphyrotheca*. This includes the presence of large rough curved chaetae on the head vertex and dorsal large abdomen and only two transversal lines of ventral dens chaetae (Betsch and Weiner 2009). However, since we observed some species of *Szeptyckitheca* with reduced ventral dens chaetotaxy, and taxa listed as *Sphyrotheca* species without the dorsal rough curved mac, we provisionally transfer *Sphyrotheca koreana* to *Szeptyckitheca*. Nevertheless, this intermediate species placement should be better investigated in the light of modern phylogenetic methods to better understand its position within the Sphyrothecinae. Further data on the boundaries of *Szeptyckitheca* and *Sphyrotheca* are discussed ahead in the text.

**Habitat.** Specimens were found in forested areas (Betsch and Weiner 2009).

**Known distribution.** North Korea (Betsch and Weiner 2009).

#### ***Szeptyckitheca machadoi* (Delamare-Deboutteville & Massoud, 1964)**

*Sminthurotheca machadoi* Delamare-Deboutteville & Massoud, 1964a: 80.

**Diagnosis.** Specimens with transversal stripes and spots of dark pigment. Ant. IV with ten subsegments; Ant. III with 21 chaetae other than the sensory clubs, two of them as small sensilla in individual cavities; Ant. II with 15 chaetae, one of them as a small sensillum in cavity, four of the regular chaetae clearly longer than the others. Head vertex with a total of 16 large spines, two of them unpaired; unpaired chaeta **A1** present; secondarily reduced chaetae near the spines absent. Trochanters I–III with 1,0,1 spines, respectively, trochanters I and III spines blunt; trochanter III with four regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and surpassing the tip of the unguis III. Female with a long subanal appendage (surpassing the ventral anal valves), spatulated, apically serrated on both edges. Dens ventral chaetotaxy formula from the apex to the base as: 2,2...1, dorsal chaetotaxy with 24 chaetae; mucronal notch prominent (adapted from Delamare-Deboutteville and Massoud 1964a).

**Remarks.** The genus *Sminthurotheca* Delamare-Deboutteville & Massoud, 1964 was erected based on a supposedly unique combination of Ant. III and large abdomen chaetotaxy. It was posteriorly synonymized with *Sphyrotheca* by Betsch (1980), due to the overlapping morphology of both genera. Later, *Sphyrotheca machadoi* was transferred to *Szeptyckitheca* by Betsch and Weiner (2009), especially due to the presence of a spine on the trochanter I and the ventral dens chaetotaxy with three whorls of chaetae. Further data on the species are presented in Table 2.

**Habitat.** Specimens were found in gallery forests, in plant debris (Delamare-Deboutteville and Massoud 1964a).

**Known distribution.** Angola, Congo (Delamare-Deboutteville and Massoud 1964a).

### ***Szeptyckitheca mucroserrata* (Snider, 1978)**

*Sphyrotheca mucroserratus* Snider, 1978: 236.

**Diagnosis.** Antennae, legs, and furca purplish, head purplish near the mouth, with purple bands between the antennae and on its vertex, trunk laterally with pale purple bands, posterior abdomen purplish. Ant. IV with nine or ten subsegments. Eyepatches with two interocular chaetae modified into strong spines each. Head vertex with a total of 16 large spines, two of them unpaired; unpaired chaeta **A1** absent. Trochanter III spine blunt, with five extra regular chaetae. Ungues with a single inner tooth, with tunica but lacking pseudonychia; unguiculus I without the internal tooth; unguiculus III filament thin and reaching the tip of the unguis III. Large abdomen lacking capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), spatulated or acuminate, serrated at the tip. Manubrium with 7+7 dorsal chaetae; dens ventral chaetotaxy formula from the apex to the base as: 3,2...1, dorsal chaetotaxy with 14 chaetae; mucronal notch discrete (adapted from Snider 1978).

**Remarks.** The short description of Snider (1978) of *Sphyrotheca mucroserratus* limits the comparison of this species with its congeners. It lacks many important useful taxonomical features (see Table 1), including some recognized as diagnostic of the genus, like the presence of the trochanter I spine and even the pair of neosminthuroid chaetae which are diagnostic of the Sphyrothecinae. The wide distribution of the species, recorded from all Americas, combined with its generic and imprecise diagnosis, support the hypothesis that the name *Szeptyckitheca mucroserrata* has possibly been used to circumscribe a complex of species. In this scenario its redescription is urgent, to improve the comprehension of the morphology and distribution of *Szeptyckitheca* species from the New World.

**Habitat.** Specimens listed in the original description were found associated with Australian pine needles, leaf mold, and forest debris in Florida, USA (Snider 1978). In Brazil, specimens of *S. mucroserrata* were found associated to forest litter and on sand dunes (Abrantes et al. 2012).

**Known distribution.** Brazil, Mexico, and USA (Snider 1978; Mari-Mutt and Bellinger 1996; Abrantes et al. 2012).

### ***Szeptyckitheca nepalica* (Yosii, 1966)**

*Sphyrotheca nepalica* Yosii, 1966: 527.

**Diagnosis.** Pale ground, body diffusely pigmented with brownish violet patches between the eyes and lateral sides of the large abdomen, antennae distally dark pigmented. Ant. IV with ten subsegments. Head vertex with a total of 11 large spines, three of them unpaired, including chaeta **A1**. Trochanters I–III with 1,1,1 spines, respectively, all blunt. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I with or without the internal tooth; unguiculus III filament not reaching the tip of the unguis III. Large abdomen without capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), acuminate, apically serrated on both edges. Manubrium with 6+6

dorsal chaetae; dens ventral chaetotaxy formula from the apex to the base as: 3,2...1, dorsal chaetotaxy with 13 chaetae; mucronal notch prominent (adapted from Yosii 1966).

**Remarks.** *Szeptyckitheca nepalica* is the only species of the genus with 11 spines on head vertex. Although the species fits *Szeptyckitheca*, especially due to the presence of the spines on trochanters I–III, its description is quite limited considering the current taxonomy of Symphypleona (see Table 2), and the species needs a formal redescription, as already noted by Betsch and Weiner (2009).

**Habitat.** Unknown.

**Known distribution.** Nepal (Yosii 1966).

### ***Szeptyckitheca peteri* (Palacios-Vargas, Vázquez & Cuéllar, 2003), comb. nov.**

*Sphyrotheca peteri* Palacios-Vargas, Vázquez & Cuéllar, 2003: 298–302, figs 1–3, Mexico, Quintana Roo, Reserva de la Biosfera de Sian Ka'na (orig. descr.).

**Diagnosis.** Males pale bluish, with purple or blue spots on antennae, dorsal head, dorsal trunk and furca, females yellowish, with brown spots with the same distribution of males. Ant. IV with nine or ten subsegments, with some proximal chaetae capitate; Ant. II with 12 chaetae, two of them clearly longer than the others. Eyepatches with one interocular chaeta each. Head vertex with a total of 16 large and rough spines, two of them unpaired; unpaired chaeta **A1** absent; secondarily reduced chaetae near the spines present. Trochanters I–III with 1,1,1 spines respectively, trochanter II spine knobbed and III blunt; trochanter III with five regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and surpassing the tip of the unguis III. Large abdomen with capitate chaetae. Female with a long subanal appendage (surpassing the ventral anal valves), spatulated, apically serrated on both faces. Dens ventral chaetotaxy formula from the apex to the base as: 3,2...1, dorsal chaetotaxy with 17 chaetae; mucronal notch discrete (adapted from Palacios-Vargas et al. 2003).

**Remarks.** *Sphyrotheca peteri* is herein transferred to *Szeptyckitheca* due to the presence of robust and somewhat erect spines on the head vertex and dorsal large abdomen, presence of three transversal rows of dental ventral chaetae and presence of spines on all trochanters, all features originally listed by Betsch and Weiner (2009) as diagnostic of *Szeptyckitheca*.

**Habitat.** Specimens were found in low flooded jungle (Palacios-Vargas et al. 2003).

**Known distribution.** Mexico (Palacios-Vargas et al. 2003).

### ***Szeptyckitheca santiagoi* (Yosii, 1959)**

*Sphyrotheca santiagoi* Yosii, 1959: 58; Lawrence 1968: 333.

**Diagnosis.** Color pattern variable, usually mostly dark with a pale dorsum. Ant. IV with ten subsegments; Ant. III with at least 15 chaetae other than the sensory clubs; Ant. II with at least 15 chaetae, one of them clearly longer than

the others; Ant. I with five chaetae. Eyepatches with one interocular somewhat spine-like chaeta each. Head vertex with a total of 14 or 16 spines, two of them unpaired; unpaired chaeta **A1** absent. Trochanters I–III with 1,1,1 spines, respectively, all blunt; trochanter III with five regular chaetae other than the spine. Ungues without the inner tooth, with tunica and weak pseudonychia; unguiculus I with or without the internal tooth; unguiculus III filament thin and reaching the tip of the unguis III. Large abdomen without capitate mac. Female with a long subanal appendage, bidentate at the apex, smooth or apically serrated. Dens ventral chaetotaxy formula from the apex to the base as: 2,2...1, dorsal chaetotaxy with 13 chaetae; mucronal notch prominent (adapted from Yosii 1959 and Lawrence 1968).

**Remarks.** *Szeptyckitheca santiagoi* is the sole species of the genus without the unguis inner tooth. However, the variability of color patterns reported by Lawrence (1968), discrepancies in its redescription compared to the original one of Yosii (1959), like differences in unguiculus morphology and dental chaetotaxy, and the wide distribution of the species in different islands of Asia and Oceania (Lawrence 1968) suggest the name *S. santiagoi* hides a species complex. In this sense, the diagnosis herein provided and the data listed in Table 2 for this species should be taken as provisional until the species can be redescribed.

**Habitat.** Specimens were found in forest moss and litter, beach debris, up palms and in native gardens (Lawrence 1968).

**Known distribution.** Australia, Papua New Guinea, Singapore, Solomon Islands (Yosii 1959; Lawrence 1968; Greenslade 2023).

### ? *Szeptyckitheca spinimucronata* (Itoh, 1993)

*Sphyrotheca spinimucronata* Itoh, 1993, in Itoh and Zhao 1993: 33.

**Diagnosis.** White ground with diffuse purple pigment on anterior and dorsal head and dorsal and lateral trunk, antennae darker. Ant. IV with ~ 10 subsegments. Head vertex with a total of 16 large spines, two of them unpaired; unpaired chaeta **A1** present; secondarily reduced chaetae near the spines absent. Trochanter III spine blunt, with five extra regular chaetae. Ungues with a single inner tooth, with tunica and lacking pseudonychia; unguiculus I without the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen without capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), acuminate, apically serrated on both edges. Manubrium with 7+7 dorsal chaetae; dens ventral chaetotaxy formula from the apex to the base as: 3,2...1, dorsal chaetotaxy with 17 chaetae; mucronal notch prominent (adapted from Itoh and Zhao 1993).

**Remarks.** *Sphyrotheca spinimucronata* is listed as a *Szeptyckitheca* species in Bellinger et al. (1996–2023), possibly due to its resemblance with *S. nepalica* (Itoh and Zhao 1993), and that is why we give its diagnosis here. However, it was not cited in the original description of the genus or in the most recent key to the group (Betsch and Weiner 2009; Zeppelini et al. 2018). Without the confirmation of the presence of trochanters I and II spines and many other relevant morphological traits (see Table 2), the positioning of *S. spinimucronata* within *Szeptyckitheca* is doubtful, and we considered it as a *species inquirenda*.

**Habitat.** Specimens were found in a coniferous forest of *Cryptomeria fortunei* (Itoh and Zhao 1993).

**Known distribution.** China (Itoh and Zhao 1993).

***Szeptyckitheca vanderdrifti* (Delamare-Deboutteville & Massoud, 1964)**

*Sphyrotheca vanderdrifti* Delamare-Deboutteville & Massoud, 1964b: 64.

**Diagnosis.** Yellowish ground color, with indistinct pigmented fields and purplish spots on the dorsum, antennae purplish. Ant. IV with ~ 10 subsegments; Ant. III with 19 chaetae other than the sensory clubs; Ant. II with 14 chaetae, two or three of them clearly longer than the others; Ant. I with six chaetae. Eyepatches with two interocular chaetae modified into strong spines each. Head vertex with a total of 16 large spines, two of them unpaired; unpaired chaeta **A1** absent; secondarily reduced chaeta near the spines present. Trochanters I–III with 1,1,1 spines, respectively, trochanter I spine acuminate and III capitate; trochanter III with four regular chaetae other than the spine. Ungues with a single inner tooth, with tunica and strong pseudonychia; unguiculus I with the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen lacking capitate mac. Female with a long subanal appendage (surpassing the ventral anal valves), acuminate, and apically or almost entirely serrated on its both edges. Dens ventral chaetotaxy formula from the apex to the base as: 2,1...1, dorsal chaetotaxy with 16 chaetae; mucronal notch discrete (adapted from Delamare-Deboutteville and Massoud 1964b).

**Habitat.** Specimens were found on marshy wood on sandy loam and shrubs on a ridge (Delamare-Deboutteville and Massoud 1964b).

**Known distribution.** Suriname (Delamare-Deboutteville and Massoud 1964b).

***Szeptyckitheca andrzeji* Medeiros, Bellini & Weiner, sp. nov.**

<https://zoobank.org/35417D56-6F55-4B77-A90F-576C05A36816>

Figs 1–6, Table 1

**Type material.** *Holotype* male on slide, Brazil, Piauí state, Altos municipality, “Floresta Nacional de Palmares” (5°3'12.53"S, 42°35'36.95"W), in sandy soil, Cerrado biome, 06/IV/2022, Mesquita C.P. col., pitfall traps. *Paratypes* on slides: one male, two females, one juvenile, with the same data as the holotype.

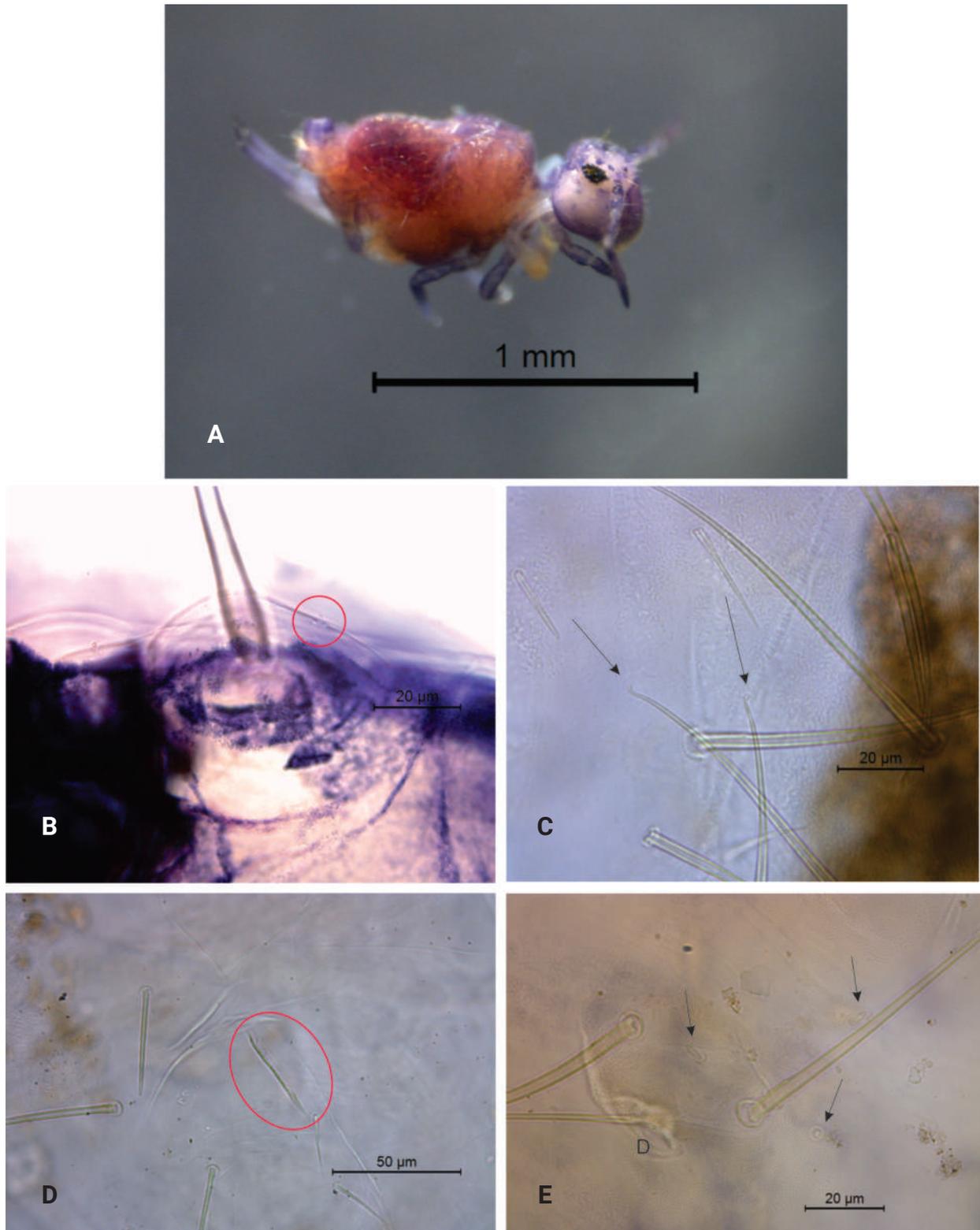
**Diagnosis.** Ground color pinkish, with purple spots on head, dorso-anterior large abdomen, and dorsal small abdomen. Ant. IV with 11 subsegments, with five capitate chaetae; Ant. III with 19 chaetae other than the sensory clubs, two of them clearly longer than the others; Ant. II subdivided, with 13 chaetae, three of them clearly longer than the others; Ant. I with six chaetae. Eyepatches lacking interocular chaetae. Head vertex with a total of 14 large spines, two of them unpaired; unpaired chaeta **A1** absent; secondarily reduced chaetae near the spines present. Trochanters I–III with 2,1,1 spines, respectively, trochanter I spines capitate, II and III spines blunt; trochanter III with five regular chaetae other than the spine. Ungues with one internal tooth, with tunica and weak pseudonychia; unguiculus I without the internal tooth; unguiculus III filament thin and

reaching the tip of the unguis III. Large abdomen dorsally with 15+15 long capitate mac. Female with a short subanal appendage (not reaching the apex of the ventral anal valves), spoon-like, and apically serrated on both faces. Manubrium with 7+7 dorsal chaetae; dens ventral chaetotaxy formula from the apex to the base as: 3...1, dorsal chaetotaxy with 17 chaetae; mucronal notch discrete.

**Description.** Body (head + trunk) length of the type series ranging between 900  $\mu\text{m}$  and 1400  $\mu\text{m}$ , holotype with 900  $\mu\text{m}$ , male average size = 900  $\mu\text{m}$ , female average size = 1300  $\mu\text{m}$ , entire type series average size = 1100  $\mu\text{m}$ . Ground color pinkish, with purple spots on head, Ant. I–III, dorso-anterior large abdomen and dorsal small abdomen. Ant. IV, legs, and dens uniformly purplish (Fig. 1A).

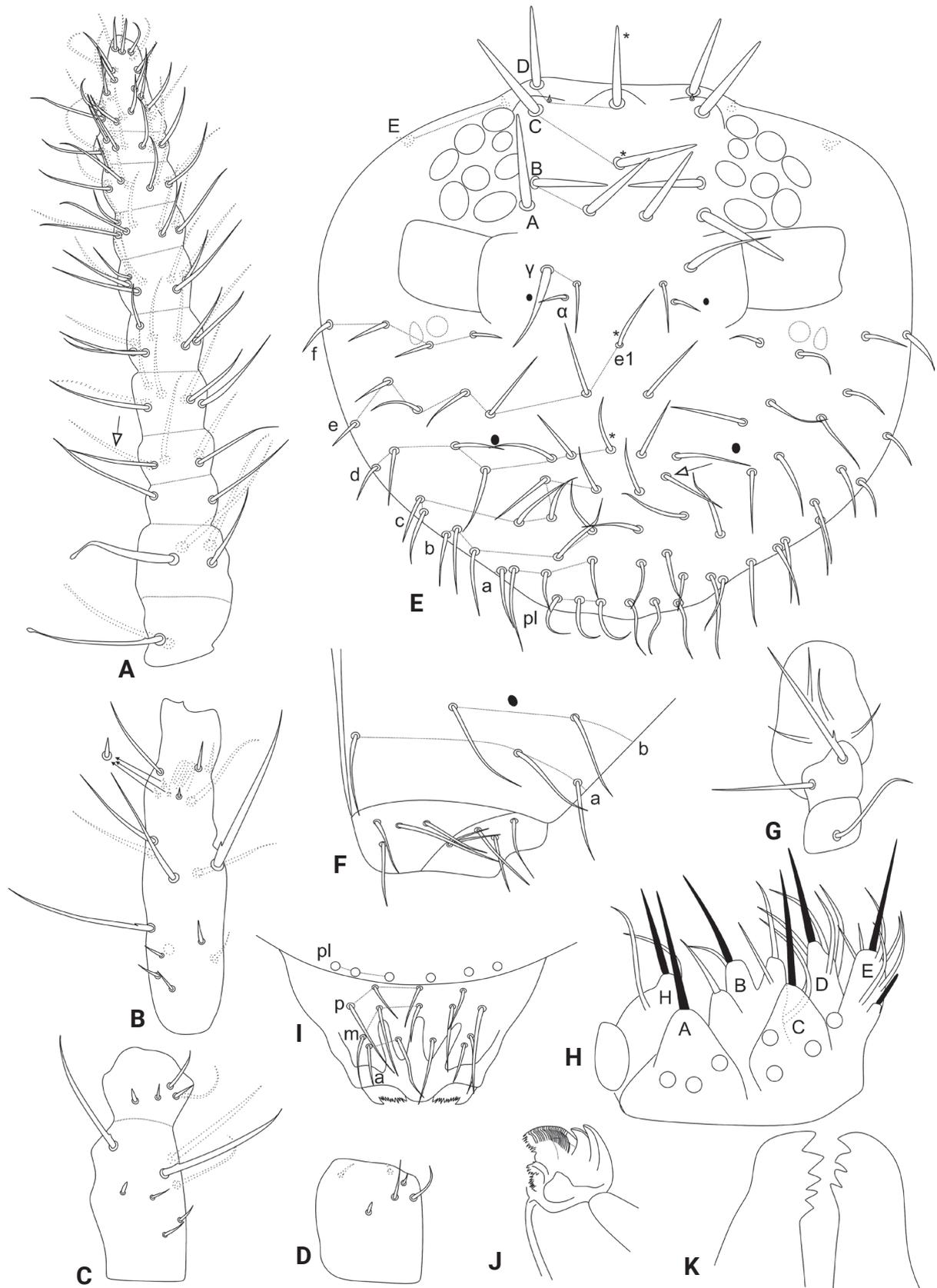
Head (Figs 1B, 2). Antennae length 433  $\mu\text{m}$  in the holotype. Holotype antennal segment ratio I:II:III:IV as 1:1.7:2.1:4.2. Ant. IV with 11 subsegments, subsegment I with two, II with four, III with two, IV with four or five, V with seven, VI with nine, VII–X with ten each, and XI with ~ 24 chaetae, respectively; subsegment I with two, subsegment II with three capitate chaetae respectively, some of them basally barbed (Fig. 2A). Ant. III with 19 chaetae other than the sensory rods, two of them clearly longer than the others and basally barbed, 11 reduced to some extent, one baso-ventral oval organ, sensory rods inside distinct shallow cavities (Fig. 2B). Ant. II subdivided, basal subsegment with eight, apical with five chaetae, respectively, basal subsegment with three enlarged chaetae, two of them basally barbed, Ant. II with seven chaetae reduced to some extent (Fig. 2C). Ant. I with six small chaetae, two of them ventral (Fig. 2D). Eyes 8+8, interocular chaetae absent, head capsule normal (not elongated) (Fig. 2E). Clypeal area **a–f** lines with 7/7/5–6/6(+1)/6(+1)/4 dorsal + ventral chaetae, respectively, **e1** chaeta present, 2+2 zones without cuticular granulation next to **f** line (Fig. 2E). Interantennal area  **$\alpha$**  and  **$\gamma$**  lines with 1/2 chaetae, respectively, 1+1 spine-like, plus 1+1 oval organs; frontal area **A–E** lines with a total of 12 large smooth spines, chaetotaxy following the formula: 1/2/1(+1)/2(+1)/2, respectively, **D** and **E** lines with secondarily reduced spines, **A1** chaeta absent (Figs 1B, 2E). Labial basomedian field with four, basolateral field with five chaetae, respectively; cephalic groove with 1+1 surrounding chaetae from **a** line (Fig. 2F). Maxillary outer lobe with apical chaeta basally barbed, longer than the basal chaeta, sublobal plate with four chaeta-like appendages (Fig. 2G). Labial palp with seven proximal chaetae, formula of the guards: **H**(2), **A**(0), **B**(5), **C**(0), **D**(4), **E**(6) plus the lateral process (Fig. 2H). Six prelabral chaetae present (Fig. 2E); labral **p**, **m**, and **a** lines with 5, 5, 4 chaetae, respectively, **p2** longer than the others, labral intrusions present, labral papillae absent, labrum apically toothed (Fig. 2I). Mandibles normal (not elongated), with 5+4 incisive apical teeth (Fig. 2K). Maxilla capitulum spherical, without any clear modification (Fig. 2J).

Trunk (Figs 1C–E, 3). Large abdomen: male blunt chaetae on dorsal large abdomen and all chaetae of the parafurcal area shorter than in females. Thorax continuous with the abdomen, without segmentations. Th. II with one **a** small chaeta and three blunt spines on papillae on **m** line; Th. III with one **a**, two **m** and one **p** blunt spines; Abd. I with one **a**, one **m**, and one **p** short blunt chaetae; Abd. II bothriotracha **A**, **B**, and **C** slightly misaligned, all short, with four **a**, five **m**, and five **p** chaetae of different shapes near the bothriotracha, Abd. II with four long capitate mac. Abd. III–IV with one unpaired dorsal chaeta plus four main lines of chaetae above the bothriotrachum **C**: **dl-1** with four, **dII-1** with four, **dIII-1**

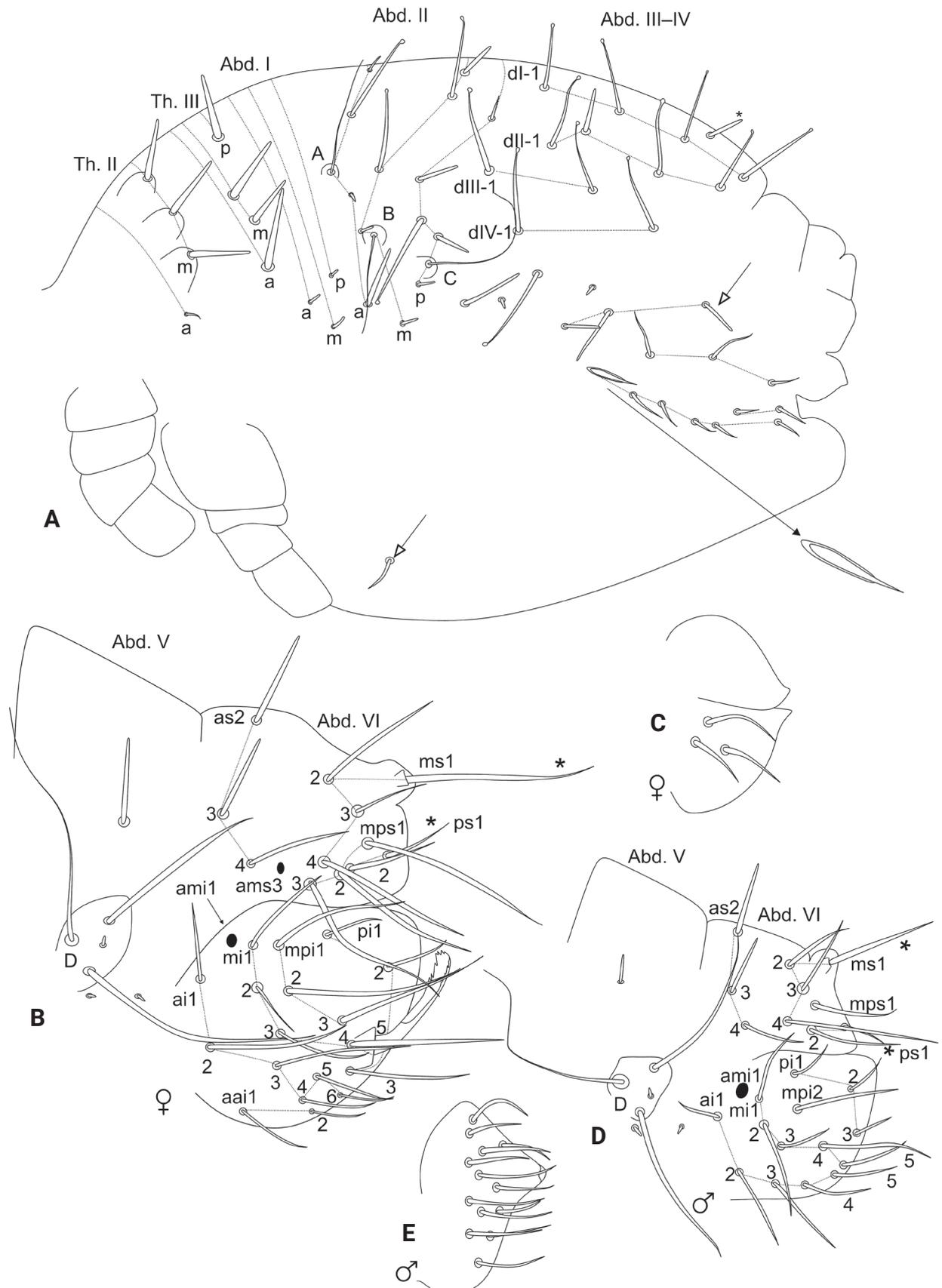


**Figure 1.** *Szeptyckitheca andrzejii* sp. nov. **A** habitus in ethanol (dorsal view) **B** frontal head spine on papilla, red circle marks a secondarily reduced spine **C** large abdomen capitata mac **D** parafurcal area, red circle marks the neosminthuroid chaeta **E** bothriotrichum D, black arrows indicate small blunt accessory chaetae.

with two, and **div-1** with two chaetae, respectively, 11 of them as four long capitata mac (Figs 1C, 3A). Parafurcal area with four rows of chaetae, with three, three, two and seven chaetae, respectively, neosminthuroid chaeta present



**Figure 2.** *Szeptyckitheca andrzeji* sp. nov. head **A** dorsal Ant. IV (white arrow points to chaeta present or absent) **B** dorsal Ant. III **C** dorsal Ant. II **D** dorsal Ant. I **E** anterior head chaetotaxy and eyes **F** labial and postlabial (ventral) chaetotaxy (right side) **G** maxillary outer lobe and sublobal plate (right side) **H** labial papillae and proximal chaetae alveoli (right side) **I** labrum **J** maxilla capitulum (right side) **K** mandibles apices.



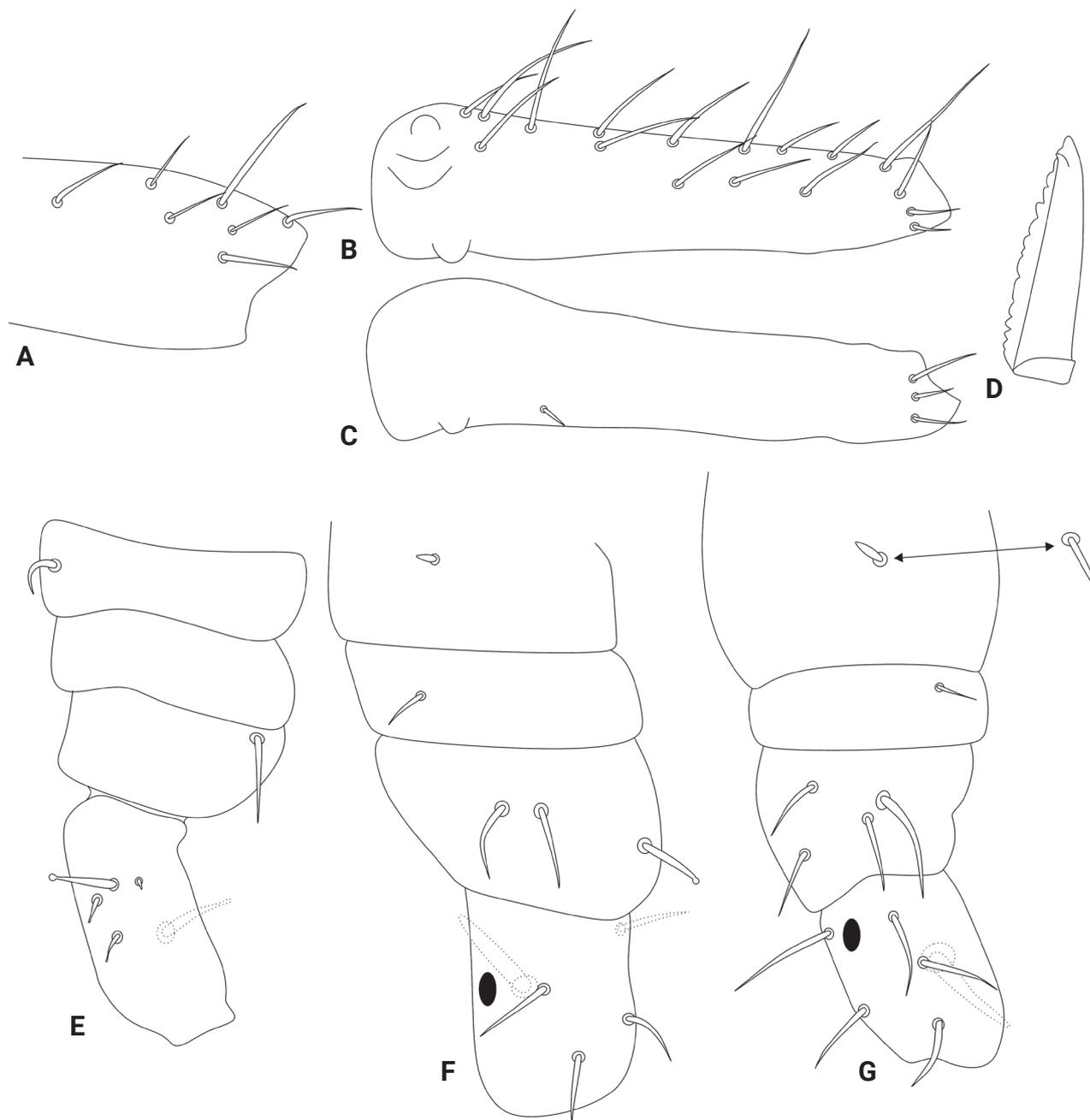
**Figure 3.** *Szeptyckitheca andrzeji* sp. nov. trunk chaetotaxy **A** male large abdomen, detail shows the neosminthroid chaeta and its alveolus, chaeta marked with a white arrow can be missing **B** female small abdomen **C** female genital plate **D** male small abdomen **E** male genital plate.

(Figs 1D, 3A). Small abdomen: including Abd. V–VI in both sexes. Abd. V chaetae smooth, with bothriotrichum **D** surrounded by three small blunt plus two long chaetae, a blunt chaeta above bothriotrichum **D** elongate in females, short in males (Figs 1E, 3B, D), Abd. VI chaetae discretely serrated (not represented in the drawings). Female Abd. VI: dorsal anal valve with **as2–4**, **ms1–4**, **mps1–3**, and **ps1–2** chaetae, **ms1** and **ps1** unpaired, **ams3** as an oval organ; each ventral anal valve with **aa1–2**, **ai1–6**, **ami1** (as an oval organ), **mi1–5**, **mpi1–3**, and **pi1–3** chaetae, **mi5** as the subanal appendage, short (not reaching the apex of the ventral anal valves), spoon-like, and apically serrated on both faces (Fig. 3B). Female genital plate with 3+3 ventral chaetae (Fig. 3C). Male Abd. VI: dorsal anal valve with **as2–4**, **ms1–4**, **mps1**, and **ps1–2** chaetae, **ms1** and **ps1** unpaired; each ventral anal valve with **ai1–5**, **ami1** (as an oval organ), **mi1–5**, **mpi2**, and **pi1–3** chaetae (Fig. 3D). Male genital plate with 13+13 chaetae (Fig. 3E).

Abdominal appendages (Fig. 4A–D). Ventral tube with 1+1 chaetae on the lateral flaps, sacs long and warty. Tenaculum ramus with three teeth each plus an apically rounded basal appendix, corpus with 2+2 chaetae. Manubrium with 7+7 dorsal chaetae (Fig. 4A); dens dorsally with two basal rounded appendages and 17 dorsal (posterior) chaetae (Fig. 4B); dens ventrally (anteriorly) with four chaetae, following the formula from the apex to the basis: 3...1 (Fig. 4C). Mucro short, apically split, external lamella serrated (with 10–17 serrations), internal smooth, ending in a discrete tooth-like apical notch (Fig. 4D). Manubrium:dens:mucro ratio of the holotype = 1.1:2.5:1.

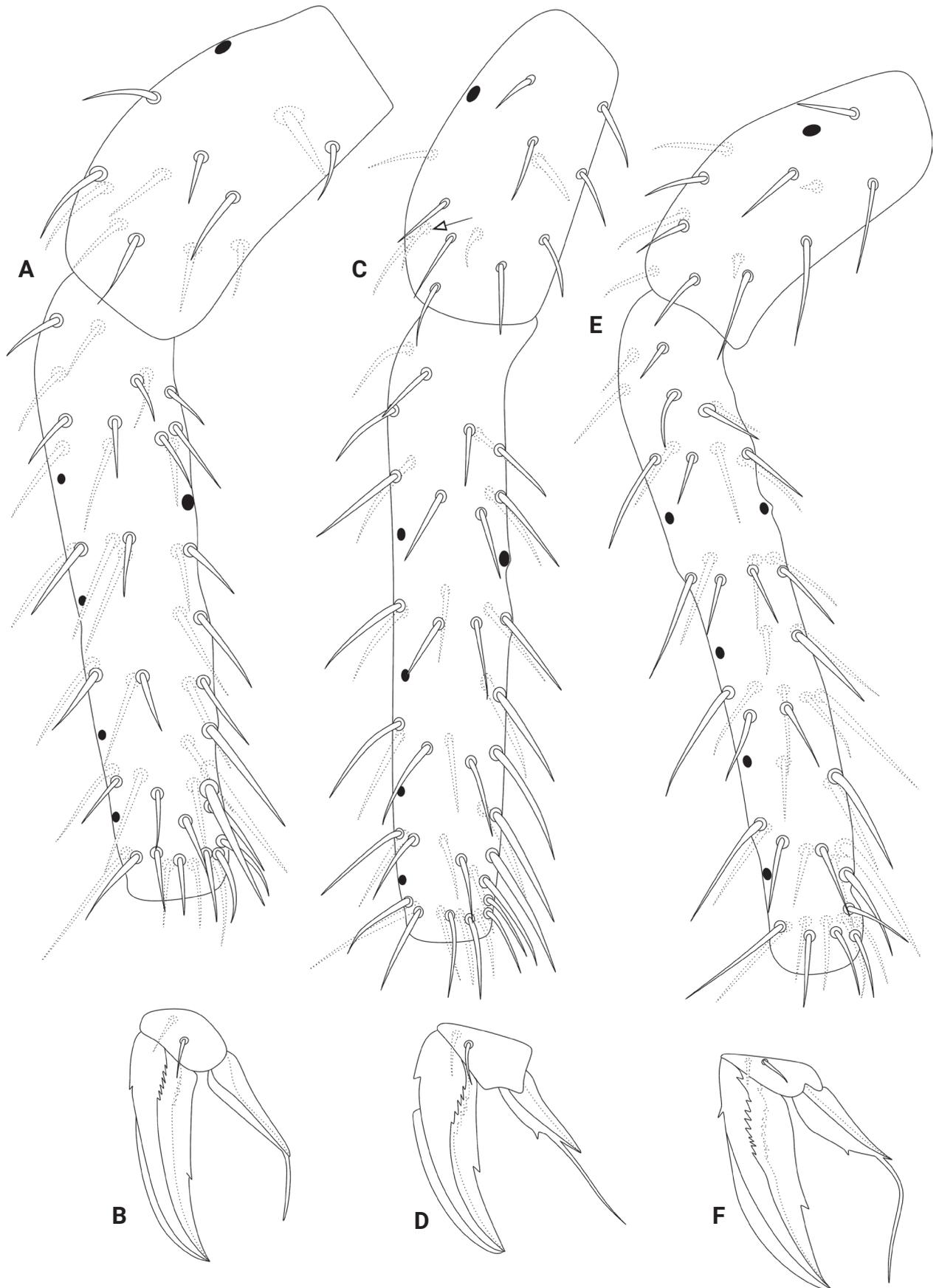
Legs (Figs 4E–G, 5). Leg I: epicoxa and coxa with one chaeta each, subcoxa without chaeta; trochanter with two capitate spines plus two capitate and one reduced acuminate chaetae (Fig. 4E); femur with one oval organ, one acuminate spine and 11 regular chaetae; tibiotarsus with five oval organs and 49 chaetae, ten of them in the apical whorl (Fig. 5A); pretarsus with one anterior and one posterior chaetae with similar sizes, unguis with one internal and one dorsal teeth, lateral teeth absent, with tunica and weak pseudonychia, unguiculus without tooth, apical filament thin and not reaching the tip of the unguis (Fig. 5B). Leg II: epicoxa and subcoxa with one chaeta each, coxa with three chaetae, one of them capitate; trochanter with one thick blunt spine plus one oval organ and four regular chaetae (Fig. 4F); femur with one oval organ, one acuminate spine and 12 or 13 regular chaetae; tibiotarsus with five oval organs and 47 chaetae, 10 of them in the apical whorl (Fig. 5C); pretarsus with one anterior and one posterior chaetae with similar sizes, unguis with one internal and one dorsal teeth, lateral teeth absent, with tunica and weak pseudonychia, unguiculus with an internal tooth, apical filament thin and reaching the tip of the unguis (Fig. 5D). Leg III: epicoxa and subcoxa with one chaeta each, coxa with four chaetae; trochanter with one thick blunt spine, one oval organ and five regular chaetae (Fig. 4G); femur with one oval organ, two reduced and 10 regular chaetae; tibiotarsus with five oval organs and 50 chaetae, ten of them in the apical whorl (Fig. 5E); pretarsus with one anterior and one posterior chaetae with similar sizes, unguis with one internal and one dorsal teeth, lateral teeth absent, with tunica and weak pseudonychia, unguiculus with one internal tooth, apical filament thin and reaching the tip of the unguis (Fig. 5F); tibiotarsi oval organs without reduced inner chaetae. Ratio of unguis I–III in the holotype = 1:1.08:1.19.

**Etymology.** The species honors Dr. Andrzej Szeptycki for his important contributions to the taxonomy and systematics of springtails.



**Figure 4.** *Szeptyckitheca andrzeji* sp. nov. trunk appendages **A** manubrium **B** dorsal dens **C** ventral dens **D** micropyle **E** epicoxa, subcoxa, and coxa of leg I **F** epicoxa, subcoxa, and coxa of leg II **G** epicoxa, subcoxa, and coxa of leg III, double arrow points to alternative morphology of the epicoxal chaeta.

**Habitat.** Specimens of *Szeptyckitheca andrzeji* sp. nov. were collected in the National Forest of Palmares, a small federal conservation unit, with a total area of 168.21 hectares, and altitudes ranging between 154 m and 250 m, located in Altos municipality, Piauí state, close to Teresina, the state’s capital (Fig. 6). The conservation unit is inserted in the Parnaíba River Sedimentary Basin, within the limits of the Cerrado biome, with influence of the Caatinga e Amazon biomes (ICMBio 2022). The vegetation of the region consists of a seasonal semideciduous forest, locally known as “Forest Cerrado” or “Cerradão” (Miranda et al. 2005), with a tree layer of medium to large size, reaching ~ 15–20 meters in height.



**Figure 5.** *Szeptyckitheca andrzeji* sp. nov. legs **A** femur and tibiotarsus I **B** foot complex I **C** femur and tibiotarsus II, chaeta marked with a white arrow can be missing **D** foot complex II **E** femur and tibiotarsus III **F** foot complex III.

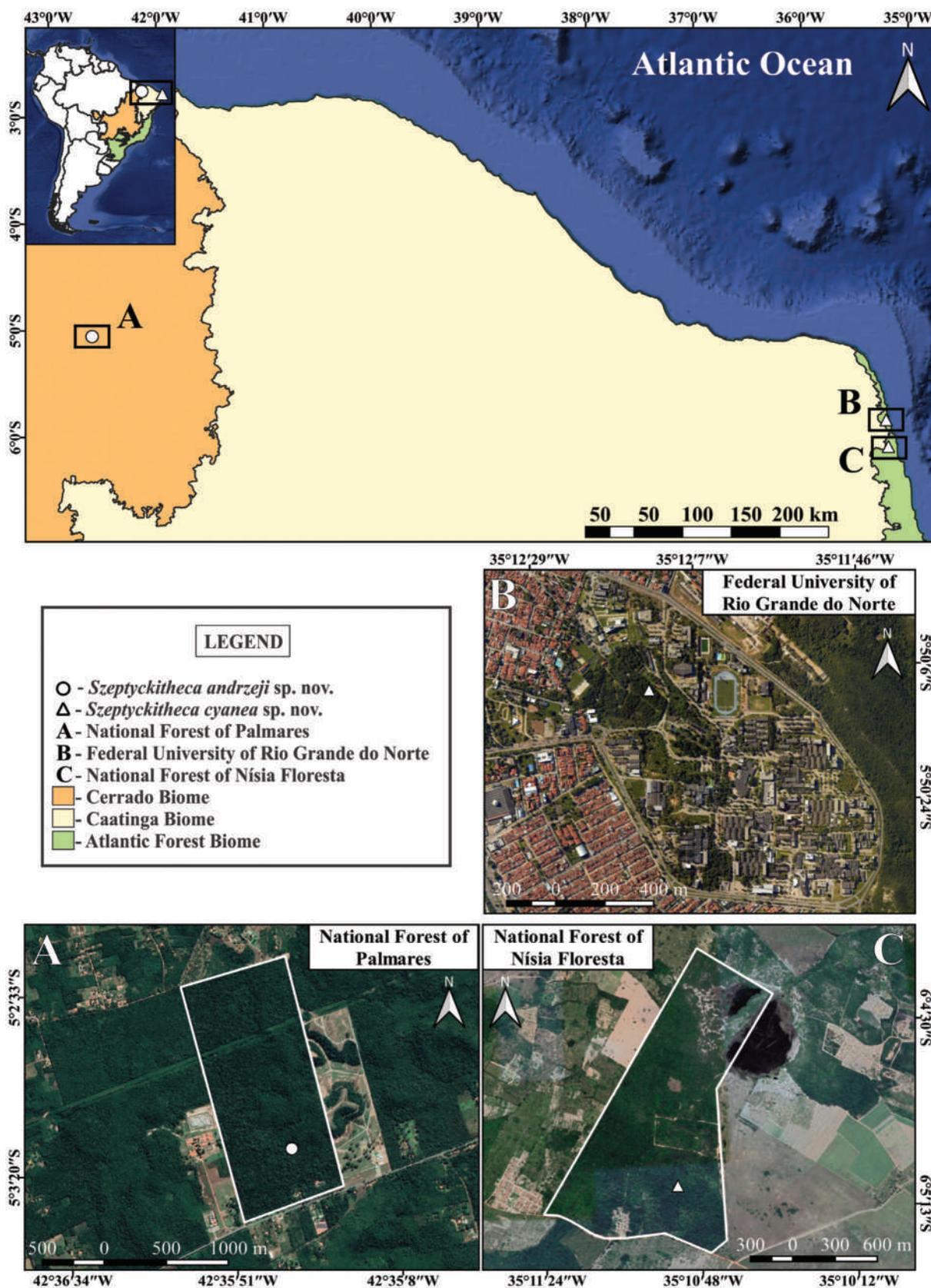


Figure 6. Known distribution of *Szeptyckitheca andrzeji* sp. nov. and *Szeptyckitheca cyanea* sp. nov. in Brazil **A** National Forest of Palmares, in Altos municipality, Piauí state **B** Federal University of Rio Grande do Norte, in Natal city, Rio Grande do Norte state **C** National Forest of Nísia Floresta, in Nísia Floresta municipality, Rio Grande do Norte state. In **A** and **C**, the white line polygons delimit the sampled areas boundaries.

The climate in the region, according to the Köppen–Geiger climate classification system, is Aw, megathermic tropical with a long dry season and rainy summer, with high temperatures throughout the year (Kottek et al. 2006). Between 1991 and 2020, the average annual temperature was 28–30 °C, with the minimum between 22–24 °C and the maximum between 33–35 °C, with an average annual insolation of 2800–3000 hours. The average annual precipitation was 1400–1600 mm, with February, March, and April as the rainiest quarter and July, August, and September as the driest one. The annual potential evapotranspiration was 2400–2600 mm, with a marked water deficit, which is typical of this Brazilian region (INMET 2023). The soil in the site where the specimens were collected is of the Latosol type, deep and well evolved, with some gravel on the surface, coming from nearby slopes, where young, shallow, and rocky soils predominate (ICMBio 2022).

**Remarks.** *Szeptyckitheca andrzeji* sp. nov. is unique among the Neotropical taxa due to its reduced ventral dens chaetotaxy, with only four chaetae distributed in two transversal rows, following the formula from the apex to the basis: 3...1. All other Neotropical *Szeptyckitheca* species have three transversal lines of dental ventral chaetae. Also, this is the only species of the genus with a short subanal appendage, not reaching the apex of the ventral anal valves (Tables 1, 2). The new species is somewhat similar to *S. kac* Zeppelini, Lopes & Lima, 2018 in color pattern, Ant. III chaetotaxy, and absence of interocular chaetae, but differs in the above-mentioned features, Ant. I chaetae (6 in the new species, 7 in *S. kac*), absence of lateral mac on f line on *S. andrzeji* sp. nov. (vs presence), shape of the trochanteral spines and number of regular chaetae on trochanter III (5 vs 3) and on dorsal dens (17 vs 15). Further comparisons are presented in Tables 1 and 2.

***Szeptyckitheca cyanea* Oliveira, Medeiros & Bellini, sp. nov.**

<https://zoobank.org/7A5516D9-4A67-40CA-B470-4EEAFA975D47>

Figs 7–11, Table 1

**Type material.** *Holotype* male on slide, Brazil, Rio Grande do Norte state, Nísia Floresta municipality, “Floresta Nacional de Nísia Floresta” (6°5'9.132"S, 35°10'53.857"W), 02/VI/2022, Xavier M.D. col., pitfall traps. **Paratypes** on slides: one male and one female, with the same data as the holotype.

**Other examined material.** Two males and one female on slides, Brazil, Rio Grande do Norte state, Natal municipality, “Mata da CAERN – UFRN” (5°50'9.665"S, 35°12'12.953"W).

**Diagnosis.** Specimens mostly bluish. Ant. IV with 11 subsegments, with at least six capitate chaetae; Ant. III with 21 chaetae other than the sensory clubs, including two peculiar small sensilla within cavities; Ant. II undivided, with 15 chaetae, four of them slightly longer than the others; Ant. I with six chaetae. Eyepatches with two small interocular chaetae. Head vertex with a total of 18 large spines, two of them unpaired; unpaired chaeta **A1** present and regular (not spine-like); secondarily reduced chaetae near the spines absent. Trochanters I–III with 2,1,1 spines, respectively, trochanters I and II spines capitate, III blunt; trochanter III with five regular chaetae other than the spine. Ungues with a one inner tooth, with tunica and weak pseudonychia; unguiculus

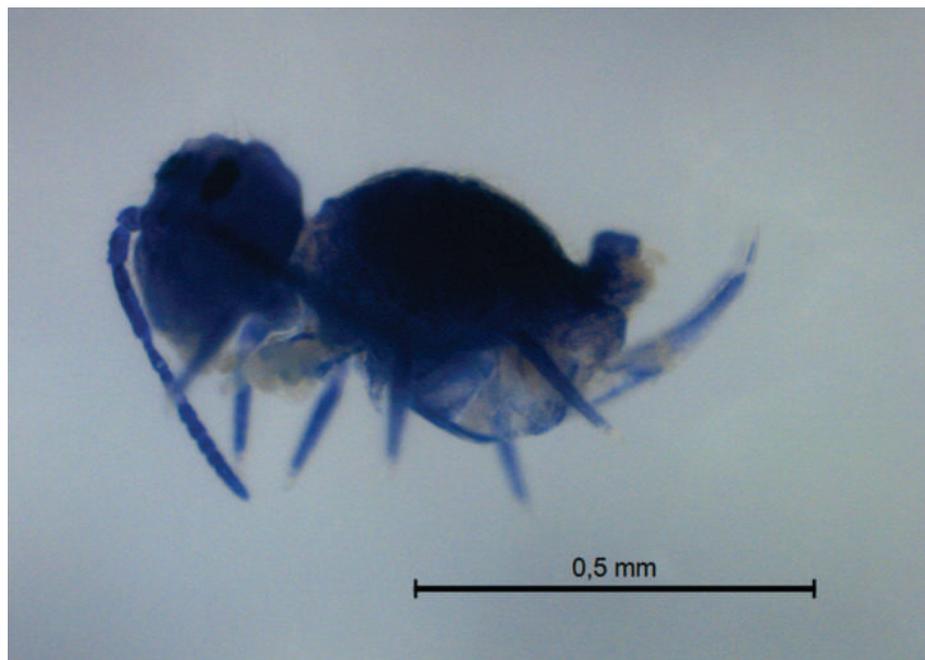
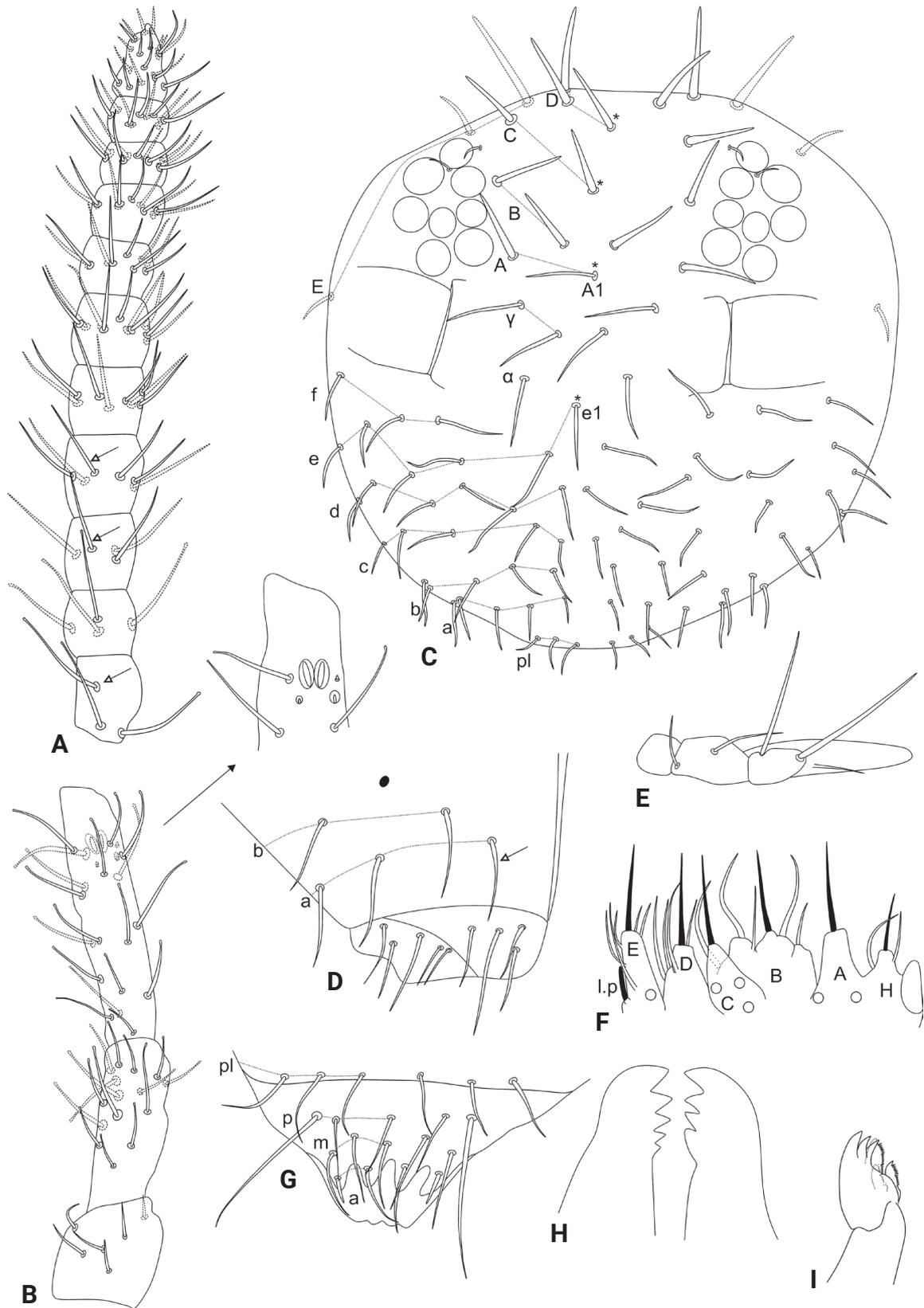


Figure 7. *Szeptyckitheca cyanea* sp. nov. habitus in ethanol (lateral view).

I with the internal tooth; unguiculus III filament thin and not reaching the tip of the unguis III. Large abdomen dorsally with ~ 26+26 long capitate mac. Female with a long subanal appendage (surpassing the apex of the ventral anal valves), slightly curved at the apex, acuminate, and apically serrated on its internal face. Manubrium with 7+7 dorsal chaetae; dens ventral chaetotaxy formula from the apex to the base as: 3,2...1, dorsal chaetotaxy with 16 chaetae; mucronal notch prominent.

**Description.** Body (head + trunk) length of the type series ranging between 551 and 818  $\mu\text{m}$ , holotype with 624  $\mu\text{m}$ , males' average size = 588  $\mu\text{m}$ , females' average size = 818  $\mu\text{m}$ , entire type series' average size = 664.5  $\mu\text{m}$ . Specimens entirely dark bluish (Fig. 7).

Head (Fig. 8). Antennae length 445.4  $\mu\text{m}$  in the holotype. Holotype antennal segment ratio I:II:III:IV as 1:1.3:2.3:5.2. Ant. IV with 11 subsegments, subsegment I with two or three, II with four, III with three or four, IV with five or six, V with eight, VI–X with ten each, and XI with ~ 19 chaetae, respectively, subsegments I+II with six or seven capitate chaetae (Fig. 8A). Ant. III with 21 chaetae other than the sensory clubs, including two peculiar small sensilla within cavities and one small sensillum without cavity, regular chaetae sizes variable but none remarkably longer than the others, most chaetae capitate, sensory rods inside two separate shallow cavities (Fig. 8B). Ant. II undivided, with 15 capitate chaetae, four of them slightly longer than the others (Fig. 8B). Ant. I with six chaetae, one of them ventral (Fig. 8B). Eyes 8+8, with 2+2 small interocular chaetae, head capsule normal (not elongated) (Fig. 8C). Clypeal area **a–f** lines with 7–8/7/5/6/5(+1)/3 dorsal + ventral chaetae, respectively, **e1** chaeta present, zones without cuticular granulation and oval organs only seen in the ventral side (Fig. 8C, D). Interantennal area **α** and **γ** lines with 1/2 regular chaetae, respectively; frontal area **A–E** lines with a total of 18 large smooth spines, chaetotaxy following the formula: 1(+1)/2/1(+1)/2(+1)/3, respectively, without secondarily reduced spines, **A1** chaeta present (Fig. 8C). Labial basomedian



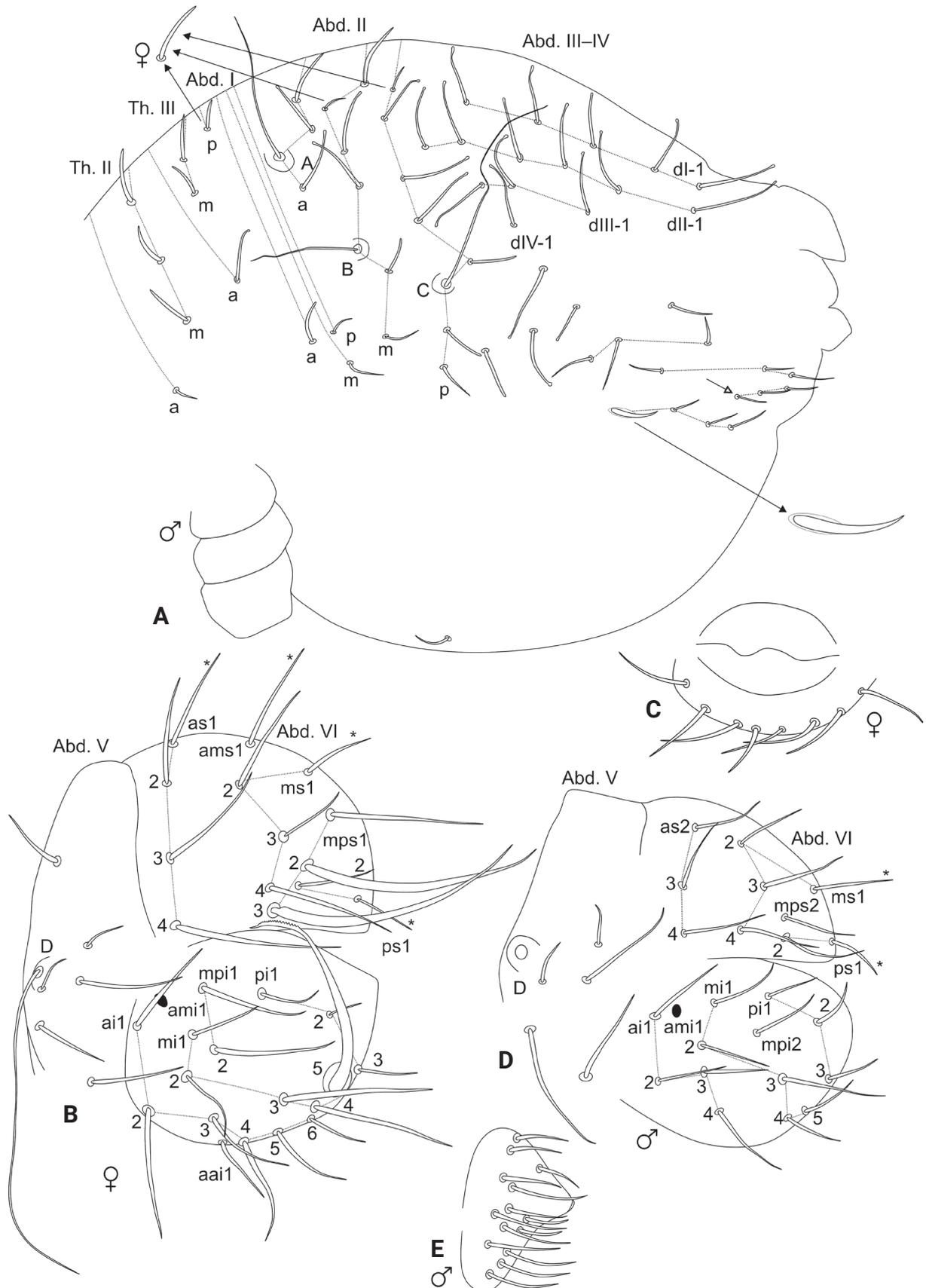
**Figure 8.** *Szeptyckitheca cyanea* sp. nov. head **A** dorsal Ant. IV (white arrows point to chaetae present or absent) **B** dorsal Ant. I–III, detail shows the ventral apical organ and the small sensilla in cavities on Ant. III **C** anterior head chaetotaxy and eyes **D** labial and postlabial (ventral) chaetotaxy (left side), white arrow points to chaeta present or absent **E** maxillary outer lobe and sublobal plate (left side) **F** labial papillae and proximal chaetae alveoli (left side) **G** labrum **H** mandibles apices **I** maxilla capitulum (left side).

field with four, basolateral field with five chaetae, respectively (Fig. 8D). Maxillary outer lobe with apical chaeta subequal to the basal chaeta, none barbed, sublobal plate with one chaeta-like appendage (Fig. 8E). Labial palp with six proximal chaetae, formula of the guards: **H**(2), **A**(0), **B**(5), **C**(0), **D**(4), **E**(5) plus the lateral process (Fig. 8F). Six prelabral chaetae present (Fig. 8C); labral **p**, **m**, and **a** lines with 5, 5, 4 chaetae, respectively, **p2** longer than the others, labral intrusions present, labral papillae absent, labrum apically without clear modifications (Fig. 8G). Mandibles normal (not elongated), with 5+4 incisive apical teeth (Fig. 8H). Maxilla capitulum elongate (Fig. 8I).

Trunk (Fig. 9). Large abdomen: thorax continuous with the abdomen, without segmentations. Th. II with one **a** small chaeta and three blunt spines on **m** line; Th. III with one capitate **a**, two **m** and one **p** chaetae, **p** reduced in males and elongate in females; Abd. I with one **a**, one **m** and one **p** chaetae; Abd. II bothriotracha **A**, **B**, and **C** slightly misaligned, **C** clearly longer than **A** and **B**, with three **a**, five **m**, and seven **p** chaetae of different shapes near the bothriotracha, at least two of them clearly shorter in males; Abd. II with seven long capitate mac. Abd. III and IV with four main lines of chaetae above the bothriotrachum **C**: **dI-1** with five, **dII-1** with six, **dIII-1** with three and **dIV-1** with one capitate chaetae, respectively, female with some chaetae longer than on male (Fig. 9A). Parafurcal area with four rows of chaetae, with three, three, two or three and four chaetae, respectively, neosminthuroid chaeta present. Three extra capitate chaetae between the bothriotrachum **C** and the parafurcal area (Fig. 9A). Small abdomen: including Abd. V–VI in both sexes. Abd. V chaetae smooth, with bothriotrachum **D** with one small accessory chaeta, chaeta above bothriotrachum **D** elongate in females, absent in males (Fig. 9B, D), Abd. VI chaetae apparently smooth. Female Abd. VI: dorsal anal valve with **as1–4**, **ams1**, **ms1–4**, **mps1–3**, and **ps1–2** chaetae, **as1**, **ams1**, **ms1**, and **ps1** unpaired; each ventral anal valve with **ai1–6**, **ami1** (as an oval organ), **mi1–5**, **mpi1–2**, and **pi1–3** chaetae, **mi5** as the subanal appendage, long (surpassing the apex of the ventral anal valves), slightly curved at the apex, acuminate, and apically serrated on its internal face (Fig. 9B). Female genital plate with 4+4 ventral chaetae (Fig. 9C). Male Abd. VI: dorsal anal valve with **as2–4**, **ms1–4**, **mps2**, and **ps1–2** chaetae, **ms1** and **ps1** unpaired; each ventral anal valve with **ai1–4**, **ami1** (as an oval organ), **mi1–5**, **mpi2**, and **pi1–3** chaetae (Fig. 9D). Male genital plate with 14+14 chaetae (Fig. 9E).

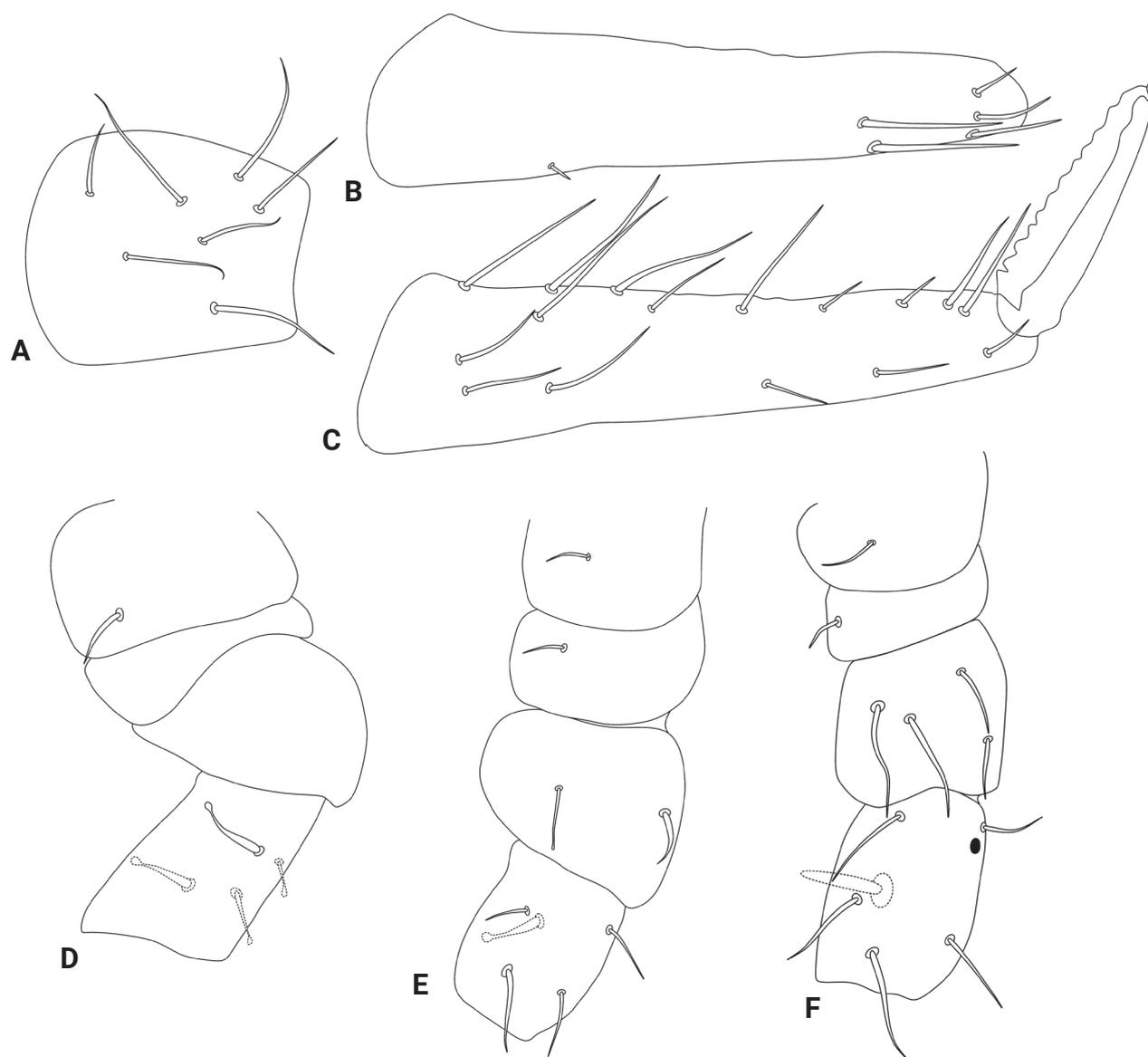
Abdominal appendages (Fig. 10A–C). Ventral tube with 1+1 chaetae on the lateral flaps, sacs long and warty. Tenaculum ramus with three teeth each plus an apically rounded basal appendix, corpus with 2+2 chaetae. Manubrium with 7+7 dorsal chaetae (Fig. 10A); dens ventrally (anteriorly) with six chaetae, following the formula from the apex to the basis: 3,2...1 (Fig. 10B); dens dorsally without basal appendages, with 16 dorsal (posterior) chaetae (Fig. 10C); Mucro short, apically split, external lamella serrated (with 12–18 serrations), internal with two distal weak crenulations, ending in a prominent apical notch (Fig. 10C). Manubrium:dens:mucro ratio of the holotype = 1.05:2.4:1.

Legs (Figs 10D–F, 11). Leg I: epicoxa with one chaeta, subcoxa and coxa without chaetae; trochanter with two capitate spines plus two capitate chaetae (Fig. 10D); femur with one oval organ, one acuminate large curved spine and ten regular chaetae; tibiotarsus with two oval organs and 46–47 chaetae, nine or ten of them in the apical whorl (Fig. 11A); pretarsus with anterior chaeta longer than the posterior one, unguis with one internal, two lateral and one dorsal

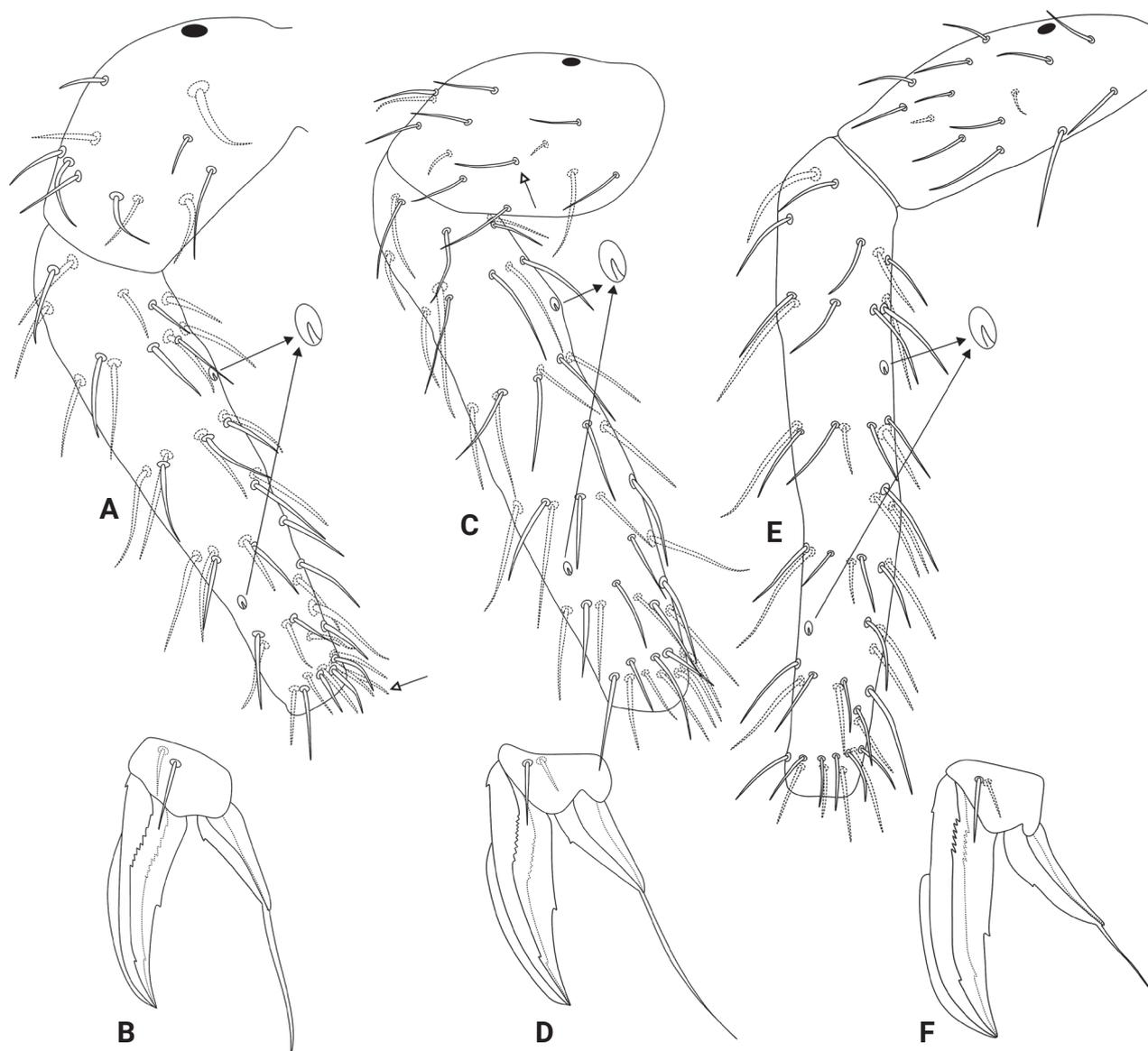


**Figure 9.** *Szeptyckitheca cyanea* sp. nov. trunk chaetotaxy **A** male large abdomen, details show the neosminthroid chaeta and its alveolus and longer chaetae of the female **B** female small abdomen **C** female genital plate **D** male small abdomen **E** male genital plate.

teeth, with tunica and weak pseudonychia, unguiculus with the tooth, apical filament thin and surpassing the tip of the unguis (Fig. 11B). Leg II: epicoxa and subcoxa with one chaeta each, coxa with two chaetae, one of them curved, other capitate; trochanter with one thick capitate spine and four regular chaetae (Fig. 10E); femur with one oval organ, two reduced and 10–11 regular chaetae; tibiotarsus with two oval organs and 47 chaetae, ten of them in the apical whorl (Fig. 11C); pretarsus with anterior chaeta longer than the posterior one, unguis with one internal, two lateral and one dorsal teeth, with tunica and weak pseudonychia, unguiculus with the tooth, apical filament thin and surpassing the tip of the unguis (Fig. 11D). Leg III: epicoxa and subcoxa with one chaeta each, coxa with four chaetae; trochanter with one thick blunt spine, one oval organ and five regular chaetae (Fig. 10F); femur with one oval organ, two reduced and 12 regular chaetae; tibiotarsus with two oval organs and 50 chaetae, ten of them in the apical whorl (Fig. 11E); pretarsus with anterior chaeta longer than



**Figure 10.** *Szeptyckitheca cyanea* sp. nov. trunk appendages **A** manubrium **B** ventral dens **C** dorsal dens and mucro **D** epicoxa, subcoxa, and coxa of leg I **E** epicoxa, subcoxa, and coxa of leg II **F** epicoxa, subcoxa, and coxa of leg III.



**Figure 11.** *Szeptyckitheca cyanea* sp. nov. legs **A** femur and tibia I (detail shows oval organs with internal small sensillum) **B** foot complex I **C** femur and tibia II (detail shows oval organs with internal small sensillum) **D** foot complex II **E** femur and tibia III (detail shows oval organs with internal small sensillum) **F** foot complex III.

the posterior one, unguis with one internal, two lateral and one dorsal teeth, with tunica and weak pseudonychia, unguiculus with the tooth, apical filament thin and not reaching the tip of the unguis (Fig. 11F); tibiotarsi oval organs with reduced inner sensilla (Fig. 11A, C, E). Ratio of unguis I–III in the holotype = 1:1.05:1.04.

**Etymology.** The species was named after its color pattern; *cyanea* from Latin means dark blue.

**Habitat.** Specimens of *S. cyanea* sp. nov. were found in two localities ~ 30 km apart in the Rio Grande do Norte state, Brazil: in the central campus of the Federal University of Rio Grande do Norte, Natal municipality, and the National Forest of Nísia Floresta, Nísia Floresta municipality (Fig. 6). Both localities are inserted in the Atlantic Forest phytogeographic domain. The specimens were collected from the forest litter covering sandy soils in shady areas. The climate of the sampled municipalities is “As” following the Köppen-Geiger system,

which means an equatorial climate with dry summer (Kottek et al. 2006). The specimens were collected during the raining season of 2022.

**Remarks.** *Szeptyckitheca cyanea* sp. nov. is the sole species of the genus with 18 spines on the head vertex. It is also the only Neotropical *Szeptyckitheca* with the frontal head A1 chaeta (see Table 1). Considering the Neotropical fauna, it is somewhat similar to *S. andrzeji* sp. nov. in the number of Ant. IV sub-segments (11), Ant. I chaetae (6), and the presence of two capitate trochanteral spines on the leg I. However, they differ in the previously mentioned features, as well as the presence of secondarily reduced chaetae on the head frons of *S. andrzeji* sp. nov. (absent in *S. cyanea* sp. nov.), female subanal appendage morphology (short and spoon-like in *S. andrzeji* sp. nov., long and acuminate in *S. cyanea* sp. nov.) and ventral dens chaetotaxy formula (3...1 in *S. andrzeji* sp. nov., 3,2...1 in *S. cyanea* sp. nov.), among other characteristics. Further comparisons are presented in Tables 1 and 2.

### Identification key of *Szeptyckitheca* species\*

- 1 Head vertex with a set of 9 rough curved mac plus 4 large erect spines....  
..... ***S. koreana* (Betsch & Weiner, 2009), comb. nov. (North Korea)**
- Head vertex without rough curved mac, with 6 or more large erect spines ... **2**
- 2 Head vertex with 15 large spines, ventral dens chaetotaxy formula as: 3,1...1, unguis pseudonychia absent ..... ***S. formosana* (Yosii, 1965) (Taiwan)**
- Head vertex with 6–14 or 16–18 large spines, ventral dens chaetotaxy formula otherwise, unguis pseudonychia usually present ..... **3**
- 3 Head vertex with 10 large spines, unguis absent or at most vestigial, female subanal appendage very long, surpassing the dorsal anal valve ..... ***S. boneti* (Denis, 1948) (Vietnam)**
- Head vertex with 6 or 11–18 spines, unguis present, female subanal appendage not reaching the apex of the dorsal anal valve ..... **4**
- 4 Head clypeal **f** line with 4+4 mac, ventral dens chaetotaxy formula as: 4,1...1, dorsal dens with 15 chaetae .....  
..... ***S. kac* Zeppelini, Lopes & Lima, 2018 (Brazil)**
- Head clypeal **f** line without mac, ventral dens chaetotaxy formula otherwise, dorsal dens with 12–14 or 16–24 chaetae ..... **5**
- 5 Trochanter II without the spine, dorsal dens with 24 chaetae .....  
.. ***S. machadoi* (Delamare-Deboutteville & Massoud, 1964) (Angola, Congo)**
- Trochanter II usually with the spine, dorsal dens with ≤ 20 chaetae ..... **6**
- 6 Head interantennal area with bifid chaetae, female anal valve with **mps1** winged, dorsal dens with 20 chaetae.....  
..... ***S. kesongensis* Betsch & Weiner, 2009 (North Korea)**
- Head interantennal area without bifid chaetae, female anal valve with **mps1** not winged, dorsal dens with < 20 chaetae ..... **7**
- 7 Head vertex with 6 large spines, ventral dens chaetotaxy formula as: 4,2...1 ..... ***S. coerulea* (Bretfeld 2005) (Yemen - Socotra Island)**
- Head vertex with 11 or more large spines, ventral dens chaetotaxy formula otherwise ..... **8**
- 8 Specimens pale, with diffused pigment and violet patches, head vertex with 11 large spines ..... ***S. nepalica* (Yosii, 1966) (Nepal)**
- Specimens color pattern otherwise, head vertex with 14–18 large spines.... **9**

- 9 Specimens with lateral weak purple bands, posteriorly purple, dorsal dens with 14 chaetae ..... ***S. mucroserrata* (Snider, 1978) (Brazil, Mexico, USA)**
- Specimens color pattern otherwise, dorsal dens with 12–13 or 16–19 chaetae ..... **10**
- 10 Trochanter II without the spine, dorsal dens with 12 chaetae..... ***S. karlarum* (Palacios-Vargas, Vázquez & Cuéllar, 2003), comb. nov. (Mexico)**
- Trochanter II with the spine, dorsal dens with > 12 chaetae ..... **11**
- 11 Ungues without the inner tooth, dorsal dens with 13 chaetae..... ***S. santiagoi* (Yosii, 1959) (Australia, Papua New Guinea, Singapore, Solomon Isl)**
- Ungues with 1 inner tooth, dorsal dens 16–19 chaetae ..... **12**
- 12 Head frontal chaeta **A1** present ..... **13**
- Head frontal chaetae **A1** absent ..... **14**
- 13 Head vertex with 18 large spines, eyepatches with 2 interocular chaetae, ventral dens chaetotaxy formula as: 3,2...1, dorsal dens with 16 chaetae ..... ***S. cyanea* Oliveira, Medeiros & Bellini, sp. nov. (Brazil)**
- Head vertex with 16 large spines, eyepatches with 1 interocular chaeta, ventral dens chaetotaxy formula as: 2,2...1, dorsal dens with 19 chaetae ..... ***S. implicata* (Hüther, 1967) (South Sudan)**
- 14 Female subanal appendage short (not reaching the apex of the ventral anal valves), ventral dens chaetotaxy formula as: 3...1 ..... ***S. andrzeji* Medeiros, Bellini & Weiner, sp. nov. (Brazil)**
- Female subanal appendage long (surpassing the apex of the ventral anal valves), ventral dens chaetotaxy formula as: 3–2,2...1 ..... **15**
- 15 Head vertex with 14 large spines, trochanter I with 2 spines, unguiculus I without the internal tooth, mucronal notch prominent ..... ***S. bellingeri* (Betsch, 1965) (Jamaica)**
- Head vertex with 16 large spines, trochanter I with 1 spine, unguiculus I with the internal tooth, mucronal notch discrete..... **16**
- 16 Ant. II with 14 chaetae, trochanter III spine capitate, with 4 regular chaetae, ventral dens chaetotaxy formula as: 3,2...1, dorsal dens with 17 chaetae ..... ***S. peteri* (Palacios-Vargas, Vázquez & Cuéllar, 2003), comb. nov. (Mexico)**
- Ant. II with 12 chaetae, trochanter III spine blunt, with 5 regular chaetae, ventral dens chaetotaxy formula as: 2,2...1, dorsal dens with 16 chaetae ..... ***S. vanderdrifti* (Delamare-Deboutteville & Massoud, 1964) (Suriname)**

\*We did not include *S. spinimucronata* as we considered it a *species inquirenda*. Further details on the species are listed in its diagnosis.

## Discussion

The boundaries between *Szeptyckitheca* and *Sphyrotheca* are quite narrow (Betsch and Weiner 2009). Many chaetotaxic features are variable within *Szeptyckitheca*, like the presence or absence of frontal chaeta **A1** on head, unguinal tunica and even the ventral dental chaetae formula (see Tables 1, 2), and so are not unequivocal to separate these genera. The same occurs in *Sphyrotheca*, and for instance, the presence of apically curved mac (spines) on head's frontal area and dorsal large abdomen listed in Betsch and Weiner (2009) differs within the genus, being present in species like *Sphyrotheca multifasciata* (Reuter, 1881), and absent in Neotropical taxa like *Sphyrotheca caputalba* Bretfeld 2002

(Bretfeld 2000, 2002). With the description of *Szeptyckitheca andrzeji* sp. nov., with its *Sphyrotheca*-like reduced ventral dens chaetotaxy, and the transfer of *Sphyrotheca koreana* to *Szeptyckitheca* due to the presence of trochanteral spines on all legs, the ventral dens chaetotaxy and the presence of curved mac on frontal head and dorsal large abdomen do not allow to separate the two genera, and the sole morphological trait which clearly differs between them is the presence of the trochanteral spine on leg 1 of *Szeptyckitheca* species. This observation supports a close relationship between these genera and it is possible one of them emerged from the other. A molecular phylogenetic analysis of the Sphyrothecinae is in need to corroborate or reject this hypothesis and to reinforce (or dismiss) the validity of *Szeptyckitheca*.

After the comparison of both new species described here, we observed other features which may be useful to diagnose *Szeptyckitheca* taxa, such as the shape of the maxilla capitulum (globose vs elongate), the shape of **E** chaetae on frontal head, the presence of an extra basal appendix on proximal dens and the number, and homology of clypeal unpaired chaetae. These features were not used in this study to separate the species due to the lack of information or uncertainties about such data in most (or all) previously described species. Nevertheless, with the expansion of the knowledge of the genus they may be useful to identify closely related taxa.

After our study, there are now 18 species assigned to *Szeptyckitheca*. Even so, many of them are in need of revision or full redescription.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

Ethical review and approval were waived for this study due to Brazilian laws which do not require permission from an institutional ethics committee on the use of animals for taxonomical studies with microarthropods.

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### Author contributions

Conceptualization: BCB, GDSM. Data curation: BCB, MFDO, GDSM. Formal analysis: MFDO, GDSM. Project administration: BCB. Resources: BCB. Software: RCN, GDSM. Supervision: BCB, WMW. Validation: GDSM, BCB. Visualization: BCB, WMW, GDSM. Writing - original draft: BCB, RCN. Writing - review and editing: GDSM, RCN, BCB, MFDO, WMW.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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# A survey of pholcid spiders (Araneae, Pholcidae) from Guiyang, Guizhou Province, China

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## Abstract

The family Pholcidae C.L. Koch, 1850 is highly diverse in Guizhou Province, southwestern China, and currently contains four genera and 22 species. Nevertheless, the distribution of pholcid spiders is conspicuously patchy in Guizhou. Species from Guiyang are poorly studied, and only *Pholcus spilis* Zhu & Gong, 1991 has been recorded. A survey was undertaken for the first time to study the pholcids in Guiyang. A total of four species are reported, comprising *Belisana yuhaoi* Yang & Yao, **sp. nov.** and three other species: *Leptopholcus tanikawai* Irie, 1999 (new record for Guiyang), *Pholcus spilis* Zhu & Gong, 1991 and *Spermophora senoculata* (Dugès, 1836) (new record for Guizhou).

**Key words:** Biodiversity, fauna, new record, new species, taxonomy



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## Introduction

The species-rich spider family Pholcidae currently contains 97 genera and 1937 species (World Spider Catalog 2023). It comprises five subfamilies: Arteminae Simon, 1893; Modisiminae Simon, 1893; Ninetinae Simon, 1890; Smeringopinae Simon, 1893; and Pholcinae C.L. Koch, 1850 (Huber 2011a; Dimitrov et al. 2013; Eberle et al. 2018), and has a worldwide distribution. Recently, a series of surveys of pholcid spiders have been undertaken in northern China. For instance, the expedition to Changbai Mountains revealed 26 species recorded from Liaoning Province (Lu et al. 2021; Yao et al. 2021; World Spider Catalog 2023; Zhao et al. 2023a). The expeditions to Yanshan-Taihang Mountains and Lüliang Mountains brought the fauna of pholcids from Hebei Province, Beijing and Shanxi Province to 31 species, six species, and 21 species, respectively (Lu et al. 2022a, b; World Spider Catalog 2023; Zhao et al. 2023b). To date, 17 genera and 271 species of pholcids have been recorded from China (World Spider Catalog 2023), of which nine genera and 99 species were collected in caves or at cave entrance ecotones from karst regions.

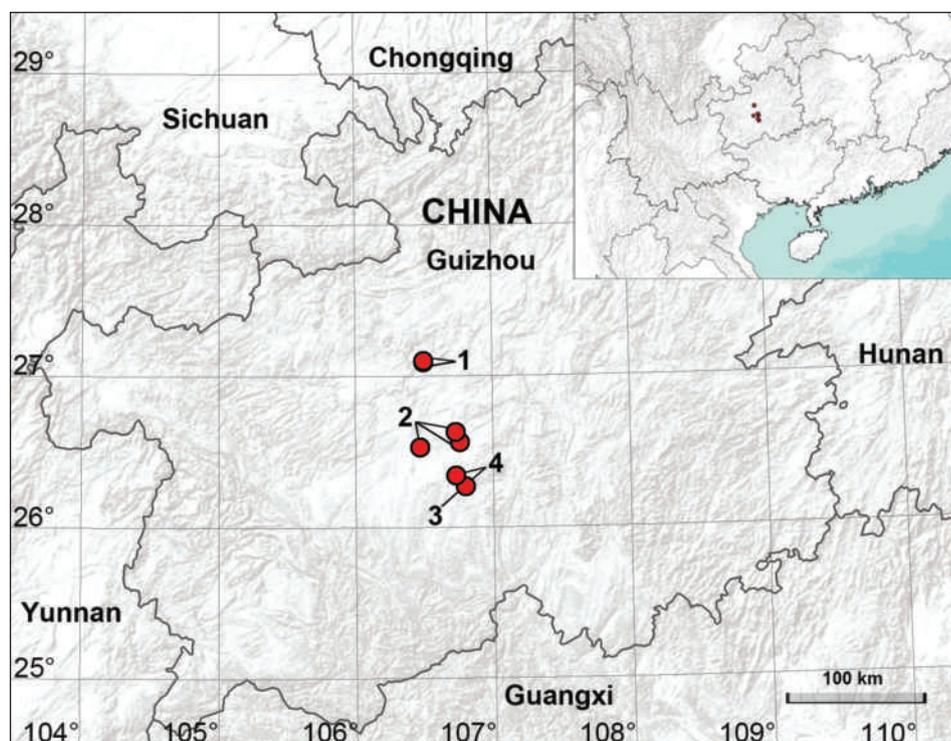
Guizhou Province, in the southwest of China, is one of the most spectacular examples of humid subtropical karst landscapes, and also exhibits high diversity of pholcids. Currently, four genera (*Belisana* Thorell, 1898, *Khorata* Huber, 2005, *Leptopholcus* Simon, 1893, *Pholcus* Walckenaer, 1805) and 22 species have been record-

ed (World Spider Catalog 2023). Nevertheless, only one species, *Pholcus spilis* Zhu & Gong, 1991, has been recorded from Guiyang, the provincial capital of Guizhou. In this paper, we undertook a survey in Guiyang for the first time and report four species, comprising a new species from a cave and three known species (Figs 1, 2).

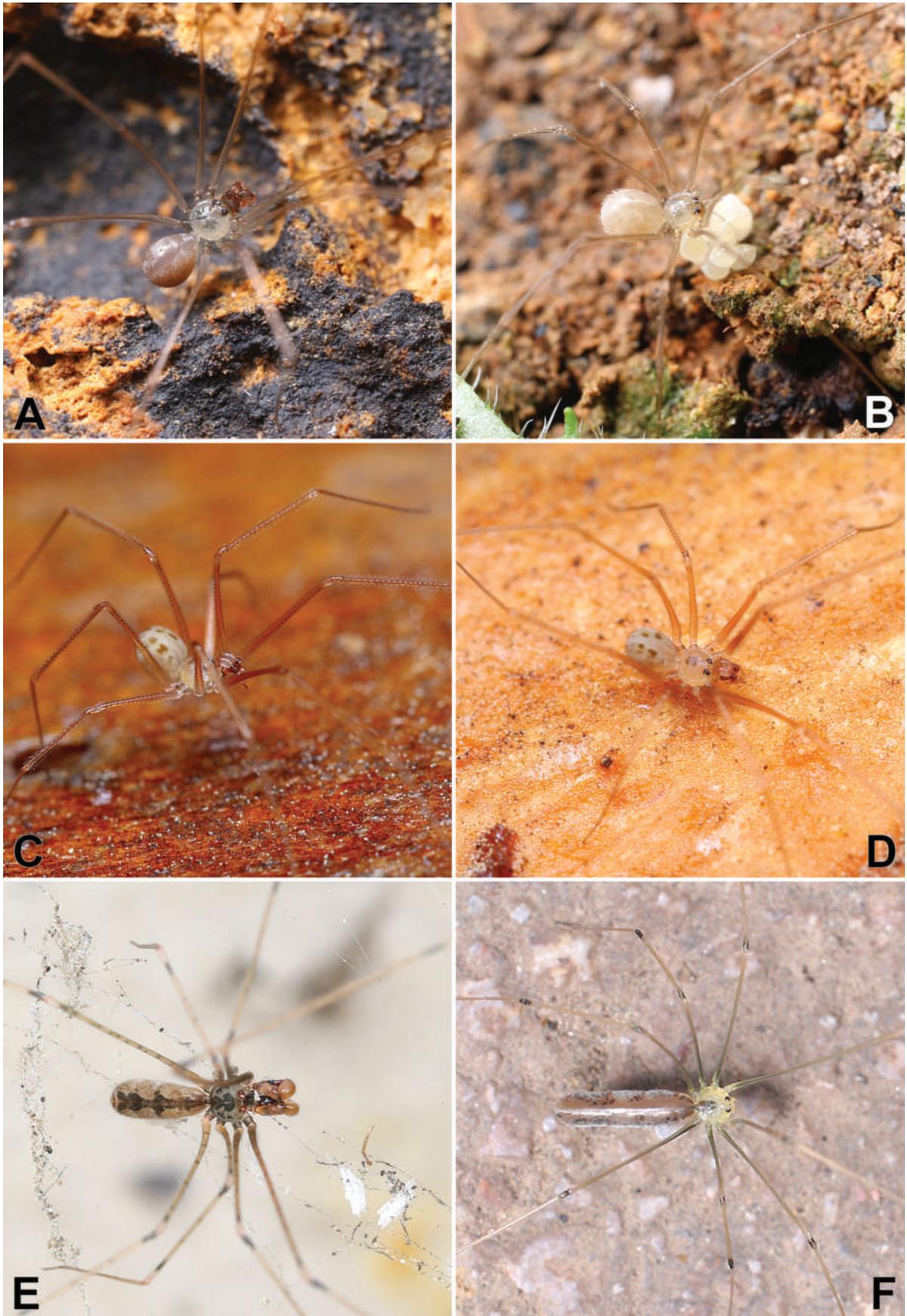
## Material and methods

Specimens were examined and measured with a Leica M205 C stereomicroscope. The left male palp was photographed. The epigyne was photographed before dissection. The vulva was treated in a 10% warm solution of potassium hydroxide (KOH) to dissolve soft tissues before illustration. Images were captured with a Canon EOS 750D wide zoom digital camera (24.2 megapixels) mounted on the stereomicroscope mentioned above and assembled using Helicon Focus v.3.10.3 image stacking software (Khmelik et al. 2005). All measurements are given in millimeters (mm). Leg measurements are shown as: total length (femur, patella, tibia, metatarsus and tarsus). Leg segments were measured on their dorsal sides. The distribution map was generated with ArcGIS v. 10.2 (ESRI Inc. 2002). The specimens studied are preserved in 75% ethanol and deposited in the College of Life Science, Shenyang Normal University (SYNU) in Liaoning, China and Guizhou Normal University (GZNU) in Guizhou, China.

Terminology and taxonomic descriptions follow Huber (2005) and Yao et al. (2015). The following abbreviations are used in the descriptions: **ALE** = anterior lateral eye, **AME** = anterior median eye, **PME** = posterior median eye, **L/d** = length/diameter; used in the illustrations: **b** = bulb, **ba** = bulbal apophysis, **da** = distal apophysis, **e** = embolus, **ep** = epigynal pocket, **f** = flap, **pa** = proximo-lateral apophysis, **pp** = pore plate, **pr** = procurus.



**Figure 1.** Distribution of Pholcidae treated in this paper 1 *Belisana yuhai* sp. nov. 2 *Leptopholcus tanikawai* 3 *Pholcus spilis* 4 *Spermophora senoculata*.



**Figure 2.** Living specimens of Pholcidae treated in this paper **A, B** *Belisana yuhai* sp. nov. (♂♀) **C, D** *Spermophora senoculata* (♂) **E** *Pholcus spilis* (♂) **F** *Leptopholcus tanikawai* (juvenile). Photographs by Q Lu (Shenzhen).

## Taxonomic accounts

Family Pholcidae C.L. Koch, 1850

Subfamily Pholcinae C.L. Koch, 1850

Genus *Belisana* Thorell, 1898

**Type species.** *Belisana tauricornis* Thorell, 1898.

***Belisana yuhaoi* Yang & Yao, sp. nov.**

<https://zoobank.org/35D4BC52-53E7-47D4-9E54-2FC5291E33DC>

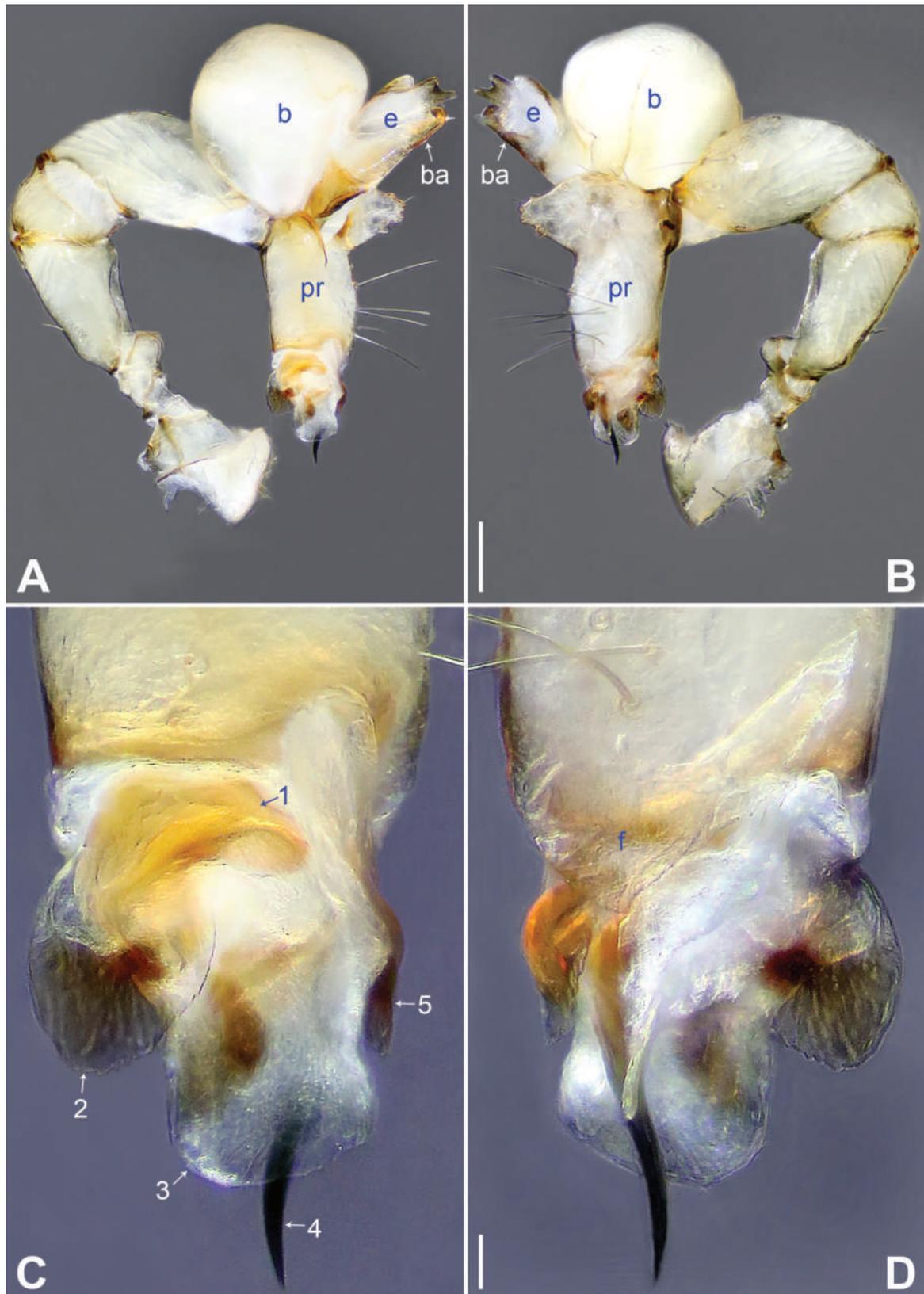
Figs 3, 4

**Type material. Holotype:** ♂ (SYNU-Ar00301), cave without a name (27°5.40'N, 106°30.00'E, 1109 m), Liutong Town, Xiuwen County, Guiyang, **Guizhou, China**, 5 June 2022, H Yu & Q Lu leg. **Paratypes:** 2♂ (SYNU-Ar00302, Ar00303) and 3♀ (SYNU-Ar00304–00306), same data as for the holotype. 1♀ (SYNU-Ar00307), Duobing Cave (27°6.00'N, 106°30.00'E, 1026 m), other data as for the holotype.

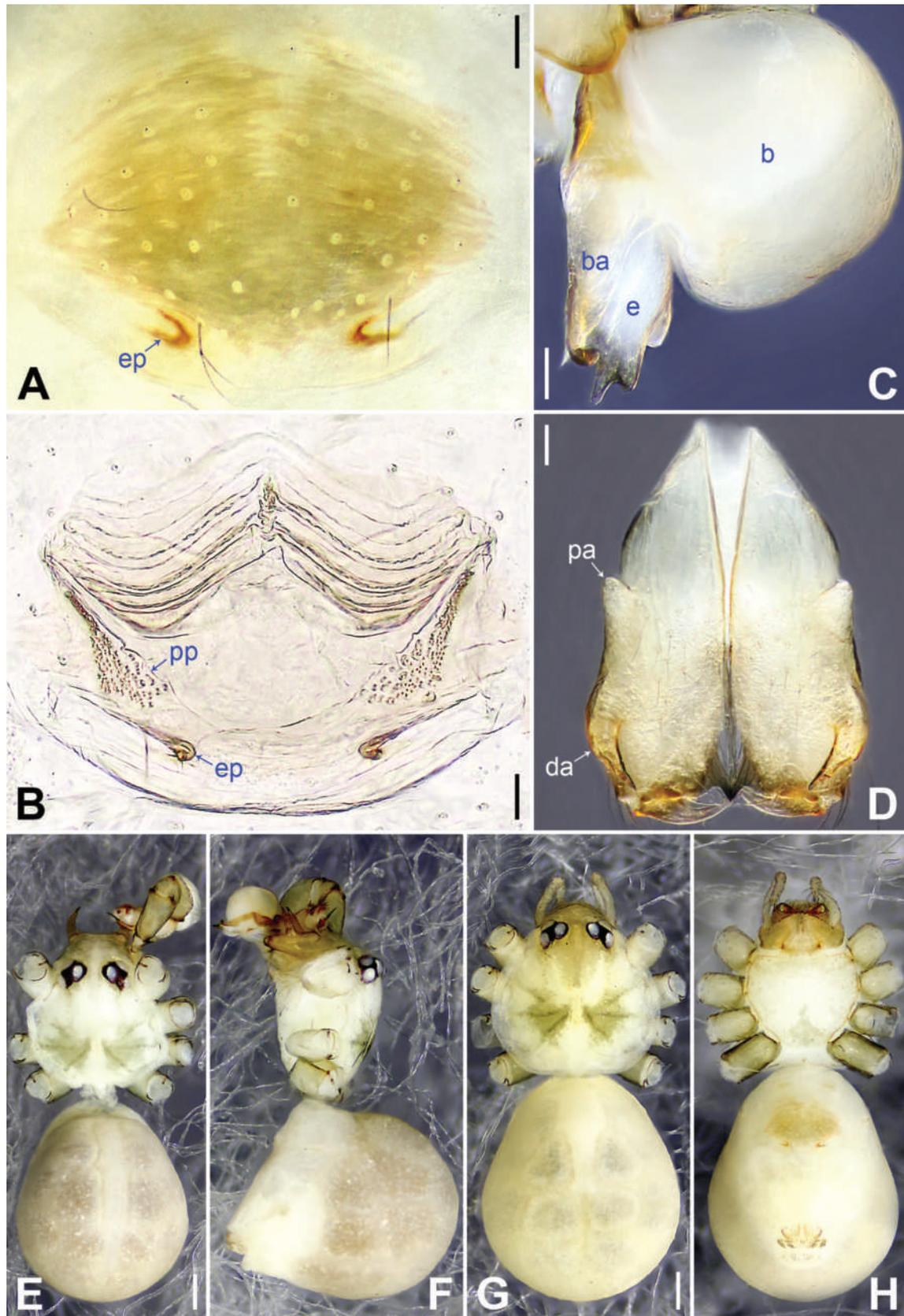
**Etymology.** The specific name is a patronym in honor of the collector Hao Yu; noun (name) in genitive case.

**Diagnosis.** The new species resembles *B. galeiformis* Zhang & Peng, 2011 (Zhang and Peng 2011: 52, fig. 1A–F) with similar male chelicerae and bulbular apophysis (Fig. 4C, D), but it can be distinguished by prolatero-ventral lamella of procurus nearly round (arrow 2 in Fig. 3C; nearly angular in *B. galeiformis*), by distal membranous lamella of procurus laterally weak sclerotized (sclerotized part nearly half-round, arrow 3 in Fig. 3C; with triangular sclerite in *B. galeiformis*), by procurus with triangular retrolateral membranous flap (f in Fig. 3D; flap large and half-round in *B. galeiformis*), by epigynal plate nearly round, posteriorly strongly curved (Fig. 4A; hat-shaped, posteriorly straight in *B. galeiformis*), and by vulval pore plates nearly triangular (Fig. 4B; long elliptical in *B. galeiformis*).

**Description. Male (holotype, SYNU-Ar00301):** total length 1.93 (2.03 with clypeus), prosoma 0.74 long, 0.78 wide, opisthosoma 1.19 long, 0.96 wide. Legs I and IV missing, femur II: 3.92 (other segments missing), leg III: 10.18 (2.97, 0.33, 2.48, 3.56, 0.84). Eye interdistances and diameters: PME–PME 0.13, PME 0.10, PME–ALE 0.04, AME absent. Sternum width/length: 0.65/0.62. Habitus as in Fig. 4E, F. Dorsal shield of prosoma yellowish, with large, brown radiating marks; ocular area and clypeus yellowish, with brown marks; sternum yellowish, with triangular posterior brown marks. Legs whitish, without darker rings. Opisthosoma yellowish, with dorsal and lateral brown spots. Ocular area not elevated. Thoracic furrow absent. Clypeus unmodified. Chelicerae as in Fig. 4D, with a pair of proximo-lateral apophyses and a pair of curved distal apophyses (distance between tips of distal apophyses: 0.21). Palp as in Fig. 3A, B; trochanter with short retrolatero-ventral apophysis; femur with small retrolatero-proximal protrusion; procurus simple proximally but complex distally, with prolatero-subdistal sclerite (arrow 1 in Fig. 3C), sclerotized prolatero-ventral lamella (arrow 2 in Fig. 3C), distal membranous lamella (arrow 3 in Fig. 3C), curved distal spine (arrow 4 in Fig. 3C), sclerotized dorsal apophysis (arrow 5 in



**Figure 3.** *Belisana yuhaoi* sp. nov., holotype male **A, B** palp: **A** prolateral view **B** retrolateral view **C, D** distal part of proscus: **C** prolateral view, arrow 1 indicates prolatero-subdistal sclerite, arrow 2 indicates sclerotized prolatero-ventral lamella, arrow 3 indicates distal membranous lamella, arrow 4 indicates curved distal spine, arrow 5 indicates sclerotized dorsal apophysis **D** retrolateral view. Abbreviations: b = bulb, ba = bulbal apophysis, e = embolus, f = flap, pr = procurus. Scale bars: 0.10 mm (**A, B**); 0.02 mm (**C, D**).



**Figure 4.** *Belisana yuhaoi* sp. nov., holotype male (C–F) and paratype female (A, B, G, H) **A** epigyne, ventral view **B** vulva, dorsal view **C** bulbal apophyses, prolateral view **D** chelicerae, frontal view **E–H** habitus: **E**, **G** dorsal view **F** lateral view **H** ventral view. Abbreviations: b = bulb, ba = bulbal apophysis, da = distal apophysis, e = embolus, ep = epigynal pocket, pa = proximo-lateral apophysis, pp = pore plate. Scale bars: 0.05 mm (A–D); 0.20 mm (E–H).

Fig. 3C), and angular retrolateral membranous flap (f in Fig. 3D); bulb (Fig. 4C) with hooked apophysis and simple embolus.

**Female (paratype, SYNU-Ar00304):** similar to male, habitus as in Fig. 4G, H. Total length 2.03 (2.13 with clypeus), prosoma 0.83 long, 0.91 wide, opisthosoma 1.20 long, 1.04 wide; tibia I: 4.02; tibia I L/d: 50. Eye interdistances and diameters: PME–PME 0.12, PME 0.08, PME–ALE 0.03, AME absent. Sternum width/length: 0.58/0.51. Epigyne (Fig. 4A) simple and flat, with brown marks and a pair of posterior pockets, 0.17 apart (ep in Fig. 4A, B). Vulva (Fig. 4B) with ridge-shaped anterior arch and a pair of nearly triangular pore plates.

**Variation.** In one male paratype (SYNU-Ar00302), leg I: 23.28 (6.02, 0.39, 5.71, 9.36, 1.80); tibia I L/d: 60. Retrolateral trichobothrium of tibia I at 6% proximally; legs with short vertical setae on metatarsi, without spines and curved setae; tarsus I with 22 distinct pseudosegments. Tibia I in another male paratype (SYNU-Ar00303): 5.38. Tibia I in the other two female paratypes (SYNU-Ar00305, Ar00306): 3.28, 3.75 (leg I missing in SYNU-Ar00307).

**Habitat.** The species was found inside cave.

**Distribution.** China (Xiuwen County in Guizhou; type locality, Fig. 1).

### Genus *Leptopholcus* Simon, 1893

**Type species.** *Leptopholcus signifer* Simon, 1893.

#### *Leptopholcus tanikawai* Irie, 1999

*Leptopholcus tanikawai* Irie, 1999: 37, figs 1–5 (♂♀).

*Leptopholcus tanikawai* Irie, 2009: 108, figs 9–11 (♂♀). Huber 2011b: 97, figs 228–231, 273, 274, 395–401, 426, 427 (♂♀). Fu and Chen 2017: 18, figs 2A–E, 3A–E (♂♀).

**New material examined.** 1♂ (GZNU), Taoyuanhe (26°31.80'N, 106°28.20'E, 1237 m), Xiuwen County, Guiyang, **Guizhou, China**, 4 June 2022, H Yu leg. 3♀ (GZNU), a forest park (26°33.60'N, 106°45.60'E, 1165 m), Nanming District, Guiyang, **Guizhou, China**, 10 August 2021, H Yu, H Zhang, D Wang, L Li & J Xin leg. 1 juvenile (GZNU), Guizhou Botanical Garden (26°37.80'N, 106°43.80'E, 1249 m), Yunyan District, Guiyang, **Guizhou, China**, 4 June 2022, H Yu & Q Lu leg.

**Distribution.** China (Xiuwen County, Nanming District and Yunyan District in Guizhou; Fig. 1).

### Genus *Pholcus* Walckenaer, 1805

**Type species.** *Aranea phalangoides* Fuesslin, 1775.

#### *Pholcus spilis* Zhu & Gong, 1991

*Pholcus spilis* Zhu & Gong, 1991: 22, fig. 4A–G (♂♀).

*Pholcus spilis* Song, Zhu and Chen 1999: 59, fig. 24E–H (♂♀). Zhang and Zhu 2009: 83, fig. 47A–G (♂♀). Huber 2011b: 359, figs 1654, 1727, 1728 (♂). Yao and Li 2012: 33, figs 161A–D, 162A–C (♂♀). Yin et al. 2012: 171, fig. 35a–g (♂♀). Zhang 2018: 4, figs 1–3A–F, pl. 1 (♂♀).

**New material examined.** 1♂ (GZNU), Sanchahe (26°16.20'N, 106°48.00'E, 1162 m), Gaopo Town, Huaxi District, Guiyang, **Guizhou, China**, 20 May 2022, H Yu & Q Lu leg.

**Distribution.** China (Huaxi District in Guizhou; Fig. 1).

## Genus *Spermophora* Hentz, 1841

**Type species.** *Spermophora meridionalis* Hentz, 1841.

### *Spermophora senoculata* (Dugès, 1836)

See World Spider Catalog 2023

**New material examined.** 2♂ (GZNU) and 2♀ (GZNU), Sanchahe (26°16.20'N, 106°48.00'E, 1162 m), Gaopo Town, Huaxi District, Guiyang, **Guizhou, China**, 20 May 2022, H Yu & Q Lu leg. 1♂ (GZNU), Laobanghe (26°20.40'N, 106°43.80'E, 1022 m), Qiantao Town, Huaxi District, Guiyang, **Guizhou, China**, 18 May 2022, H Yu & Q Lu leg.

**Distribution.** China (Huaxi District in Guizhou; Fig. 1).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

ZY and QH designed and funded the study. LY and ZY performed morphological species identification. LY and FZ finished the species descriptions and took the photos. LY and ZY drafted the manuscript. QH and ZY revised the manuscript. All authors read and approved the final version of the manuscript.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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# On *Psalmopoeus* Pocock, 1895 (Araneae, Theraphosidae) species and tarantula conservation in Ecuador

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## Abstract

Two novel species of *Psalmopoeus* Pocock, 1895 are described from the north-western and central-western slopes of the Cordillera Occidental of the Andes mountain range in Ecuador. The new species are easily differentiated from other congeners of *Psalmopoeus* by spermathecae and male palpal bulb morphology and a comparatively distant distribution to the type localities of the geographically nearest known congeners. The diagnosis of *P. ecclesiasticus* Pocock, 1903 is revised and updated, considering the novel species and observations on spermatheca of this species. Likewise, an evaluation is provided for the new species in terms of conservation due to the various threats impacting ecosystems and ecosystem services of their type localities. Finally, the importance of theraphosid spiders in Ecuador and South America and their possible conservation requirements are discussed and assessed.

**Key words:** Andes, Choco, pet trade, Psalmopoeinae, smuggling, taxonomy, western Ecuador



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## Introduction

*Psalmopoeus* Pocock, 1895 includes arboreal Psalmopoeinae spiders Samm & Schmidt, 2010 diagnosed from all other members of the subfamily Psalmopoeinae mainly by the presence of maxillary lyra with one row of thick and rough stridulatory setae (Cifuentes and Bertani 2022). This arboreal clade currently has ten valid species distributed largely in Central America down to South America; Ecuador is the southernmost record for the genus with *Psalmopoeus ecclesiasticus* Pocock, 1903, with the Antilles (Cifuentes and Bertani 2022; WSC 2023).

Literature on Ecuadorian Psalmopoeinae spiders is scarce, with only three valid species described from the country (WSC 2023). These are distributed along the western slopes of Cordillera Occidental of the Andes, the eastern slopes of the Cordillera Real Oriental of the Andes, and lowland Amazonia, *Psalmopoeus ecclesiasticus* Pocock, 1903, *Amazonius elenae* (Schmidt, 1994),

and *Tapinauchenius cupreus* Schmidt & Bauer, 1996 respectively (Pocock 1903; Hüsser 2018; Gabriel and Sherwood 2019; Cifuentes and Bertani 2022). This paper aims to describe two new species of *Psalmopoeus* recently discovered in the Province of Cotopaxi in the central-western region of Ecuador, the Province of Santo Domingo de Los Tsachilas, and the Province of Pichincha in western Ecuador. Additionally, we provide the first commentaries and suggestions about the conservation of tarantulas in Ecuador and the possible threats that Theraphosidae may face in terms of extinction.

## Materials and methods

Examined specimens are deposited at Museo de Zoología, Universidad San Francisco de Quito, Ecuador (**ZSFQ-i**) and Museo de Zoología, Pontificia Universidad Católica del Ecuador (**QCAZ-I**). Information on species for comparative diagnoses were obtained from actual redescriptions and descriptions of *Psalmopoeus* species (Mendoza 2014; Gabriel and Sherwood 2019; Cifuentes and Bertani 2022).

Specimens from ZSFQ-i were examined and measured under an Olympus SZX16 stereomicroscope with an Olympus DP73 digital camera. Specimens from QCAZ-I were examined and measured under a Nikon SMZ745T stereomicroscope with an Mshot MS60. All measurements are presented in millimetres. Female genitalia were excised using a syringe tip; soft tissue was digested with a solution of 15% KOH, washed in distilled water and 75% ethanol, and examined under an Olympus SZX16 stereomicroscope. Compound images were obtained by stacking a series of photographs taken at different depths processed with the stacking software of Photoshop and editing tools.

Biogeographic classification follows the proposal by Morrone (2014), with modifications proposed by Cisneros-Heredia and Yáñez-Muñoz (2007) and Cisneros-Heredia (2006, 2007, 2019). Ecuadorian classification of ecosystems follows MAE (2013). Shape files of cropland use and mining concessions were extracted from Potapov et al. (2022) and Peñaherrera-Romero (2023). Conservation categories and criteria follow IUCN (2001).

General description and measurements follow standards proposed by Gabriel and Sherwood (2019) and Cifuentes and Bertani (2022) for the genus *Psalmopoeus*. Bulb length proportion follows measurements proposed by Cifuentes and Bertani (2022). Leg spination description follows Cifuentes and Bertani (2022). The term longitudinal folds follows, in part, Dupérré and Tapia (2020).

The type locality and historical distribution of *P. ecclesiasticus* was obtained from the original description of Pocock (1903) with additional information from Peters (1955), Lynch and Duellman (1997), and Gabriel and Sherwood (2019). Additional records of *P. ecclesiasticus* distribution were obtained from the examined specimen deposited in the QCAZ-I collection and Cifuentes and Bertani (2022) records.

Morphological somatic abbreviations: **AME**, anterior median eyes; **ALE**, anterior lateral eyes; **PME**, posterior median eyes; **PLE**, posterior lateral eyes. Female: **iLB**, ill-defined lobe; **wLB**, well-defined lobe.

## Taxonomic account

**Theraphosidae Thorell, 1869**

**Psalmopoeinae Samm & Schmidt, 2010**

***Psalmopoeus* Pocock, 1895**

***Psalmopoeus chronoarachne* sp. nov.**

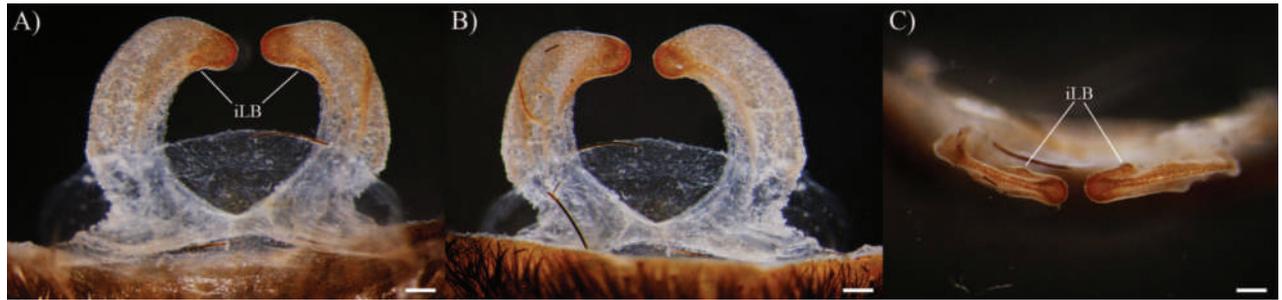
<https://zoobank.org/F87ABBB2-B8AB-44F0-A788-BA0DFFC51342>

Figs 1–3

**Material examined. Holotype:** REPUBLIC OF ECUADOR • 1 ♀; Province of Cotopaxi, Canton Pangua, Parish of El Corazón, Hacienda La Mariela; -1.0856, -79.1841, 760 m a.s.l.; 27 February 2023; M. López-García, J. Montalvo, D. Brito-Zapata and C. Reyes-Puig leg.; ZFSQ-i11704.

**Diagnosis.** *Psalmopoeus chronoarachne* sp. nov. can be distinguished from its known congeners by spermathecal morphology, specifically: from *Psalmopoeus satanas* sp. nov. by having only a single ill-defined lobe on each receptacle, absence of well-defined lobes, apical digitiform lobe, comparatively receptacles more curved towards the centre (Fig. 1) (two ill-defined lobe and a single domed well-defined lobe in receptacles, apical digitiform lobe present and receptacles comparatively less curved towards the centre in *P. satanas* sp. nov.; Fig. 8); from *P. ecclesiasticus* by having comparatively less curved receptacles towards the centre and distant to each other, distal apex more curved and not overlapping, receptacles with only a single ill-defined lateral lobe on apical-inner and apical digitiform lobe absent (Fig. 1) (comparatively more curved receptacles towards the centre, distal apex less curved and overlapping, receptacles with apical digitiform lobe, one to four protruding well-defined lobes, and a single ill-defined lobe in *P. ecclesiasticus*; Fig. 10; see also Gabriel and Sherwood 2019: fig. 1; Cifuentes and Bertani 2022: figs 224, 229); from *P. cambridgei*, *P. irminia*, *P. pulcher*, *P. langenbucheri*, *P. reduncus*, and *P. victori* by having elongated and curved receptacles towards the centre with distal apex curved with only a single ill-defined lateral lobe on apical-inner and apical digitiform lobe absent (elongated and straight receptacles with distal apex straight with apical digitiform lobe and various central lobes in *P. cambridgei*, *P. irminia*, *P. pulcher*; elongated and triangular receptacles with distal apex straight, comparatively more sclerotised, thinner, and shorter apical digitiform lobe pointing upwards, not overlapping but very close and two to three well-defined lateral lobes in *P. langenbucheri*; short and triangular receptacles with distal apex straight, comparatively more elongated apical digitiform lobe pointing upwards but not overlapping and only a single ill-defined lateral lobe in *P. reduncus*; elongated and straight receptacles with distal apex slightly curved upwards or straight without receptacles in *P. victori*; see figures in Mendoza 2014: figs 27, 28; Cifuentes and Bertani 2022: figs 125, 170–175, 190–191, 215, 245, 268–271, 283, 300, 309).

**Description. Female holotype** (ZSFQ-i11704): Total length including chelicerae: 30.48. Carapace: length 11.84, width 10.50. Caput: slightly raised. Ocular tubercle: slightly raised, length 1.34, width 3.01. Eyes: ALE > AME, AME > PLE, PLE > PME, anterior eye row straight, posterior row slightly recurved. Clypeus: wide; clypeal fringe long. Fovea: straight. Chelicera: length 6.45, width 2.70.



**Figure 1.** *Psalmopoeus chronoarachne* sp. nov. female holotype (ZSFQ-i11704), spermatheca **A** dorsal view **B** ventral view **C** apical view. Abbreviation: iLB, ill-defined lobe. Scale bars: 0.2 mm.



**Figure 2.** *Psalmopoeus chronoarachne* sp. nov. female holotype (ZSFQ-i11704): Maxillae (left side) showing maxillary lyra. Scale bar: 0.2 mm.

Abdomen: length 12.19, width 6.84. Maxilla with 147–224 cuspules covering approximately 30% of the proximal edge. Labium: length 2.05, width 1.65, with 163 cuspules most separated by 1.0–2.0× the width of a cuspule. Labio-sternal mounds joined along the entire base of the labium. Sternum: length 5.33, width 4.42, with two pairs of sigilla. Tarsi I–IV fully scopulate, tarsi I and II divided by narrow strip of longer and thicker setae, Tarsus III–IV divided by wide strip of longer and wider setae. Metatarsal scopulae: I 90%; II 90%; III 65%; IV 50%. For lengths of legs and palpal segments see Table 1; legs 4, 1, 2, 3. Spination: Leg IV: metatarsus v 0-0-0 (2ap). Palp: tibia v 0-0-0 (2ap). Posterior lateral spinnerets with three segments, basal 2.07, median 0.86, digitiform apical 1.61. Lateral median spinnerets with one segment. Stridulation organ with eight primary lyra on left maxilla, eight on right; primary lyra wider from base to apex (Fig. 2). Spermatheca (Fig. 1) with two elongate asymmetrical receptacles and distant to each other, curved towards the centre and distal apex more curved and more sclerotised; apex constricted but wide. Two dorsal longitudinal folds and three ventral longitudinal folds on left receptacle, three dorsal longitudinal folds and



**Figure 3.** *Psalmopoeus chronoarachne* sp. nov. female holotype (ZSFQ-i11704), live habitus **A** dorsolateral view **B** dorsal view. Scale bars: 10 mm.

**Table 1.** *Psalmopoeus chronoarachne* sp. nov. female holotype (ZFSQ-i11704), podomere measurements.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
<b>I</b>	11.31	6.16	9.63	7.35	5.44	39.89
<b>II</b>	10.58	5.34	9.21	7.05	5.40	37.58
<b>III</b>	8.68	4.55	7.62	6.92	5.01	32.78
<b>IV</b>	11.03	4.85	10.03	9.74	5.03	40.68
<b>Palp</b>	7.68	4.47	5.44	–	6.44	24.03

two ventral longitudinal folds on right receptacle. Each receptacle with a single ill-defined lateral lobe on apical-inner, each lobe disposed on the most inner longitudinal fold. Colouration: carapace and legs covered with short and long bright golden setae, abdomen covered with short black setae and long reddish setae (Fig. 3).

**Etymology.** The specific epithet is a noun in apposition referring to the combination of the Greek words *chrono* (χρόνο), in reference to time, and *arachne* (Αράχνη), meaning spider. The compound word refers to the adage that these spiders could “have their time counted” or reduced by impactful anthropogenic activities. The name addresses conservation concerns about the survival and prevalence of spider species in natural environments.

**Distribution.** *Psalmopoeus chronoarachne* sp. nov. is only known from its type locality, Hacienda La Mariela at 760 m, Province of Cotopaxi, in the central area of the Cordillera Occidental of the Andes of Ecuador (Figs 11, 12).

**Ecology.** The holotype of *Psalmopoeus chronoarachne* sp. nov. (Fig. 3) was found in the foothill evergreen forest of the Cordillera Occidental of the Andes in the Western Ecuador biogeographic province (Figs 11, 12). The spider was observed on a tree at approximately 1.5 m up from the forest floor.

***Psalmopoeus satanas* sp. nov.**

<https://zoobank.org/A69A2394-F71C-43E6-9414-891D4601F542>

Figs 4–8

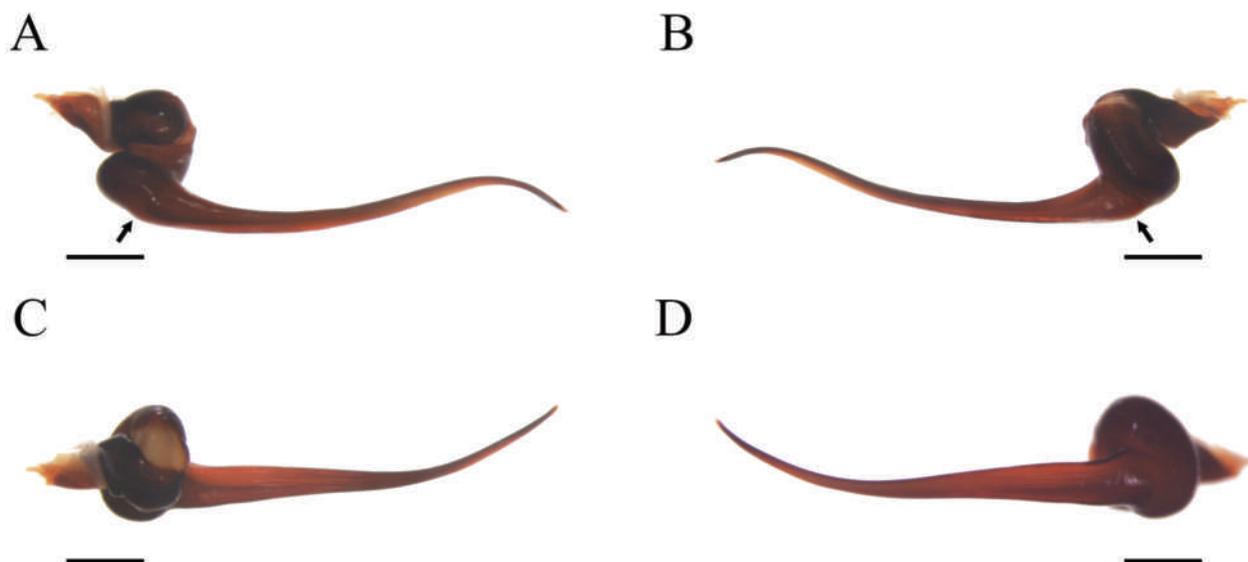
*Psalmopoeus ecclesiasticus* Pocock, 1903: Cifuentes and Bertani 2022: 217–235 (in part, misidentification).

**Material examined. Holotype:** REPUBLIC OF ECUADOR • 1 ♂; Province of Santo Domingo de los Tsáchilas, Canton Santo Domingo, Parish of San José de Alluriquín, Reserva Otongachi - Fundación Otonga; -0.3209, -78.9513, 866 m a.s.l.; 24 May 2021; R. J. León-E, R. F. Valencia, and S. Cortese leg.; ZSFQ-i12150 (Field code: OG-Satanas).

**Paratypes:** REPUBLIC OF ECUADOR • 1 ♀; Province of Pichincha [= Province of Santo Domingo de los Tsáchilas], Canton Santo Domingo, Parish of San José de Alluriquín, La Magdalena; -0.2647, -79.0256, 920 m a.s.l.; 02 November 1995; B. Yangari leg.; QCAZ-i274324 (Field code: MYGA 08). REPUBLIC OF ECUADOR • 1 ♀; Province of Pichincha, Canton San Miguel de Los Bancos, Parish of Mindo, Los Bancos; 0.0166, -78.8833, 909 m a.s.l.; 17 December 1988; V. Navarrete leg.; QCAZ-i274323 (Field code: MYGA 40).

**Additional material.** REPUBLIC OF ECUADOR • 1 sub ♀; Province of Santo Domingo de los Tsáchilas, Canton Santo Domingo, Parish of San José de Alluriquín, Reserva Otongachi - Fundación Otonga; -0.3209, -78.9517, 937 m a.s.l.; 05 October 2017; A. Tadashima leg.; ZSFQ-i12156 (Field code: AT16).

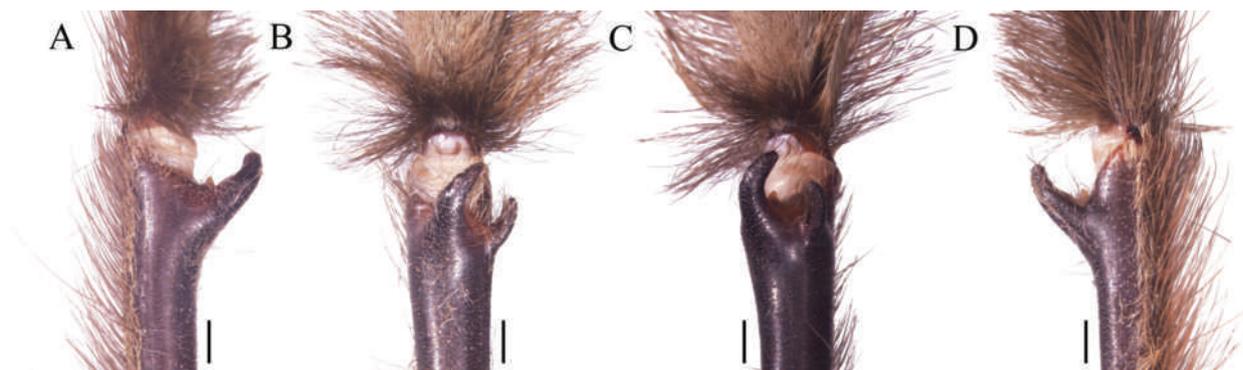
**Diagnosis.** *Psalmopoeus satanas* sp. nov. can be distinguished from known congeners by the morphology of male palpal bulb and by female spermathecal morphology. Males of *Psalmopoeus satanas* sp. nov. can be distinguished from all other male congeners by having a slender embolus slightly curved, almost straight at distal part (Fig. 4A–D), the presence of a prominent ventral dilatation (Fig. 4A, B), and the embolus being ~ 4× the tegulum length in retrolateral view (Fig. 4B) (ventral dilatation unknown in all other congeners, for comparative measurements see Cifuentes and Bertani 2022). Additionally, males of *Psalmopoeus satanas* sp. nov. can be distinguished from *P. cambridgei*, *P. reduncus*, *P. pulcher*, *P. irminia*, *P. victori* by the absence of a distal thickening in retrolateral branch of tibial apophysis (distal thickening present in retrolateral branch of tibial apophysis in *P. cambridgei*, *P. reduncus*, *P. pulcher*, *P. irminia*, *P. victori*; see also Gabriel and Sherwood 2020: figs 13, 14; Cifuentes and Bertani 2022: figs 150–152, 187–189, 209–211, 242–244, 253–255, 280–282). Females of *Psalmopoeus satanas* sp. nov. can be distinguished from *Psalmopoeus chronoarachne* sp. nov. by having two ill-defined lobes and a single domed well-defined lobe in receptacles, apical digitiform lobe present and comparatively receptacles less curved towards the centre (Fig. 8) (only a single ill-defined lobe on each receptacle, absence of well-defined lobes, apical digitiform lobe, comparatively receptacles more curved towards the centre in *Psalmopoeus chronoarachne* sp. nov.; Fig. 1); from *P. ecclesiasticus* by having straight receptacles, distal apex curved towards the centre and overlapping, comparatively less sclerotised wider and longer apical digitiform lobe pointing upwards and only two ill-defined lateral lobe and a single domed well-defined lateral lobe on apical-inner (Fig. 8) (curved receptacles towards the centre, distal apex more curved and overlapping, receptacles comparatively with more



**Figure 4.** *Psalmopoeus satanas* sp. nov. male holotype (ZSFQ-i12150), palpal bulb (right hand side), arrow indicates ventral dilatation of embolus **A** prolateral view **B** retrolateral view **C** dorsal view **D** ventral view. Scale bars: 1.5 mm.

sclerotised, thin and shorter apical digitiform lobe pointing downwards, one to four protruding well-defined lobes and a single ill-defined lobe in *P. ecclesiasticus* (Fig. 10); see also Gabriel and Sherwood 2019: fig. 1; Cifuentes and Bertani 2022: figs 224, 229); from *P. cambridgei*, *P. irminia*, *P. pulcher*, *P. langenbuchi*, *P. reduncus*, and *P. victori* by having elongated and straight receptacles with distal apex curved with the combination of only two ill-defined lateral lobe and a single domed well-defined lateral lobe on apical-inner and narrow apical digitiform lobe overlapping each other (elongated and straight receptacles with distal apex straight, comparatively more sclerotised narrow apical digitiform lobe pointing upwards but not overlapping and two to three protruding and well-defined lobes at centre in *P. cambridgei*; elongated and straight receptacles with distal apex straight, comparatively more sclerotised wider apical digitiform lobe pointing upwards but not overlapping and a single well-defined lobe at centre of each receptacle in *P. irminia*, elongated and straight receptacles with distal apex straight, comparatively more sclerotised thin apical digitiform lobe pointing upwards but not overlapping and numerous lobes at centre or lateral which reduce in size from apex to centre in *P. pulcher*; elongated and triangular receptacles with distal apex straight, comparatively more sclerotised wider and shorter apical digitiform lobe pointing upwards, not overlapping but very close and two to three well-defined lateral lobes in *P. langenbuchi*, short and triangular receptacles with distal apex straight, comparatively more sclerotised wider and shorter apical digitiform lobe pointing upwards but not overlapping and only a single ill-defined lateral lobe in *P. reduncus*; comparatively more elongated and straight receptacles with wider distal apex without receptacles in *P. victori*; see also Mendoza 2014: figs 27, 28; Cifuentes and Bertani 2022: 125, 170–175, 190, 191, 215, 245, 268–271, 283, 300, 309).

**Description. Male holotype** (ZSFQ-i12150): Total length including chelicerae: 29.10. Carapace: length 12.60, width 11.62. Caput: slightly raised. Ocular tubercle: slightly raised, length 2.21, width 3.14. Eyes: ALE > PLE, PLE < AME, AME > PME, anterior eye row recurved, posterior row recurved. Clypeus: wide; clypeal

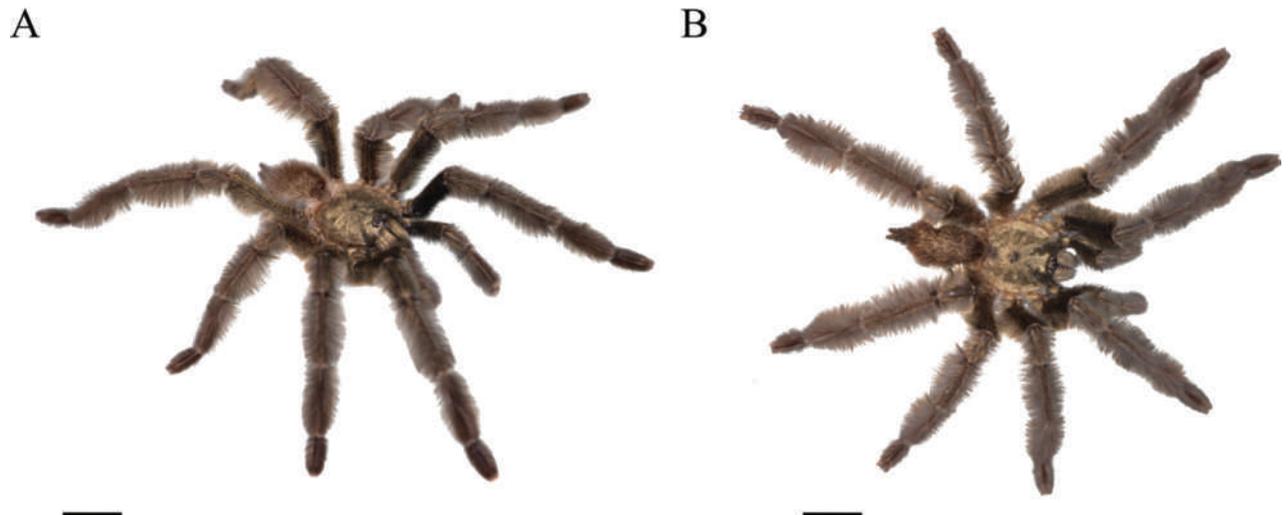


**Figure 5.** *Psalmopoeus satanas* sp. nov. male holotype (ZSFQ-i12150), tibial apophysis (left hand side) **A** retrolateral view **B** dorsal view **C** dorso-retrolateral view **D** prolateral view. Scale bars: 5 mm.

fringe long. Fovea: recurved. Chelicera: length 4.25, width 2.57. Abdomen: length 11.35, width 6.32. Maxilla with 137 cuspules covering approximately 20% of the proximal edge. Labium: length 1.77, width 2.25, with 131 cuspules most separated by 1.0–2.0× the width of a single cuspule. Labio-sternal mounds joined along the entire base of the labium. Sternum: length 6.57, width 4.53, with two pairs of elongated sigilla. Tarsi I–IV fully scopulate, Metatarsal scopulae: I 95%; II 90%; III 80%; IV %, metatarsi I divided by up to half of the segment, metatarsi IV divided by a strip of longer and wider setae. For lengths of legs and palpal segments see Table 2; legs 1, 4, 2, 3. Spination: Leg II: tibia v 0-0-0 (1ap). Leg III: metatarsus v 0-0-0 (3ap). Leg IV: metatarsus v 0-0-0 (1ap). Tibia I with principal paired tibial apophysis and a short, irregular, and triangular central third apophyses, RB longer than PB, RB and PB with one megaspine (Fig. 5). Posterior lateral spinnerets with three segments, basal 3.32, median 1.14, digitiform apical 2.01. Lateral median spinnerets with one segment. Stridulation organ with 12 primary lyra on left maxilla (two of them widely separated and proximal to basal section of maxilla), ten on right (one of them slightly thinner, separated, and proximal to basal section of maxilla, slight scar on individual); other primary lyra wider from base to apex (Fig. 7). Palp (Fig 4): tegulum length 1.53, width 0.713, embolus proximal width 0.66, length 6.41. Embolus proximal portion slightly curved with a prominent ventral dilatation in medial section. Embolus length to tegulum length: 4.18. Embolus distal third slightly curved to ventral and retrolateral sides; retrolateral curvature almost straight. Embolus tapers to the tip ending in a straight tip. Colouration: abdomen, carapace, and legs covered with short and long pale golden setae (Fig. 6). After two years in preservative, with pale grey colouration and brown setae.

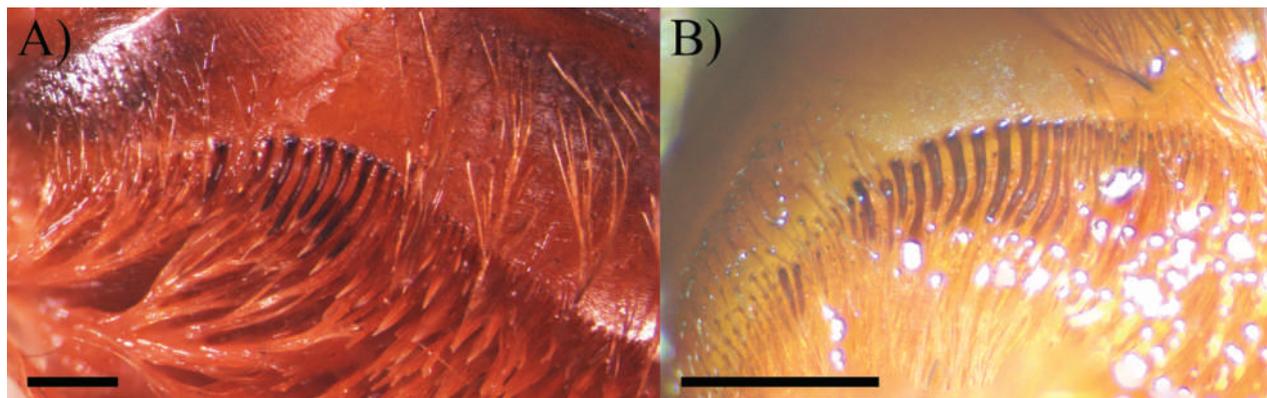
**Table 2.** *Psalmopoeus satanas* sp. nov. male holotype (ZSFQ-i12150), podomere measurements.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
<b>I</b>	16.14	6.91	14.11	13.34	6.66	56.69
<b>II</b>	14.89	6.20	13.02	12.34	5.87	52.42
<b>III</b>	12.58	5.16	10.01	11.69	6.02	44.97
<b>IV</b>	15.18	5.53	13.12	14.87	6.44	55.16
<b>Palp</b>	9.20	4.55	8.51	–	2.81	25.21

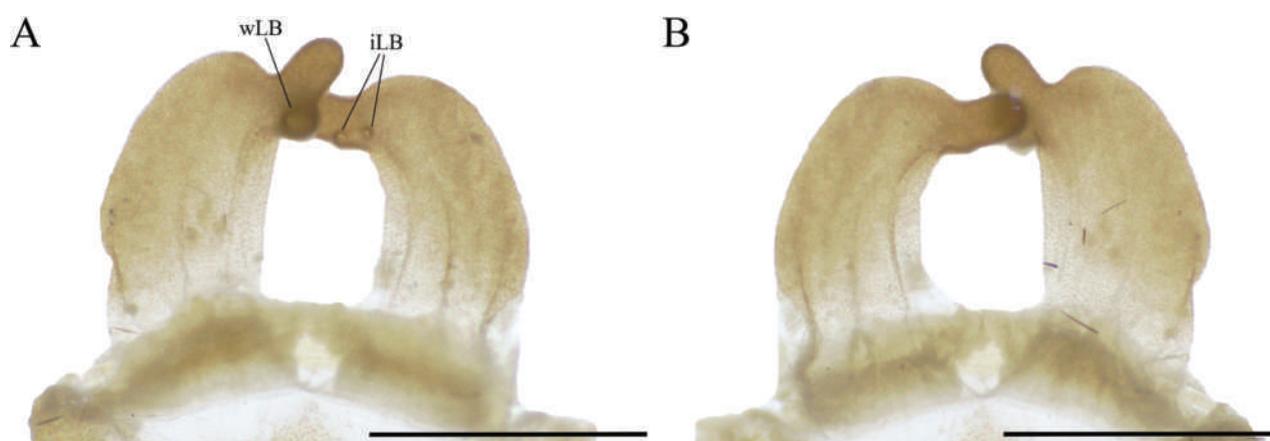


**Figure 6.** *Psalmopoeus satanas* sp. nov. male holotype (ZSFQ-i12150), live habitus **A** dorsolateral view **B** dorsal view. Scale bars: 10 mm.

**Female paratype** (QCAZ-i274324): Total length including chelicerae: 46.26. Carapace: length 16.45, width 15.27. Caput: slightly raised. Ocular tubercle: slightly raised, length 1.34, width 3.01. Eyes: AME > ALE, AME > PLE, PLE > PME, anterior eye row straight, posterior row slightly recurved. Clypeus: wide; clypeal fringe long. Fovea: straight. Chelicera: length 7.18, width 3.88. Abdomen: length 22.63, width 14.09. Maxilla with 170–183 cuspules covering approximately 50% of the proximal edge. Labium: length 2.46, width 2.68, with 157 cuspules most separated by 1.0–2.0× the width of a cuspule. Labio-sternal mounds joined along the entire base of the labium. Sternum: length 9.32, width 7.86, with two pairs of elongated sigilla. Tarsi I–IV fully scopulate, tarsi IV divided by wide strip of longer and thicker setae, Metatarsus IV divided by wide strip of longer and wider setae up to the half of the segment. Metatarsal scopulae: I 100%; II 100%; III 75%; IV 25%. For lengths of legs and palpal segments see Table 3; legs 4, 1, 2, 3. Spination: Leg II: tibia v 0-0-0 (1ap). Leg III: metatarsus v 0-0-0 (2ap). Leg IV: metatarsus v 0-0-0 (2ap). Palp: tibia v 0-0-0 (1ap). Posterior lateral spinnerets with three segments, basal 3.24, median 2.03, digitiform apical 2.37. Lateral median spinnerets with one segment. Stridulation organ with 15 primary lyra on left maxilla (two of them considerably thinner, widely separated, and proximal to basal section of maxilla), 13 on right (two of them considerably thinner, widely separated, and proximal to basal section of maxilla) (Fig. 7B); other primary lyra wider from base to apex. Spermatheca (Fig. 8) with two elongate asymmetrical receptacles overlapping each other, usually straight and distal apex curved towards the centre and more sclerotised; apex constricted with narrow apical lobe pointing upwards. Three dorsal longitudinal folds and ventral longitudinal folds absent on left receptacle, three dorsal longitudinal folds and ventral longitudinal folds absent on right receptacle. Left receptacle with a single well-defined lobe on apical-inner disposed on the most inner longitudinal fold. Right receptacle with two ill-defined lateral lobes on apical-inner, each lobe disposed on the most inner longitudinal fold. Colouration: after 30 years in preservative, with a dark brown colouration and pale brown setae (Fig. 9).



**Figure 7.** *Psalmopoeus satanas* sp. nov. Maxillae showing maxillary lyra **A** male holotype (ZSFQ-i12150) (right hand side) **B** female paratype (QCAZ-i274324) (left side). Scale bars: 0.4 mm (**A**), 0.2 mm (**B**).



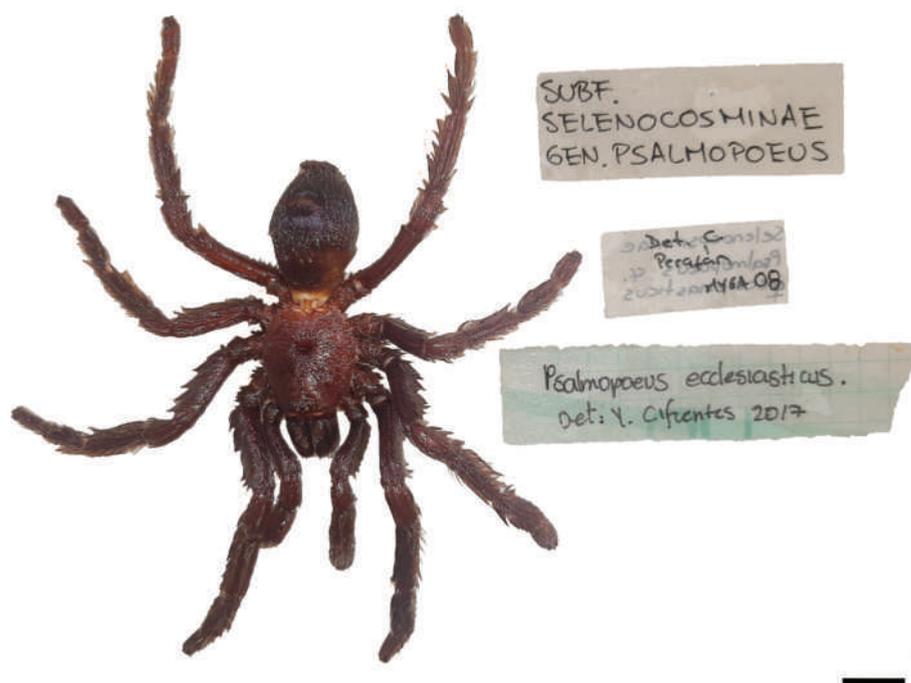
**Figure 8.** *Psalmopoeus satanas* sp. nov. female paratype (QCAZ-i274324), spermatheca **A** dorsal view **B** ventral view. Abbreviations: iLB, ill-defined lobe; wLB, well-defined lobe. Scale bars: 2 mm.

**Table 3.** *Psalmopoeus satanas* sp. nov. female paratype (QCAZ-i274324), podomere measurements.

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
<b>I</b>	13.68	10.01	10.22	8.05	6.73	48.69
<b>II</b>	10.49	8.00	10.53	9.03	5.06	43.11
<b>III</b>	7.72	6.26	8.14	7.22	6.28	35.62
<b>IV</b>	13.77	6.14	12.9	9.36	5.62	47.79
<b>Palp</b>	8.68	6.31	5.86	–	7.12	27.97

**Variation.** (QCAZ-i274323) Stridulation organ with 9 primary lyra on left maxilla (two of them considerably thinner, widely separated, and proximal to basal section of maxilla), 11 on right (one of them considerably thinner, widely separated, and proximal to basal section of maxilla).

**Etymology.** The specific epithet is a noun in apposition honouring the nickname of the holotype male *Satanas*. The members of the Mygalomorphae Research Group in the Laboratory of Terrestrial Zoology at Universidad San Francisco de Quito grew very fond of this individual during its care, in spite of the individual's bad temperament and sporadic attacks (reason for the nickname).



**Figure 9.** *Psalmopoeus satanas* sp. nov. female paratype, habitus and previous examiner labels. Scale bar: 15 mm.

**Distribution.** *Psalmopoeus satanas* sp. nov. is known from the localities La Magdalena and Reserva Otongachi in the Province of Santo Domingo de los Tsáchilas and Los Bancos in the province of Pichincha. The new species is distributed across an altitudinal range of 866–937 m, in the north of the Cordillera Occidental of the Andes of Ecuador (Figs 11, 12).

**Ecology.** *Psalmopoeus satanas* sp. nov. is found in low montane and montane evergreen forest of the Cordillera Occidental of the Andes, in the Western Ecuador biogeographic province. The male holotype was found within a bamboo fence and exhibited defensive behaviour when observed. This behaviour then transformed into fleeing, where the spider made quick sporadic movements, nearly too fast to see.

**Remarks.** Previously the female paratypes were examined by Carlos Perafán during his doctoral thesis about historical and actual distribution of Mygalomorphae from the northern Andes (Perafán 2017). During his revision he identified the female paratype (QCAZ-i274324) as *Psalmopoeus* cf. *ecclesiasticus* and the other female paratype (QCAZ-i274323) as *Psalmopoeus* sp., each one with a respective handwritten label (Fig. 9). Prior to this, Yeimy Cifuentes examined the same specimens for her taxonomic revision and cladistic of the subfamily Psalmopoeinae and concluded that both were *Psalmopoeus ecclesiasticus*, also including a new handwritten label stating the identification of each specimen and reporting each locality for the distribution of the previously mentioned species (Cifuentes and Bertani 2022).

During the recent revision of these specimens by the first author, it was observed that the spermathecae of both specimens and also a third, also examined by Carlos Perafán and Yeimy Cifuentes which certainly is *Psalmopoeus ecclesiasticus* (Fig. 10) and was collected near the type locality, were not completely

cleaned and that only the left receptacle of the female paratype (QCAZ-i274323) of *Psalmopoeus satanas* sp. nov. was properly cleaned, making it impossible to observe the complete morphology of apical lobe and number of lobes. This led to both Peráfan and Cifuentes making erroneous identifications; although Carlos opted for a more conservative approach. Additionally, the right receptacle of the female paratype (QCAZ-i274323) of *Psalmopoeus satanas* sp. nov. was broken by someone who previously examined the specimen.

Morphology of tibial apophyses has been used for cladistics analysis in *Psalmopoeinae* and in some cases for species diagnoses (e.g., *P. langenbuchi*; Cifuentes and Bertani 2022) using some characters related to spines combination, branches development, origin of each branch, and morphology of central protuberance behind the two branches (Hüsser 2018; Cifuentes and Bertani 2022). Nevertheless, intra-specific variation has not yet been fully explored and some characters may or may not be reliable for proposing synapomorphies for previously known species or new ones; we tentatively use the distal thickening of retrolateral branch as secondary character to differentiate *P. satanas* sp. nov. from *P. cambridgei*, *P. reduncus*, *P. pulcher*, *P. irminia*, *P. victori*. Cifuentes and Bertani (2022) used the shape of the central protuberance as diagnostic character for *P. langenbuchi*. However, it should be noted that significant variation of width and length of this structure have been observed between left and right tibial apophysis in the male holotype (ZSFQ-i12150). For this reason, we encourage future researchers to evaluate intra- and inter-specific variation in order to confirm the validity of these tibial apophysis characters in species diagnosis and to evaluate morphometric aspects of other structures (e.g., leg segment ratios and spermathecae measurements; Hamilton et al. 2016; Gabriel and Sherwood 2020).

### ***Psalmopoeus ecclesiasticus* Pocock, 1903**

Fig. 10

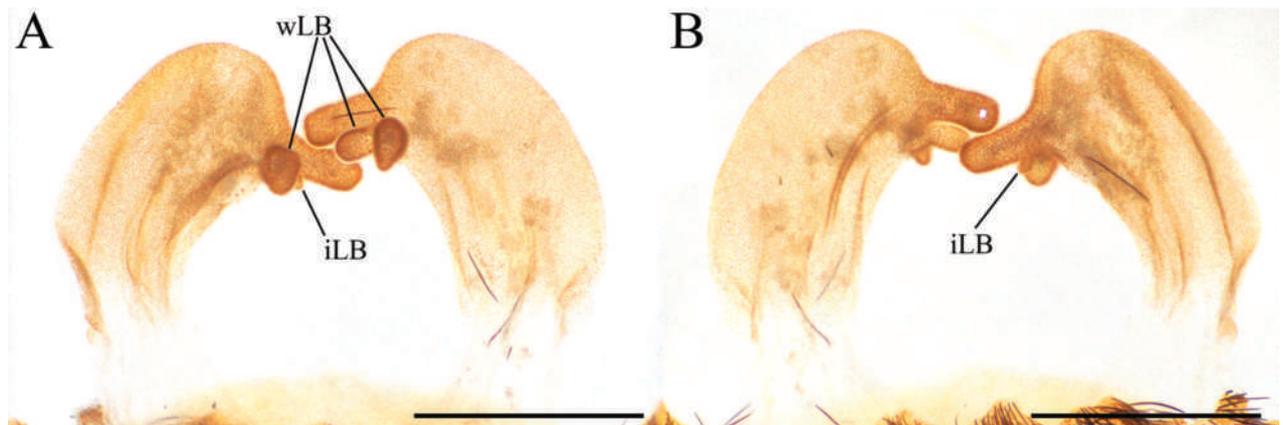
*Psalmopoeus ecclesiasticus*: Schmidt, Bullmer, and Thierer-Lutz (2006): 8, fig. 10.

*Psalmopoeus ecclesiasticus*: Gabriel and Sherwood (2019): 41, figs 1–10.

*Psalmopoeus ecclesiasticus*: Cifuentes and Bertani (2022): 77, figs 2, 217–235.

**Material examined. Non-type material:** Republic of Ecuador • 1 ♀; Province of Esmeraldas, Canton San Lorenzo, Parish of Alto Tambo, Alto Tambo; 0.9000,-78.5333, 790 m a.s.l.; 07 December 2002; D. Salazar leg.; QCAZ-i274322 (Field code: MYGA 158).

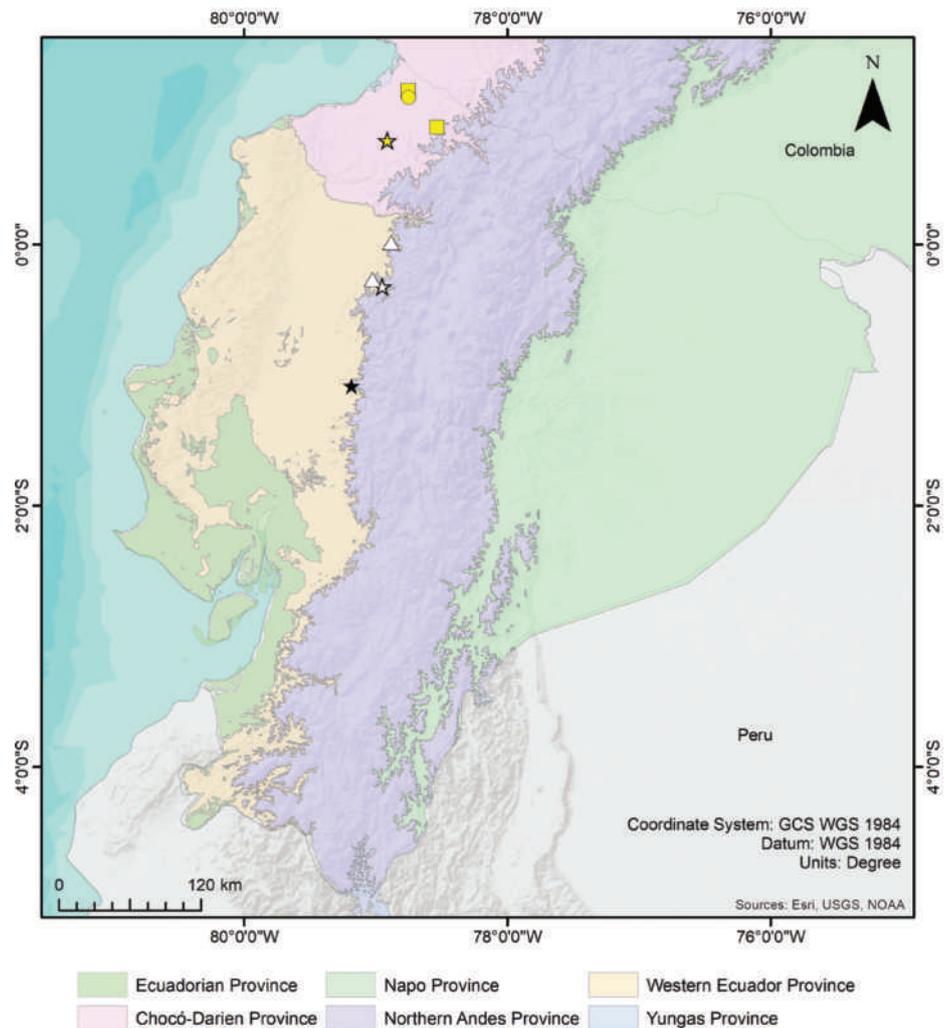
**Amended diagnosis.** Females of *Psalmopoeus ecclesiasticus* can be distinguished from *Psalmopoeus chronoarachne* sp. nov. by comparatively having more curved receptacles towards the centre, distal apex less curved, and overlapping, receptacles with apical digitiform lobe overlapping, one to four protruding well-defined lobes, and a single ill-defined lobe (Fig. 10) (comparatively less curved receptacles towards the centre and distant to each other, distal apex more curved and not overlapping, receptacles with only a single ill-defined lateral lobe on apical-inner and apical digitiform lobe absent in *Psalmopoeus chronoarachne* sp. nov.; Fig. 1); from *Psalmopoeus satanas* sp. nov. by having curved receptacles towards the centre, distal apex more curved and overlapping, receptacles comparatively with more sclerotised, thin and shorter apical digitiform



**Figure 10.** *Psalmopoeus ecclesiasticus* non-type female (QCAZ-i274322), spermatheca **A** dorsal view **B** ventral view. Scale bars: 2 mm.

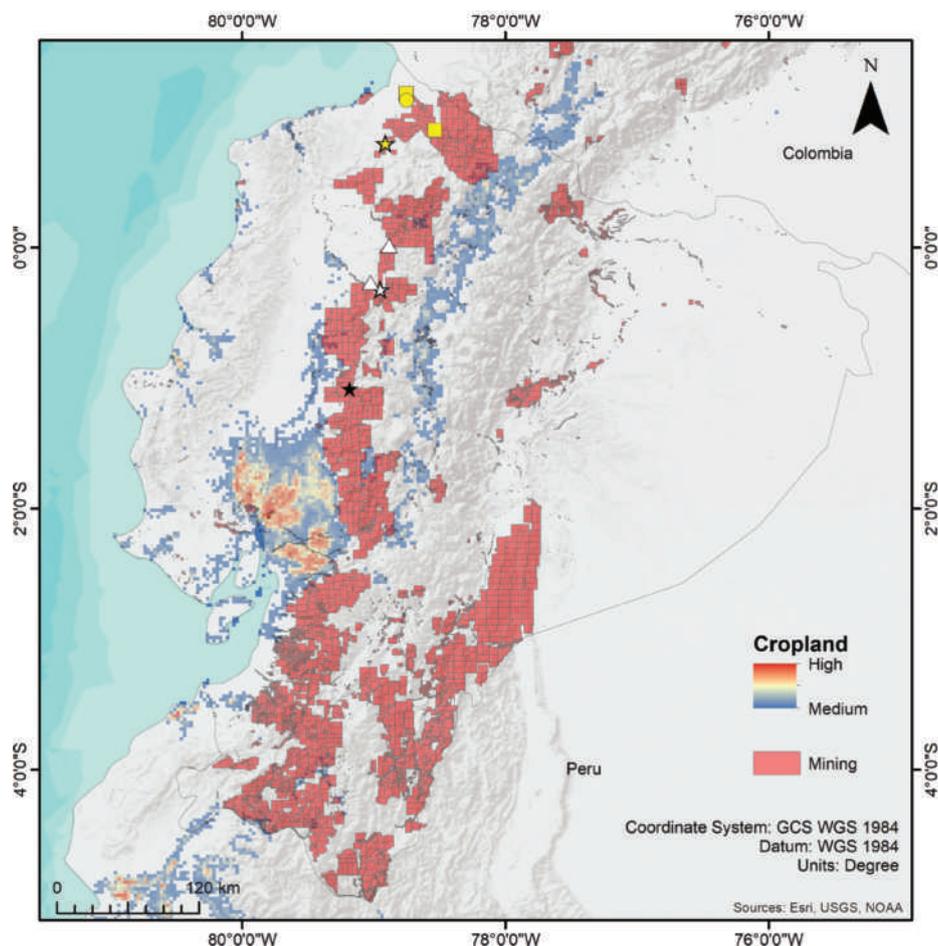
lobe pointing downwards, one to four protruding well-defined lobes and a single ill-defined lobe (straight receptacles, distal apex curved towards the centre and overlapping, comparatively less sclerotised wider and longer apical digitiform lobe pointing upwards and only two ill-defined lateral lobe and a single domed well-defined lateral lobe on apical-inner in *Psalmopoeus satanas* sp. nov.; Fig. 8); from *P. cambridgei*, *P. irminia*, *P. pulcher*, *P. langenbuchi*, *P. reduncus*, and *P. victori* by having elongated curved receptacles towards the centre, distal apex curved and overlapping, thin and shorter apical digitiform lobe pointing downwards, one to four protruding well-defined lobes, and a single ill-defined lobe disposed on the central longitudinal fold (elongated and straight receptacles with distal apex straight, comparatively more sclerotised, narrow, and elongated apical digitiform lobe pointing upwards and not overlapping and two to three protruding and well-defined lobes at central longitudinal fold in *P. cambridgei*; elongated and straight receptacles with distal apex straight, comparatively more sclerotised wider and shorter apical digitiform lobe pointing upwards but not overlapping and a single domed well-defined lobe at centre of each receptacle in *P. irminia*, elongated and straight receptacles with distal apex straight, comparatively more sclerotised thin apical digitiform lobe pointing upwards but not overlapping and numerous lobes at centre or lateral which reduce in size from apex to centre in *P. pulcher*; elongated and triangular receptacles with distal apex straight, comparatively more shorter apical digitiform lobe pointing upwards, not overlapping but very close to each other and two to three well-defined lateral lobes in *P. langenbuchi*, short and triangular receptacles with distal apex straight, comparatively more sclerotised wider and shorter apical digitiform lobe pointing upwards but not overlapping and only a single ill-defined lateral lobe in *P. reduncus*; comparatively more elongated and straight receptacles with wider distal apex without receptacles in *P. victori*; see figures in Mendoza 2014: figs 27, 28; Cifuentes and Bertani 2022: 125, 170–175, 190–191, 215, 245, 268–271, 283, 300, 309)

**Remarks.** During a recent visit to the QCAZ collection a female of *P. ecclesiasticus* was examined by PPR. The specimen was collected in the locality Alto Tambo, almost ca. 43 km SW from the type locality of this species. Herein we illustrate the spermatheca of this specimen, demonstrating intraspecific variation, not able to be shown in previous works (Gabriel and Sherwood 2019; Cifuentes and Bertani 2022); accordingly, we provide an updated diagnosis for the females of *P. ecclesiasticus*.



**Figure 11.** Distribution of the genus *Psalmopoeus* Pocock, 1895 in Ecuador, including biogeographical regions of Ecuador. White star = Reserva Otongachi, type locality of *P. satanas* sp. nov.; White triangle = Localities of *P. satanas* sp. nov. paratypes; Black star = Hacienda La Mariela, type locality of *P. chronoarachne*; Yellow star = Rio Sapayo, type locality of *P. ecclesiasticus*; Yellow circle = Carondelet, historical record of *P. ecclesiasticus*; Yellow squares = Additional records of *P. ecclesiasticus*.

**Conservation.** Little is known about the actual population density of *P. chronoarachne* sp. nov., and *P. satanas* sp. nov. Through a comparison of the known distribution of each species and the most probable overlapping anthropogenic threats, we found that mining concessions occupy the entire distribution of the species evaluated and expand further, demonstrating a severe distributional threat (Fig. 12). This, considering that these areas close to the known distribution of each species, may represent potential distributions of the populations. Likewise, another threat that was considered was agriculture and croplands. Even though it does not seem to overlap with the distribution of the species, this plausible threat is very close to the known localities. Additionally, we must remark that the data used for cropland distribution in Ecuador is not current and for many localities that occupy these species, cropland and livestock grasslands are present in larger than currently given projections (pers. obs.). In fact, the type localities of *Psalmopoeus chronoarachne* sp. nov. and *P. satanas* sp.



**Figure 12.** Distribution of the genus *Psalmopoeus* Pocock, 1895 in Ecuador, including mining concessions and cropland use. White star = Reserva Otongachi, type locality of *P. satanas* sp. nov.; White triangle = localities of *P. satanas* sp. nov. paratypes; black star = Hacienda La Mariela, type locality of *P. chronoarachne*; yellow star = Rio Sapayo, type locality of *P. ecclesiasticus*; yellow circle = Carondelet, historical record of *P. ecclesiasticus*; yellow squares = additional records of *P. ecclesiasticus*.

nov. are both surrounded by cropland and livestock grassland (Brito-Zapata pers. comm. 09 May 2023; R.JL-E pers. obs.) but this is not officially registered.

According to the IUCN (2001) criteria for the Red List Categories, in poorly known taxa and where the population statuses of species are not known in detail (see Discussion), background information on habitat deterioration and other causal factors can be used for assigning any threat category. Based on the information previously mentioned, herein we propose that *Psalmopoeus chronoarachne* sp. nov. should be placed in the *Critically Endangered* category based on the criteria combination B2abiii by taking in reference that the estimated area of occupancy estimated for this species is less than 10 km<sup>2</sup>, demonstrating severe fragmentation caused by cropland and mining concessions. Likewise, the territory is inferred to be on a steady decline in area of occupancy and quality of habitat. Similarly, *Psalmopoeus satanas* sp. nov. could also be classified in the *Critically Endangered* category based on the criteria combination B1abiii by taking in reference that the area of occupancy estimated for this species is ~ 83 km<sup>2</sup>, severely fragmented by cropland and mining concessions and following an inferred continuing decline in the available area of occupancy and quality of habitat.

## Discussion

*Psalmopoeus chronoarachne* sp. nov. appears to be endemic to its type locality in Hacienda La Mariela in the Pangua canton. Pangua is located slightly west between the Quilotoa and Chimborazo massifs geographically (Stern 2004), a region recognised for its high biodiversity, critical to numerous threatened species of amphibians, birds, mammals, among others, that rely on its ecosystems. The locality is ecologically similar to the Reserva Ecológica Ilinizas, especially its subtropical forests. However, there exists a difference in beta biodiversity because the locality is in an intermediate zone between the subtropical forests of Reserva Ecológica Ilinizas and the arid environments of Reserva de Producción de Fauna Chimborazo (Tapia Armijos 2016), relatively low in elevation (760 m), but intercepting two diametrically distinct ecological regions.

Nonetheless, although the region is relatively ecologically unique, because Pangua is not within the bounds of any governmental ecological reserve, it is highly threatened by both legal (Fig. 12) and illegal mining operations that extract metals such as copper, silver, and gold, introducing pollutants to its ecosystems (Cooper and Jolly 1970; Ngole-Jeme and Fantke 2017; Liu et al. 2020). Habitat fragmentation due to the expansion of urban and agricultural zones, accompanied by the concurrent introduction of non-native species, represents other threats the region's ecosystems face (Pimm 1987; Simberloff et al. 2005; Ricciardi and Cohen 2007). Thus, given these escalating threats to the ecosystems of Pangua, it is essential to consider that this species meets the aforementioned conservation categories and should be classified as *Critically Endangered* within the criteria combination B2abiii.

Similarly, in the case of *P. satanas* sp. nov., the species also appears to be endemic to the western foothill forest near San José de Alluriquin and Mindo. This geographic region is affected by the Toachi and Pilatón rivers which flow from the Corazón volcano and affect the terrain to form orographic formations such as Macuhi, Pasayambo, Yunguilla, and Zarpullo (Arcos Argoti 2011). Because of this topography, the region is irregular, complex, and prone to high endemism; thus, especially threatened (Sonne et al. 2022).

As aforementioned for *P. chronoarachne* sp. nov., this region is also unprotected by governmental ecological reserves. However, various private and communal protected areas, like La Hesperia, Fundación Otonga, and Yunguilla nearby, may serve as sanctuaries. This region faces habitat loss due to fragmentation, deforestation, and both legal (Fig. 12) and illegal mining (La Florida Mining Concession). Consequently, the introduction of pollutants, non-native species, or habitat fragmentation is plausible and a latent threat (Cooper and Jolly 1970; Pimm 1987; Simberloff et al. 2005; Ricciardi and Cohen 2007; Ngole-Jeme and Fantke 2017; Liu et al. 2020). As a consequence, similar to the case of *P. chronoarachne* sp. nov., it is essential to consider that *P. satanas* sp. nov. could also be classified as *Critically Endangered*, within the criteria combination B1abiii.

Consequently, it is essential to consider the potential loss of both *P. chronoarachne* sp. nov. and *P. satanas* sp. nov. and the ecological consequences that would result from their extinctions. These species are the only arboreal clades of theraphosid spiders in the region and thus may serve essential roles in the stratified micro-ecosystems in their respective areas.

To avoid this loss in Ecuadorian biodiversity, it is essential that these species be considered legally and that stricter regulations and penalties for illegal mining or other extracting-related activities, including specimen smuggling, be implemented to discourage such practices. Likewise, the engaging and educating of local communities about the importance of biodiversity conservation is essential to avoid further extinction and to educate about the potential economic benefits derived from ecotourism initiatives. Finally, it is important to consider that the areas in which these arthropods live are not under legal protection. The implementation of protected areas in these localities is essential to maintain the remaining population of these endangered species, and to encourage research on the remaining undescribed or unknown tarantula species in the area.

As a final point, we would like also to emphasise the latent threat of the illegal pet trade of wild tarantulas as a reason for wild population declines of tarantulas (Fukushima et al. 2019). This issue has been present in tarantula field collection since the peak of the tarantula pet hobby trade in the 1980s in North America and Europe (Smith 2020). Considering the publications of some hobby taxonomists who described novel species or made taxonomic treatments of Ecuadorian species based on pet-trade specimens (obviously wild caught), it is inferred that the issue has been ongoing for more than 32 years in Ecuador (Schmidt 1986, 1993, 1995a, 1995b, 2002, 2003a, 2003b; Kirk 1990; Schmidt and Bauer 1996; Bauer and Antonelli 1997; Peters 2003, 2005; Bullmer et al. 2006; Schmidt et al. 2006). Although this series of publications encouraged research on Ecuadorian tarantulas previously ignored for centuries, they also functioned as catalysts within the exotic pet-trade hobby, aiding in obtaining these species and further encouraging people to collect undescribed species. During this time, the sale, purchase, and study of these specimens in other countries was not considered illegal nor was it regulated by international institutions or hobbyist societies. In Ecuador since 1981 the fauna and flora become part of the domain of the state by the addition of Art. 74 in which implied penal actions against those who extract, commercialise, transport, and acquire wildlife and derived products (Corte Suprema de Justicia 1981), meaning that the first steps of Ecuadorian specimens present in the tarantula pet market started from illegal extractions.

“Official” evidence of illegally trafficked Ecuadorian tarantulas reported by Ecuadorian institutional authorities is limited: only two reports highlighting the confiscation of unknown species of tarantulas in Ecuador were made public in 2018 and 2021 by Ministerio del Ambiente, Agua y Transición Ecológica and local newspapers (PP-R pers. obs.). However, it is relatively easy to find Ecuadorian specimens belonging to the genera *Amazonius* Cifuentes & Bertani, 2022, *Avicularia* Lamarck, 1818, *Megaphobema* Pocock, 1901, *Cyclosternum* Ausserer, 1871, *Cymbiapophysa* Gabriel & Sherwood, 2020, *Tapinauchenius* Ausserer, 1871, *Thrixopelma* Schmidt, 1994, *Neischnocolus* Petrunkevitch, 1925, *Pamphobeteus* Pocock, 1901, and *Psalmopoeus* Pocock, 1895 being available for sale in various websites and Facebook groups (PP-R pers. obs.). It is important to understand that although some of these specimens have been bred in captivity, wild specimens are still being commercialised. It is clear that the knowledge of the ecologies and trophic dynamics of tarantulas in Ecuador, and the world, can still be improved upon. However, it is likely that when a thorough evaluation of the conservation status of each known species will be achieved, many of

these will meet the critical categories within the IUCN criteria. We encourage future work by Ecuadorian and international researchers, organisations, and governments to effectively understand the reality about the threat of tarantula smuggling and the required conservation status of each species in the country.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: PPR. Data curation: PPR. Formal analysis: PPR, RJLE. Investigation: RJLE, PPR. Methodology: PPR. Project administration: PPR. Supervision: PPR. Validation: PPR. Visualization: PPR. Writing - original draft: RJLE, PPR. Writing - review and editing: RJLE, PPR.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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# Review of the leafhopper tribe Deltocephalini Dallas, 1870 (Hemiptera, Cicadellidae, Deltocephalinae) in Pakistan with description of a new species of *Paramesodes*

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## Abstract

A review of the leafhopper tribe Deltocephalini in Pakistan is provided, including comprehensive illustrated keys to all genera and species, along with their diagnosis and distribution. A new species of *Paramesodes* is described, *P. dirensis* **sp. nov.**, which was discovered in the Upper Dir District of Khyber Pakhtunkhwa, Pakistan. A detailed description of this new species is provided together with photographs for visual reference. This tribe now has three genera and 13 species from Pakistan. The genus *Deltocephalus* Burmeister includes two species, and *Maiestas* Distant has nine species; *Paramesodes* Ishihara is now represented with two species.

**Key words:** Auchenorrhyncha, distribution, key, morphology

## Introduction

Leafhoppers of the tribe Deltocephalini can be identified by their small to moderate size, tapering or parallel-sided clypellus, narrow lorum, linear connective with closely appressed anterior arms, connective fused to the aedeagus, and dorsal sculpturing of the first valvula imbricate. This tribe includes 74 genera and over 600 species distributed worldwide (Duan et al. 2020; Zhang et al. 2023). Until now, 12 species of Deltocephalini have been known from Pakistan (Naveed et al. 2021; Shah et al. 2021).

The genus *Paramesodes* Ishihara, comprising 16 Old World species, is poorly represented in Pakistan, with only a single previous species, *P. lineaticollis* (Distant), known. Wilson (1983) revised the genus and recognized one species from Europe and the Middle East, one species from Africa, and nine species from

Asia. Five other species were subsequently included: *P. montanus* Rao, (1989) from India, *P. wilsoni* Rao & Ramakrishnan, (1990) from India, *P. iraniensis* Webb & Viraktamath, (2009) from Iran, and *P. menghaiensis* Li, Dai & Xing, (2011) and *P. cangshanae* Duan & Zhang, (2012) from China. *Paramesodes* is externally similar to *Paramesus* Fieber, *Exitianus* Ball, and *Ctenurellina* McKamey but they can be differentiated by having the forewing with the inner anteapical cell open basally (closed in *Paramesus* and *Ctenurella*), and the veins are always pale or white (usually brown in *Exitianus*). In the male genitalia the connective and aedeagus are fused (free in *Paramesus* and *Exitianus*), and the pygofer side has a dense covering of long, stout, pale macrosetae (2–6 dark or black macrosetae along apical margin in *Exitianus*) and the pygofer side has a large process (lower edge of pygofer pectinate in *Ctenurella*). This, together with the aedeagus, is the most important character for species identification (Duan and Zhang 2012).

In this paper, we provide a list of leafhoppers of the tribe Deltocephalini from Pakistan, illustrated keys to genera and species with their diagnostic characters, and a description and illustration of a new species, *P. dirensis* sp. nov.

## Material and methods

All specimens were examined with a Leica ZOOM2000 stereomicroscope. Drawings were made using an Olympus drawing tube. Photos were taken by a ZEISS SteREO Discovery.V20 stereomicroscope equipped with a ZEISS AxiocamICc 5 camera that also provided measurements. Adobe Photoshop CS was used to compile photographs. Specimens from Pakistan are deposited in the various collections as indicated in the published records. Type specimens of the new species, examined and figured for this study, are deposited in the Entomological Museum, Northwest A&F University, Yangling, Shaanxi, China.

## Taxonomy

**Family Cicadellidae Latreille, 1802**

**Subfamily Deltocephalinae Dallas, 1870**

**Tribe Deltocephalini Dallas, 1870**

### Key to genera of Deltocephalini from Pakistan

- 1 Crown with broad black transverse submarginal band between eyes (Figs 1, 9); pygofer side with large process (Figs 3, 10) ..... ***Paramesodes***
- Crown without black transverse band between eyes (Figs 12, 15, 18, 19); pygofer side without process ..... **2**
- 2 Aedeagal shaft short, robust, strongly curved dorsally, gonopore apical (Figs 13, 16) ..... ***Deltocephalus***
- Aedeagal shaft long, slightly curved dorsally, with gonopore indistinct (Figs 20–25, 27, 29) ..... ***Maiestas***

### Genus *Deltocephalus* Burmeister

#### Key to species of *Deltocephalus* from Pakistan (male)

- 1 Crown with six brown spots on anterior margin (Fig. 12); aedeagal shaft with shallow apical notch (Fig. 13) ..... *D. vulgaris*
- Crown with single brown spot on anterior margin adjacent to eyes (Fig. 15); aedeagal shaft without apical notch (Fig. 16).....*D. infirmus*

#### *Deltocephalus vulgaris* Dash & Viraktamath

Figs 12–14

*Deltocephalus (Deltocephalus) vulgaris* Dash & Viraktamath, 1998: 4, figs 1–11 (India); Zhang and Duan 2011: 3, fig. 3A–H (China); Naveed et al. 2019: 285, figs 1A, B, 3A–D (Pakistan).

**Diagnosis.** This species can easily be identified by the color pattern and the aedeagus with a shallow apical notch.

**Distribution.** China, India, Pakistan.

#### *Deltocephalus infirmus* Melichar

Figs 15–17

*Deltocephalus infirmus* Melichar, 1903: 203, pl. 5 fig. 11 (Sri Lanka); *Jassargus infirmus*: Ishihara 1961: 244, figs 53–58 (misidentification); *Deltocephalus infirmus*: Webb and Viraktamath 2009: 13, fig. 10; Naveed et al. 2019: 285, figs 1C, 3D–G (Pakistan).

**Diagnosis.** A similar species to *D. vulgaris* but differs by having the crown with only a single brown spot and the aedeagal shaft without an apical notch.

**Distribution.** India, Pakistan, Sri Lanka, Thailand.

### Genus *Maiestas* Distant

#### Key to species of *Maiestas* from Pakistan (males)

- 1 Overall color dark brown; forewing with sub basal and subapical irregular white transverse band (Fig. 18) ..... *M. albomaculata*
- Color not as above (Fig. 19) ..... **2**
- 2 Crown, face, and thorax with black patches (Fig. 19) ..... *M. maculata*
- Crown, face, and thorax without black patches ..... **3**
- 3 Forewing with extra cross-veins, at least in clavus ..... **4**
- Forewing without extra cross-veins ..... **5**
- 4 Aedeagus with a large subapical ventral process (Fig. 20).....*M. indica*
- Aedeagus with a short apical ventral process (Figs 21, 22).....*M. pruthii*
- 5 Aedeagus with pair of short lateral processes (Fig. 23).....*M. trispinosa*
- Aedeagus without lateral processes..... **6**

- 6 Aedeagus in lateral view similar in width in distal half (Fig. 24) ... ***M. subviridis***
- Aedeagus in lateral view evenly tapered from base to apex ..... **7**
- 7 Style apophysis broadest sub basally (Fig. 26); aedeagal shaft in lateral view not sinuate (Fig. 25)..... ***M. tareni***
- Style apophysis broadest at base (Fig. 28); aedeagal shaft in lateral view slightly sinuate (Fig. 27)..... ***M. sinuata***

***Maiestas albomaculata* (Dash & Viraktamath)**

Fig. 18

*Deltocephalus (Recilia) albomaculatus* Dash & Viraktamath, 1998: 12, figs 29–34 (India); *Maiestas albomaculata*: Webb and Viraktamath 2009: 21; *Maiestas albomaculata*: Naveed et al. 2019: 287, figs 1E–I, 3H, I (Pakistan); Shah et al. 2021: 403, fig. 1A–D (Pakistan).

**Diagnosis.** This species differs from other species of *Maiestas* in color and male genitalia, including the dorsolateral, laminate serrations of the aedeagal shaft.

**Distribution.** Pakistan, India.

***Maiestas indica* (Singh-Pruthi)**

Fig. 20

*Allophleps indica* Singh-Pruthi, 1936: 120, fig. 132; pl. 9 fig. 3 (Pakistan); *Allophleps delhiensis* Rao & Ramakrishnan, 1990: 111, figs 1–9 (India), synonymized by Dash and Viraktamath 1998: 35; *Deltocephalus (Recilia) indicus*: Dash and Viraktamath 1998: 35–36, fig. 305 (India); *Maiestas indica*: Webb and Viraktamath 2009: 21; Naveed et al. 2019: 287; Shah et al. 2021: 403, fig. 1E (Pakistan).

**Diagnosis.** This species can be identified by the aedeagus, which has a distinctive, large, subapical ventral process, the forewings which have accessory cross-veins, and the shorter head.

**Distribution.** Pakistan, India.

***Maiestas maculata* (Singh-Pruthi)**

Figs 19, 29

*Cicadula maculata* Singh-Pruthi, 1930: 58–59, figs 80, 81, pl. 5 fig. 2 (India); *Thamnotettix prabha* Singh-Pruthi, 1930: 62, figs 85, 86, pl. 5 figs 6, 6a (India), synonymized by Webb and Viraktamath 2009: 41; *Recilia prabha*: Ghauri 1980: 166–169, figs 1, 3–11; *Deltocephalus (Recilia) maculata*: Dash and Viraktamath 1998: 32, figs 260–269 (India); *Maiestas maculata*: Webb and Viraktamath 2009: 22; *Maiestas maculata*: Zhang and Duan 2011: 37–39, figs 33–35, pl. 4 fig. E, pl. 5 fig. P, pl. 6 fig. P (China); Shah et al. 2021: 404, fig. 2A–I (Pakistan).

**Diagnosis.** This species can be distinguished from other *Maiestas* species by its habitus, which has variable black patches on the head and thorax, and the shape of its aedeagus and style.

**Distribution.** China, India, Pakistan.

### ***Maiestas pruthii* (Metcalf)**

Figs 21, 22

*Deltocephalus notatus* Singh-Pruthi, 1936: 128–129, fig. 139, pl. 9 fig. 10 (Pakistan) (primary homonym: *Deltocephalus notatus* Melichar, 1896); *Deltocephalus pruthii* Metcalf, 1967: 1173 (nom. nov. pro *D. notatus* Singh-Pruthi, 1936); *Deltocephalus (Recilia) pruthii*: Dash and Viraktamath 1998: 22, 23, figs 150–158 (India); *Maiestas pruthii*: Webb and Viraktamath 2009: 20, new combination; Khatri and Webb 2010: 11, pl. 2a fig. 13, misidentification; Naveed et al. 2019: 286, fig. 2A–C (incorrectly cited as *M. subviridis*; H. Naveed pers. comm.), Fig. 3i, misidentification; Shah et al. 2021: 406, fig. 4F–L (Pakistan).

**Diagnosis.** This species has a relatively long, acute head, with a pair of inverted U-shaped markings basally, and forewings with extra cross veins. The identity of this species is based on the figures of Dash and Viraktamath (1998).

**Distribution.** India, Pakistan.

### ***Maiestas setosa* (Ahmed, Murtaza & Malik)**

*Recilia setosa* Ahmed et al., 1988: 412, fig. 2 (Pakistan); *Maiestas setosa*: Webb and Viraktamath 2009: 20; Naveed et al. 2019: 287; Shah et al. 2021: 406 (Pakistan).

**Diagnosis.** The identity of this species remains uncertain due to the limitations of the original description and the accompanying figures. Additionally, the type series from Karachi, which was indicated in the original account as deposited in the Zoological Museum of the University of Karachi (Ahmed et al. 1988), is unavailable (Khatri and Webb 2010: 11). Until the type material can be studied, pinpointing the precise classification of this species will be challenging.

**Distribution.** Pakistan.

### ***Maiestas sinuata* Shah & Duan**

Figs 27, 28

*Maiestas sinuata* Shah & Duan in Shah et al., 2021: 406, fig. 3A–H (Pakistan).

**Diagnosis.** This species differs in appearance of its habitus, presence of fine, apical setae on the subgenital plate, the style having a thicker apical process than other species, and the aedeagus lacking a ventrobasal “heel”.

**Distribution.** Pakistan.

***Maiestas subviridis* (Metcalf)**

Fig. 24

*Stirellus subviridis* Metcalf, 1946: 125; *Deltocephalus (Recilia) subviridis*: Dash and Viraktamath 1998: 24, figs 166–172 (India); *Maiestas subviridis*: Webb and Viraktamath 2009: 19, fig. 40; *Maiestas subviridis*: Khatri and Webb 2010: 11, pl. 2b, c, fig. 12 (Pakistan); Zhang and Duan 2011: 19, fig. 17, pl. 2 fig. H (China); Naveed et al. 2019: 287; Shah et al. 2021: 408, fig. 4A–E (Pakistan).

**Diagnosis.** This species can be distinguished by the rounded apex of the aedeagus which bears a very short apical spine.

**Distribution.** China, India, Pakistan, Pacific.

***Maiestas tareni* (Dash & Viraktamath)**

Figs 25, 26

*Deltocephalus (Recilia) tareni* Dash & Viraktamath, 1995: 74–76, figs 1–15; Dash and Viraktamath 1998: 16, figs 78–84 (India); *Maiestas tareni*: Webb and Viraktamath 2009: 22; Khatri and Webb 2010: 11, pl. 2d, fig. 11 (Pakistan); Zhang and Duan 2011: 20 (China); Naveed et al. 2019: 290, figs 2G–I, 3N, O; Shah et al. 2021: 408, fig. 5A–Z (Pakistan).

**Diagnosis.** This species can be identified by its relatively straight and stout style, apophysis with a serrated inner margin, and the aedeagus in lateral view evenly tapered from base to apex and relatively straight.

**Distribution.** China, India, Pakistan.

***Maiestas trispinosa* (Dash & Viraktamath)**

Fig. 23

*Deltocephalus (Recilia) trispinosus* Dash & Viraktamath, 1998: 35, figs 296–304 (India); *Maiestas trispinosa*: Webb and Viraktamath 2009: 38; *Maiestas trispinosa* Shah et al., 2021: 408, fig. 6A–I (Pakistan).

**Diagnosis.** This species can easily be distinguished from the others by the lateral, spine-like processes of the aedeagus.

**Distribution.** India, Pakistan.

**Genus *Parasmesodes* Ishihara**

*Parasmesodes* Ishihara, 1953: 45. Type species: *Athysanus albinervosus* Matsuura, 1902.

**Distribution.** Palearctic, Oriental, and Ethiopian regions.

**Remarks.** Previously, only 1 species was recorded from Pakistan. This study adds one more new species to the genus, bringing the total to two for the country.

### Key to species of *Paramesodes* from Pakistan (males)

- 1 Pale yellowish species (Fig. 1); pygofer broadly rounded posteriorly, with process in lateral view straight distally, apex directed posteriorly (Fig. 3); aedeagal shaft recurved distally in lateral view (Fig. 7); constricted subapically in ventral view (Fig. 6).....***P. dirensis* sp. nov.**
- Dark yellowish species (Fig. 9); pygofer oval posteriorly, with process in lateral view directed dorsally distally (Fig. 10); aedeagal shaft evenly curved dorsally in lateral view (Fig. 10), not constricted preapically in ventral view (Fig. 11).....***P. lineaticollis***

### ***Paramesodes dirensis* sp. nov.**

<https://zoobank.org/158C20E7-3752-4E06-A4CE-AED5721D0A7D>

Figs 1–8

**Description.** **Length:** male 5.2–6.1 mm, female 6.1–6.4 mm. **Coloration:** pale, with brown markings (Fig. 1). Crown with broad, black, transverse submarginal band between eyes (Fig. 1). Face pale yellow, with brown, transverse striations on clypeus (Fig. 2). Pronotum with medial dark brown longitudinal marking as well as three brown longitudinal markings on each side (Fig. 1). Scutellum with median, longitudinal, dark brown markings and pale brown lateral markings (Fig. 1). Forewings with variable brown markings; veins prominent and white (Fig. 1). Legs pale, with brown markings.

**Male genitalia:** pygofer lobe broad basally, narrowing apically, forming a rounded-oval apex, with long, yellowish-brown spines extending beyond apical margin, a large process arising near medial dorsal margin and straight apically, surpassing pygofer lobe, without any bend (Figs 3, 4). Subgenital plates triangulate; macrosetae uniseriate laterally (Fig. 5). Valve triangular (Fig. 5). Style as in Figure 5. Connective fused to aedeagus, with arms closely appressed distally (Figs 6, 7). Aedeagus tubular, tapering apically, recurved in lateral view, constricted preapically in ventral view; gonopore apical (Figs 6, 7).

**Female.** Same in appearance as male. Seventh sternum with lateral margins not extended, posterior margin with median projection, rounded (Fig. 8).

**Materials examined.** **Holotype** ♂, PAKISTAN: Khyber Pakhtunkhwa: Upper Dir, 35°9'55.89"N, 72°2'48.54"E, 1840 m, 24.07.2019, Hassan Naveed leg., sweep net. **Paratypes** 8♂, 5♀, same data as holotype.

**Etymology.** This species is named after type locality, the Upper Dir in Khyber Pakhtunkhwa.

**Remarks.** *Paramesodes dirensis* sp. nov. is similar to its congeners in general appearance, but it differs from those species in the combination of male genitalia features, i.e., the pygofer is oval posteriorly with a relatively straight process distally and the aedeagal shaft is distally recurved in lateral view and constricted preapically in ventral view. In the Wilson's (1983) key, the new species runs to couplet 7 along with *P. lineaticollis*.



**Figures 1–8.** *Paramesodes dirensis* sp. nov. **1** habitus, dorsal view **2** face **3** male pygofer, lateral view **4** male pygofer, dorsal view **5** subgenital plate, valve and style, dorsal view **6** connective and aedeagus, dorsal view **7** connective and aedeagus, lateral view **8** female abdominal tip and 7<sup>th</sup> sternum, dorsal view.

***Paramesodes lineaticollis* (Distant)**

Figs 9–11

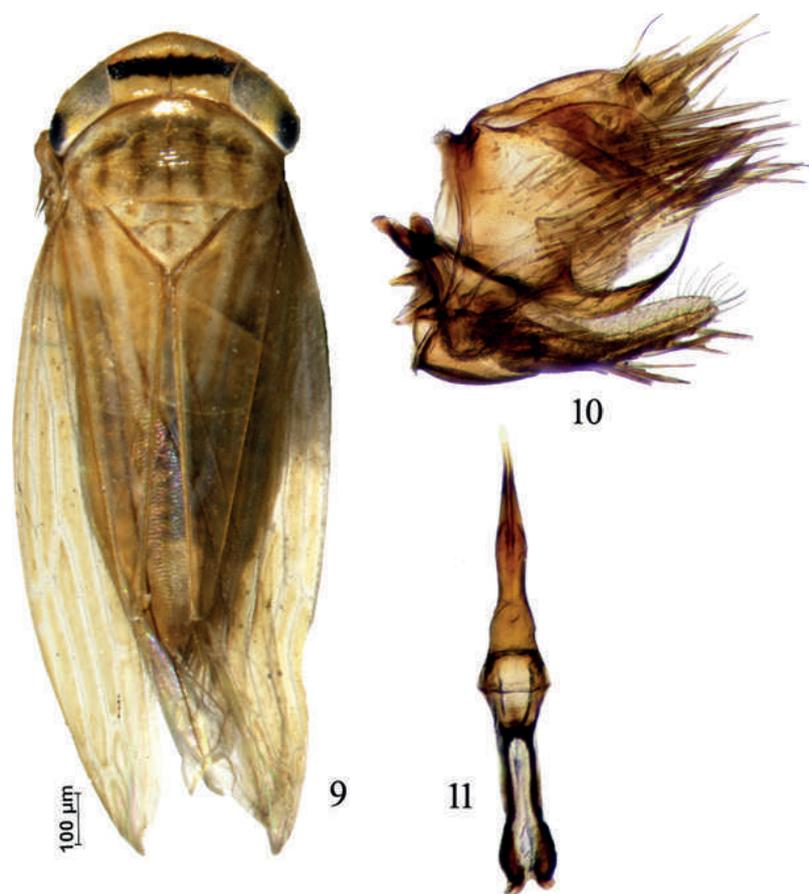
*Paramesodes lineaticollis* (Distant, 1908: 294, *Paramesus*) (India); Wilson 1983: 21, 22, figs 23–29.

*Paramesodes ishurdii* Mahmood & Meher, 1973: 135 (Pakistan), synonymized by Wilson 1983: 21.

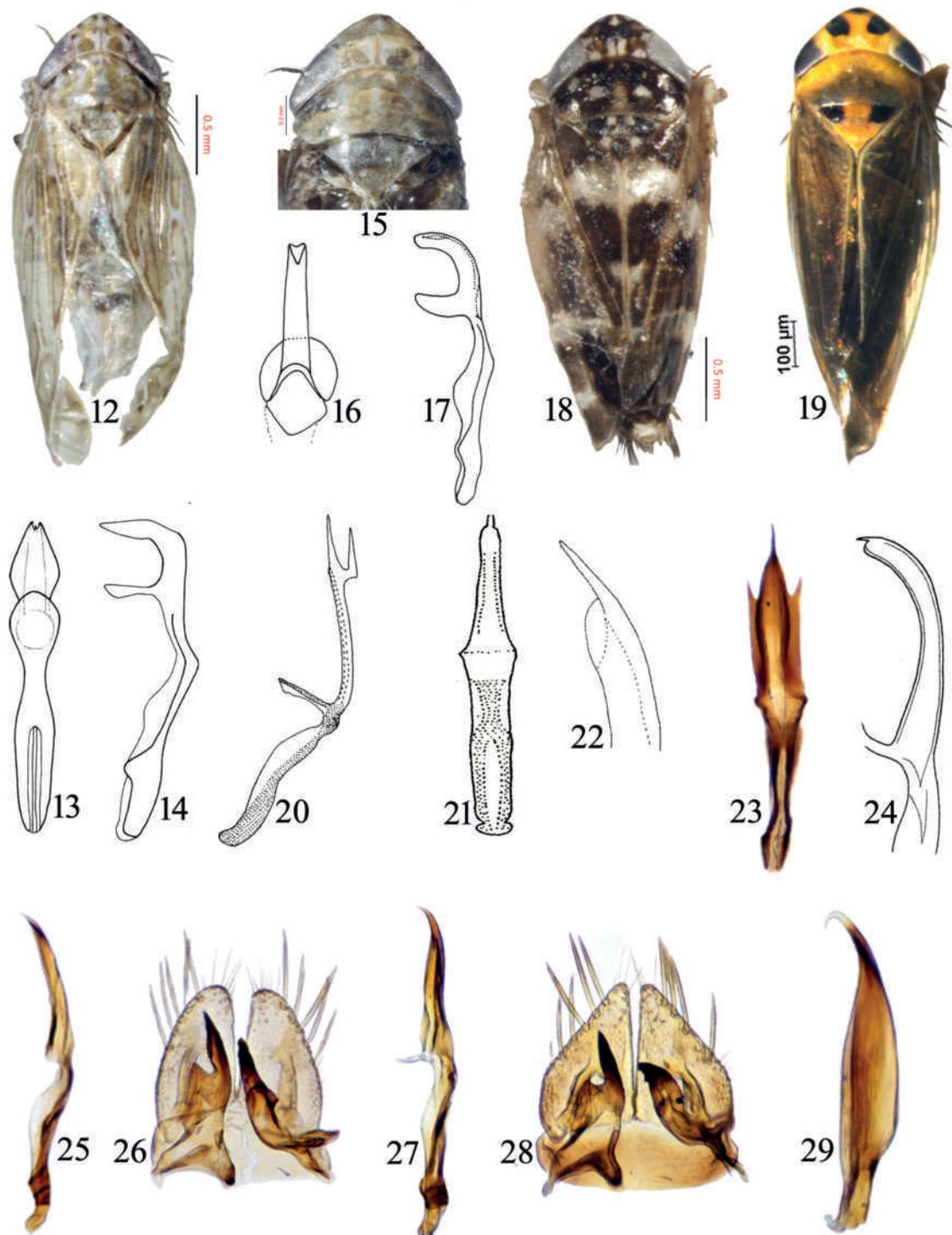
**Materials examined.** ♂, PAKISTAN: Khyber Pakhtunkhwa: Shinkiari, 34°28'19.1064"N, 73°16'14.3004"E, 22.07.2018, Bismillah Shah leg., sweep net.

**Distribution.** Bangladesh, China, India, Indonesia, Pakistan, Philippines, Taiwan, Turkey.

**Diagnosis.** The male pygofer processes is distinct, directed ventrally or postero-ventrally over basal half, and turned abruptly posteriorly immediately distad of its midlength. Mahmood and Meher (1973) reported this species as *P. ishurdii* for the first time from Pakistan, but that species was later synonymized by Wilson (1983).



**Figures 9–11.** *Paramesodes lineaticollis* 9 habitus, dorsal view 10 male pygofer, lateral view 11 connective and aedeagus, dorsal view.



**Figures 12–29.** 12–14 *Deltocephalus vulgaris* Dash & Viraktamath: 12 habitus, dorsal view 13 aedeagus and connective, dorsal view 14 aedeagus and connective, lateral view 15–17 *D. infirmus* (Melichar): 15 habitus, dorsal view 16 aedeagus and connective, dorsal view 17 aedeagus and connective, lateral view 18 *Maiestas albomaculata* (Dash & Viraktamath) habitus, dorsal view 19 *M. maculata* (Singh-Pruthi) habitus, dorsal view 20 *M. indica* (Singh-Pruthi) aedeagus and connective, lateral view (after Dash & Viraktamath, 1998) 21, 22 *M. pruthii* (Metcalf): 21 aedeagus and connective, dorsal view (after Dash & Viraktamath, 1998) 22 apex of aedeagus, lateral view 23 *M. trispinosa* (Dash & Viraktamath) aedeagus and connective, dorsal view 24 *M. subviridis* (Metcalf) aedeagus, lateral view (after Khatri & Webb, 2010) 25, 26 *M. tareni* (Dash & Viraktamath): 25 aedeagus and connective, lateral view 26 subgenital plate, valve and styles, dorsal view 27, 28 *M. sinuata* Shah & Duan: 27 aedeagus and connective, lateral view 28 subgenital plate, valve and styles, dorsal view 29 *M. maculata* (Singh-Pruthi) aedeagus, lateral view.



Figures 30, 31. *Paramesodes lineaticollis* 30 subgenital plates, valve and styles, dorsal view 31 aedeagus and connective, lateral view.

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### Additional information

#### Conflict of interest

The authors have declared that no competing interests exist.

#### Ethical statement

No ethical statement was reported.

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#### Data availability

All of the data that support the findings of this study are available in the main text.

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# A new species of pit-viper from the Ayeyarwady and Yangon regions in Myanmar (Viperidae, *Trimeresurus*)

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## Abstract

In a genomic study by Chan and colleagues, pit-vipers of the *Trimeresurus erythrurus*–*purpureomaculatus* complex from the Ayeyarwady and Yangon regions in Myanmar were demonstrated to be a distinct species based on robust population genetic and species delimitation analyses. Here, we provide morphological characterizations and a formal description of those populations as a new species. The new species, *Trimeresurus ayeyarwadyensis* **sp. nov.**, is most closely related to *T. erythrurus* and *T. purpureomaculatus* and shares morphological characteristics with both of those species. Some specimens of *T. ayeyarwadyensis* **sp. nov.** have green dorsal coloration and no distinct dorsal blotches (a trait shared with *T. erythrurus* but not *T. purpureomaculatus*), while others have dark dorsal blotches (a trait shared with *T. purpureomaculatus* but not *T. erythrurus*). The distinct evolutionary trajectory of the new species, coupled with the lack of obvious morphological differentiation, represents a classic example of the cryptic nature of species commonly found in the *Trimeresurus* group of Asian pit-vipers and underscores the need for data-rich analyses to verify species' boundaries more broadly within this genus.

**Key words:** Cryptic species complex, mangrove pit-viper, morphology, snake, systematics, taxonomy



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## Introduction

The evolutionary history of Asian pit-vipers of the genus *Trimeresurus* Lacepède, 1804 remains poorly understood and is characterized by phylogenetic uncertainty (Malhotra and Thorpe 2000, 2004a, b; Giannasi et al. 2001; Creer et al. 2003, 2006; Mulcahy et al. 2017; Mallik et al. 2021). Furthermore, complicated phenotypic variation has been a major source of taxonomic confusion (Sanders et al. 2004; Vogel et al. 2004, 2022; Wostl et al. 2016; Mulcahy et al. 2017; Chandramouli et al. 2020; Chen et al. 2020). Within this genus, mangrove

pit-vipers of the *T. purpureomaculatus*–*erythrurus* complex exhibit one of the most extreme phenotypic variations that have confounded researchers for decades (Pope and Pope 1933; Smith 1943; Chan et al. 2022).

*Trimeresurus erythrurus* is invariably green with no dorsal markings and is not a mangrove-forest obligate (Deuti et al. 2021). Contrastingly, *T. purpureomaculatus* occurs exclusively in mangroves, has highly variable dorsal coloration (ranging from purplish-gray, yellow, reddish-brown to black, but never green), and typically has distinct dorsal blotches, except for some melanistic populations in Singapore and on Sumatra. Interestingly, the melanistic variant has not been documented north of Singapore. Until recently, this species complex has not been subjected to focused studies primarily because genetic material from the type locality of *T. purpureomaculatus*, which is Singapore, had never been collected.

A topotype of *T. purpureomaculatus* from Singapore was first sequenced by Chan et al. (2022), who provided a preliminary phylogeographic framework that characterized phenotypic variants in association with genetic clades and geographic distribution. Their study revealed a genetically divergent lineage (herein referred to as *T. sp.*) from the Ayeyarwady and Yangon regions in Myanmar that occurs in the intervening region between the distributions of *T. erythrurus* and *T. purpureomaculatus*. Snakes from these regions have green dorsal coloration with varying degrees of blotchiness, which could be considered a blend between the phenotypes of *T. erythrurus* and *T. purpureomaculatus*. Based on mitochondrial DNA divergences and intermediate phenotypic attributes, Chan et al. (2022) hypothesized that the populations from Ayeyarwady and Yangon regions could represent an undescribed species that was well supported as sister to *T. purpureomaculatus*, which has hybridized, or is hybridizing, with *T. erythrurus* and/or *T. purpureomaculatus*.

A subsequent genomic study performed an in-depth investigation into the population genetics of the *Trimeresurus purpureomaculatus*–*erythrurus* complex with an emphasis on elucidating the species boundaries of *T. sp.* from the Ayeyarwady and Yangon regions in Myanmar (Chan et al. 2023). In that study, gene flow was demonstrated to be present between *T. sp.* and *T. erythrurus* and to a lesser extent, *T. purpureomaculatus* (Chan et al. 2023). Contrasting with the mtDNA, the genomic data placed the new species sister to *T. erythrurus* with strong support. Additionally, a population of *T. erythrurus* from the Patheingyi District in the Ayeyarwady Region was shown to be highly admixed between *T. erythrurus* and *T. sp.* However, despite genetic admixture, *T. sp.* was unequivocally demonstrated to be an independently evolving lineage that is not conspecific with either *T. erythrurus* or *T. purpureomaculatus* (Chan et al. 2023). In this study, we perform additional analyses and provide a morphological characterization of *T. sp.* from the Ayeyarwady and Yangon regions and its description as a new species.

## Materials and methods

A total of 24 female and 26 male vouchered specimens comprising *Trimeresurus erythrurus*, *T. purpureomaculatus*, and *T. sp.* were examined from the holdings of the California Academy of Sciences (CAS), University of Florida (UF), Naturhistorisches Museum Wien (NMW), Museum of Comparative Zoology, Harvard University (MCZ), and the Natural History Museum London (NHMUK/

**BMNH**), as well as anesthetized live specimens from Mizoram (courtesy of HT Lalremsanga, Mizoram University) and Myanmar (courtesy of Michihisa Toriba, Japan Snake Institute). Approximate locations of examined specimens are shown in Fig. 1A. The following mensural and meristic morphological characters were assessed: snout–vent length (**SVL**), tip of snout to tip of tail; tail length (**TaL**), cloaca to tip of tail; head length (**HL**), posterior margin of jaw articulation to tip of snout; head width (**HW**), widest distance across the dorsal surface of head; head width at posterior margin of supraoculars (**HW1**); eye diameter (**ED**), horizontal diameter of eye; eye–nostril–distance (**EYE2NOS**), distance between anterior margin of orbit to posterior margin of nostril; eye–pit–distance (**EYE2PIT**), distance between anterior margin of orbit to posterior margin of pit; nostril–pit–distance (**NOS2PIT**), distance between the nostril and the pit, measured between the outer edges; length of supraocular (**LSUP**) measured on the right side; width of supraocular (**WSUP**), measured on the right side; width of the internasal (**WINTNAS**), measured horizontally on the right side; ventral scales (**VEN**), number of ventral scales; subcaudals (**SC**) number of subcaudal scales; number of supralabials (**SL**) and infralabials (**IL**), average of left and right side; cephalic scales (**CEP**), minimum number of cephalic scales between left and right supraoculars; minimum number of scales between posterior margins of supraoculars (**BTWSUP**); number of scales bordering the supraoculars (**BORSUPOC**), average of right and left scales, not including post- and pre-ocular scales; the number of scales between the nasal scale and the shield bordering the pit anteriorly (**NASPIT**); the number of scales separating the internasal scales (**INTNAS**); rostral shape (**ROST**), the ratio of the dorsal margin of the rostral scale to the ventral margin; the number of paired chin shields between mental scale and first ventral scale (**CHIN**); the number of scales between the edge of the mouth and the ventral scales/chin shields, starting at and including the last sublabial (**VENTEDGE**). We also measured the position of dorsal scale row reductions from 31 to 29 scales (occurring just behind the head), 29–27 scales, 27–25, 25–23, 23–21, 21–19, and 19–17 scales (occurring just before the vent). Each reduction was recorded against the corresponding ventral scale row and converted to a percentage of VEN before analysis. Similarly, the reductions on the tail from 14–12 scale rows, 12–10, 10–8, 8–6, and 6–4 scale rows were measured against subcaudal scales and converted to a percentage of SC before analysis. A few ordinal characters were also included: keeling of body scales (**BSCK**) measured at mid-body; keeling of temporal scales (**KTEMP**); keeling of head scales (**KHEAD**), measured on a scale of 0 (no keeling) to 1.5 (sharp keel present) in 0.5 increments; raw measurements and counts for all examined specimens are listed in Suppl. material 1.

To control bias stemming from sexual dimorphism (Chan and Grismer 2021) while maximizing sample sizes, males and females were analyzed initially using a two-way analysis of variance/co-variance. Specimens were grouped for this purpose using biogeographic criteria into 5 groups: North-East India and Bangladesh (10 males, 7 females), Arakan Yoma (4 males, 4 females), Ayeyarwady delta excluding Patheingyi District (8 males, 11 females), Patheingyi District (1 male, 2 females), and Kra Isthmus (3 males, 0 females). Mensural characters were first log-transformed. Levene's test for homogeneity of variances was performed, and if significant, the Brown-Forsythe alternative to ANOVA was considered instead. Characters showing non-significant between-group variation

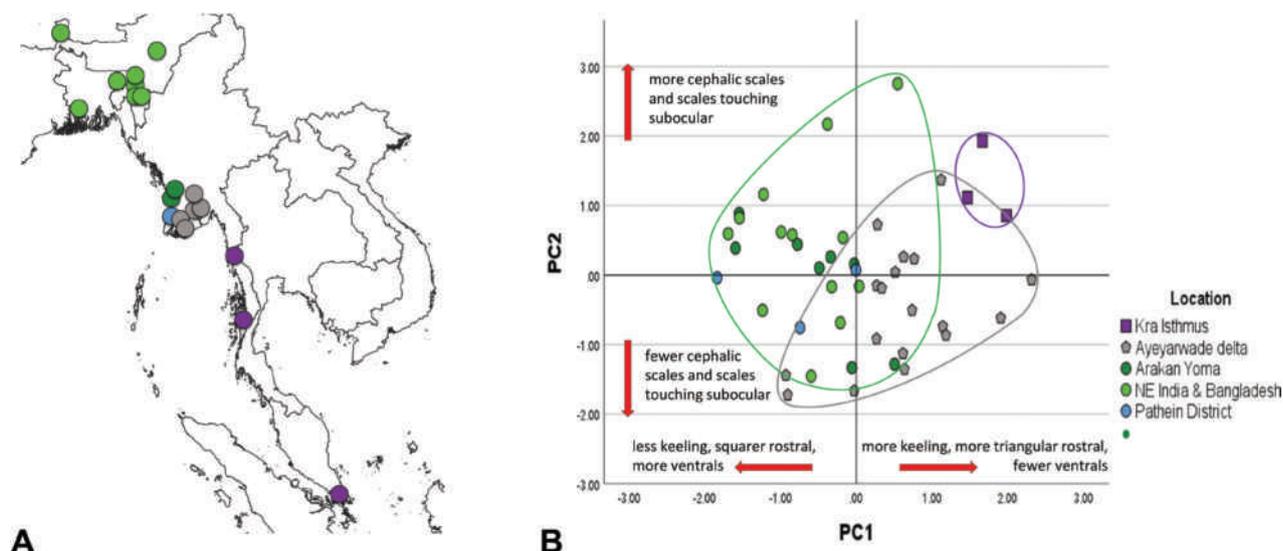
were not considered further. We performed allometric body-size correction for mensural characters using the Thorpe method (Thorpe 1983). Principal components analysis (PCA) was used to find the best low-dimensional representation of variation in the data to determine whether morphological variation could form the basis of detectable group structure. Unrotated PCs were extracted from the correlation matrix and the number of PCs to be retained was based on examination of the scree plot.

To gain greater clarity on diagnostic characters separating the two geographically proximate and introgressing species, we carried out a discriminant analysis on the same set of characters on each sex separately, but also including sexually dimorphic characters. Variables were entered in a stepwise manner, with the variable that minimized the overall Wilks' lambda entered at each step, and also included some characters reflecting the prominence of the postocular and lateral stripes: OC (number of scales covered by the postocular white stripe) and SC1 (proportion of first scale row at 50% VEN covered by white area). The minimum partial F-to-enter was 3.84. Leave-out-one classification was performed to determine the robustness of the result. All morphological analyses were performed and visualized using SPSS v. 27.0.

## Results

Summary statistics of raw mensural and meristic data are presented in Table 1. Two-way ANOVA or ANCOVA showed significant ( $P < 0.05$ ) differences between sexes but not among groups in HL, HW1, and TL. Among-group variation was significant in the following characters: VEN, SC, BSCK, SL, CEP, HW, NASPIT, ED, SOCBORD, ROST, KTEMP, KHEAD, VS%25–23, SC%10–8, SC%8–6. Of these, SC, SL, HW, SC%8–6; ED were also significantly different between sexes. ED showed a significant interaction between group and covariate (HL) and hence was discarded as the difference between within-group slopes invalidates size adjustment. Levene's test showed significant heteroscedasticity in VEN, SC, CEP, KTEMP, KHEAD, VS%25–23. Meristic characters were tested further using the Brown-Forsythe alternative in one-way ANOVA (for each sex separately) which relaxes the assumption of equal variances between groups, and if they remained significant in either sex, they were retained for further analysis. However, it was not possible to carry out robust tests for KTEMP and KHEADSC as at least one group had zero variance.

The characters that were entered into the PCA with both sexes included after this initial screening step were VEN, BSCK, CEP, NASPIT, SOCBORD, ROST, KTEMP, KHEAD, VS%25–23, and SC%10–8 (sexually dimorphic characters were not included). The resulting graph showed that *T. sp.* could be partly distinguished from *T. purpureomaculatus* (Kra Peninsula) and *T. erythrurus* (NE India and Bangladesh, Arakan Yoma, and Patheingyi District) on the first two axes. Separation can be observed between *T. purpureomaculatus* and *T. erythrurus* along PC1 (Fig. 1B), largely determined by the degree of keeling on the body, head, and temporal scales, the shape of the rostral scale, and also the reduction from 25 to 23 scale rows occurring further down the body in *T. purpureomaculatus*. However, *T. sp.* overlaps with both *T. purpureomaculatus* and *T. erythrurus* on PC1. The PC2 axis largely distinguishes *T. sp.* from *T. purpureomaculatus* on the basis of possessing a lower number of cephalic scales (CEP) and scales



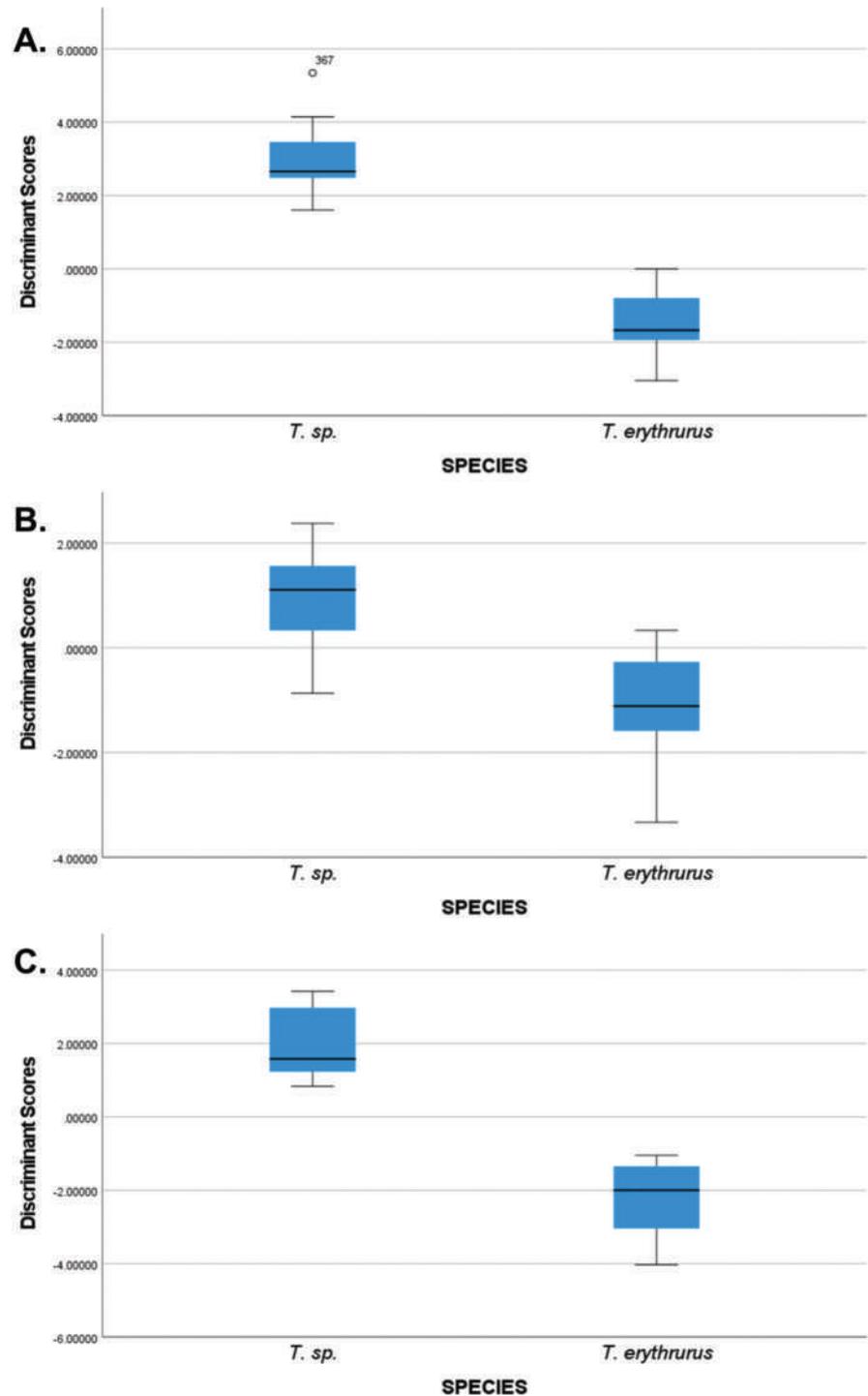
**Figure 1.** **A** approximate locations of examined specimens grouped and colored according to geographic regions; Purple (Isthmus of Kra) = *Trimeresurus purpureomaculatus*; green (Arakan Yoma, Northeast India and Bangladesh, and Patheingyi District, Myanmar) = *T. erythrurus*; gray (Ayeyarwady Delta, Myanmar) = *T. sp.* Overlapping localities are not shown **B** principal component analysis, males and females combined.

**Table 1.** Summary statistics for specimens examined in this study. Snout-vent length and tail length are measured in mm and statistics are calculated from specimens over 350 mm SVL (males) or 400 mm (females) only. Values shown are mean ± standard deviation followed by min–max in parentheses.

	<i>T. erythrurus</i>		<i>T. purpureomaculatus</i>	<i>T. sp.</i>	
	male (n = 15)	female (n = 13)	male (n = 5)	male (n = 8)	female (n = 11)
SVL	484.1 ± 55.8 (398–582)	634.4 ± 91.6 (478–746)	522.8 ± 57.7 (456–610)	477.9 ± 23.6 (432–500)	647.5 ± 177 (459–935)
TaL	120.9 ± 16.7 (97–146)	102.2 ± 2.32 (57–127)	139.6 ± 13.61 (123–158)	128.3 ± 0.85 (112–135)	112.4 ± 28.6 (84–165)
SL	10.9 ± 0.72 (9–13)	11.7 ± 0.81 (10–13)	10.8 ± 0.91 (9–12)	10.2 ± 0.7 (9–11)	10.9 ± 0.65 (10–12)
IF	12.7 ± 0.68 (12–14)	13.19 ± 1.23 (10–15)	12.9 ± 0.96 (11–14)	12.7 ± 1.04 (11–15)	13.05 ± 0.79 (12–14)
VEN	166.4 ± 5.7 (156–181)	168.4 ± 5.32 (163–180)	161 ± 3.16 (156–164)	161.7 ± 5.92 (150–170)	165.1 ± 4.89 (157–169)
SC	65.67 ± 3.22 (60–71)	56.4 ± 6.12 (49–68)	73.2 ± 2.77 (69–76)	73.3 ± 1.75 (71–76)	55.0 ± 2.14 (52–58)

bordering the subocular (SOCBORD), while the position of the scale reduction from 10 to 8 scale rows on the tail (SC%10–8) occurs closer to the vent. Thus, while a combination of these characters can distinguish most specimens of the three species, they may not be diagnostic for all specimens. Note that the Patheingyi District specimens, which showed substantial genetic introgression between *T. erythrurus* and *T. sp.* (Chan et al. 2023), appear to be morphologically identical to *T. erythrurus* and were grouped with them in the subsequent discriminant analysis.

Discriminant analysis of males *T. erythrurus* and *T. sp.* resulted in 100% correct classification and cross-validation (in which each case is classified according to the functions generated by all cases other than that case) with three variables entered: VEN, SC, and BSCK (Fig. 2A). In females, BSCK and SL were entered but discrimination was less successful, with only 90.9% and 83.3% of *T. sp.* and *T. erythrurus* respectively being accurately classified (Fig. 2B). Increasing the number of included variables by entering them together rather than in a stepwise manner increased the accuracy of classification to 100%



**Figure 2.** Box plots of discriminant scores classifying *T. erythrurus* and *T. sp.* **A** males with three variables entered (VEN, SC, and BSCK) achieved 100% success in discrimination and cross-validation **B** females with two variables entered (BSCK and SL) with lower discrimination success **C** females with an increased number of included variables (BSCK, SL, KTEMP, VS%25–23, HW1) increased discrimination success but decreased cross-validation success.

when using all cases, but cross-validation accuracy was reduced to 54.5% and 60% respectively (Fig. 2C). In this analysis, in addition to BSCK and SL, KTEMP, VS%25–23, and HW1 also contributed to the discrimination to a similar degree.

## Systematics

Evidence supporting the distinction of *Trimeresurus* sp. as a unique and independent lineage warranting species recognition was demonstrated in Chan et al. (2023) based on robust analyses of genomic data and is further substantiated by the morphological analysis presented above. Below, we provide a morphological description of the new species.

### *Trimeresurus ayeyarwadyensis* sp. nov.

<https://zoobank.org/26B634FE-3296-4227-9F46-A9942137BE75>

Fig. 3A–F

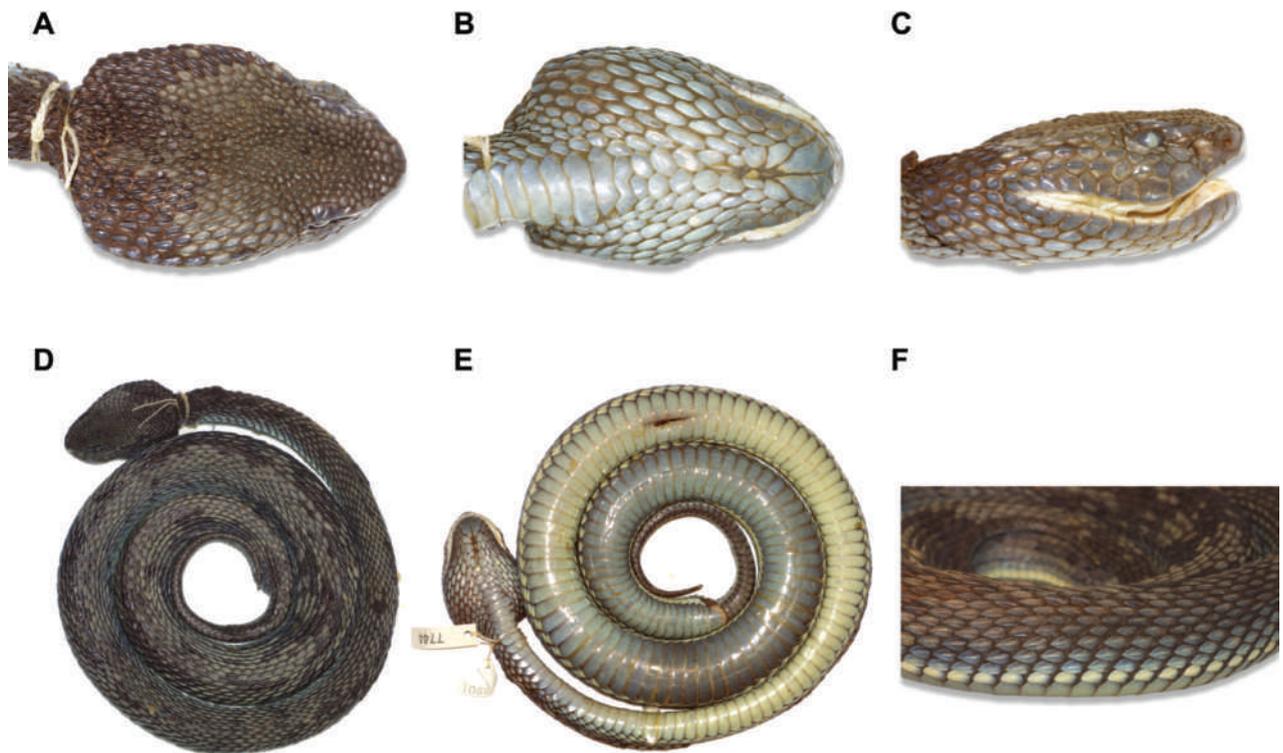
Vernacular name: Ayeyarwady pit viper

**Type material. Holotype.** CAS 219801, adult female, collected on 19 Jan. 2001 from Ayeyarwady Region, Pyapon District, Bogalay Township, Mein Ma Hla Kyun Wildlife Sanctuary, Mi Gyaung Gaung Pok Camp (16°00'45.1"N, 95°19'30.6"E) at 2025 hrs by H. Win, T. Thin, K.S. Lwin, A.K. Shein, and H. Tun.

**Paratypes.** CAS 213587, adult male, collected on 9 Jan. 2000 from Yangon Region, Hlaw Ga Park, Mingalardon Township (17°1'36.5"N, 96°5'49.4"E) at 0800 hrs by H. Win, T. Thin, S.L. Oo, and S.W. Kyi; CAS 212245, adult female, collected on 22 Apr. 2000 from Ayeyarwady Region, vicinity of Mwe Hauk Village (16°16'34.3"N, 94°45'1.3"E) at 1935 hrs by J.B. Slowinski, G.R. Zug, R.S. Lucas, and J.V. Vindum; CAS 219783, adult male, collected on 18 Jan 2001 from Ayeyarwady Region, Pyapon District, Bogalay Township, Mein Ma Hla Kyun Wildlife Sanctuary, West Htaw Pai Camp (15°56'43.7"N, 95°19'2"E) at 2105 hrs by H. Win, T. Thin, K.S. Lwin, A.K. Shein, and H. Tun.

**Diagnosis.** *Trimeresurus* can be distinguished from all other Asian pit-vipers by the condition of the first infralabial and nasal scale, which are at least partially fused. The new species can be diagnosed from other species of *Trimeresurus* by the following combination of characters: in both sexes ( $n = 19$ ), 23–25 dorsal mid-body scale rows (mean  $24.3 \pm 0.97$ ), 17 scale rows just anterior to vent (rarely 15 or 16) and body scales distinctly and sharply keeled; in males ( $n = 8$ ), 150–170 ventral scales (mean  $160.6 \pm 5.6$ ), 71–76 subcaudal scales (mean  $73 \pm 1.8$ ); a minimum of 9–11 scales between supraoculars (mean  $9.9 \pm 0.6$ ); between 5–9 scales touching the subocular scale (not counting pre- and post-oculars; mean  $6.9 \pm 0.9$ ); supralabials 9–12 (mean  $10.4 \pm 0.8$ ). In females ( $n = 11$ ), 157–174 ventral scales (mean  $165.1 \pm 4.9$ ), 52–58 subcaudal scales (mean  $55 \pm 2.1$ ); a minimum of 10–12 scales between supraoculars (mean  $10.8 \pm 0.9$ ); between 5–8 scales touching the subocular scale (not counting pre- and post-oculars; mean  $6.5 \pm 0.8$ ); supralabials 10–12 (mean  $10.9 \pm 0.6$ ).

**Description of holotype (Fig. 3A–F).** This is a large female with a total length of 1008.3 mm (SVL 935 mm, TaL 148 mm), with indistinct darker dorsal markings and a distinct pale dorsolateral stripe covering 90% of the first scale row and extending onto the second scale row. The upper part of head lacking any paler color, and a postocular stripe is lacking. The ventral surface is paler but darkens towards the infralabials, which are the same color as the upper part of the head. Heavily keeled scales are present on body and head. Only a blunt keel is present on the first scale row adjacent to the ventrals at mid-body, but



**Figure 3.** Holotype of *Trimeresurus ayeerwadyensis* sp. nov. (CAS 219801), adult female from Pyapon District, Ayeyarwady Division, Myanmar **A** dorsal view of head **B** ventral view of head **C** lateral view of right side of head **D** dorsal view of whole body **E** ventral view of whole body **F** lateral view of mid-body. Photographs by A. Malhotra.

higher scale rows become progressively more sharply keeled with an obvious ridge at the center of the scale. Temporal scales and scales on the rear of the head are similarly sharply keeled, but scales between the supraoculars are tubercular rather than obviously keeled. Ventrals 161, subcaudals 54; there are 25 scale rows at 14 ventral scales, which is maintained until the 94<sup>th</sup> ventral scale (thus there are 25 scale rows at mid-body) and reducing to 17 just anterior to the vent. There are 11 supralabials and 14 infralabials on the right side and 10 and 14, respectively, on the left side. There are a minimum of 10 tubercular scales between the supraoculars, with 16 between the inner rear edges of the supraoculars, which are relatively small and undivided. Internasals are separated by one scale; the first supralabial is almost completely fused with the scale surrounding the nostril, with only a small notch on its rear edge; there are 2 small scales between this fused scale and the fused second supralabial and loreal scale, which forms the anterior border of the pit; 1 or 2 scales are present between the supralabials and the subocular, which is bordered by 6 scales on the right and 7 scales on the left side of the head (not including the pre- and post-oculars). There are 2 postoculars and 8 paired chin shields, with the most anterior pair being the largest, between the first infralabials (which meet on the ventral side of the head) and the first ventral (defined as the first undivided scale on the ventral side of the head). The anal scale is entire, and the subcaudals are paired. The upper margin of the rostral scale is 39% the length of the lower margin.

**Variation in the paratypes.** In CAS 213587, a male, the lateral white stripe is very prominent, and there is a faint postocular streak. It has a more uniformly

colored ventral surface, which is closer to the shade of the dorsal surface, and continues on the head as far as the postocular streak, whereas in the second male, CAS 219783, the head is almost uniformly dark in coloration, being only slightly paler steel gray on the ventral surface and the supralabials. In CAS 212245 (a female), the ventral surface of body and head is distinctly paler, with this paler ventral color continuing onto the infra- and supralabials, but more patchy on the latter, and also appearing in patches on two to three scale rows above the supralabials. The internasals are in contact in both CAS 212245 and CAS 213587 but separated by one scale in CAS 219783. Ventrals are 170 in both CAS 213587 and CAS 212245 but 160 in CAS 219783; subcaudals 72 and 76 in the males and 57 in the female. Supralabials are 10/10 and 11/11 in CAS 213587 and CAS 219783, respectively, and 12/12 in the female CAS 212245, while the corresponding sublabial counts are 11/12, 12/13, and 14/14. While CAS 213587 and CAS 219783 have an almost entirely fused first supralabial and nasal, like the holotype, they lack any scales between this and the fused second supralabial/loreal scale. This scale is also fused only on the right side in CAS 213587. On the other hand, CAS 212245 has two scales between the fused first supralabial and nasal and the fused second supralabial/loreal scale as in the holotype, but the fusion of the first supralabial and nasal scale is only partial, with deep sutures extending towards the center from both sides, leaving only the area immediately below the nostril fused. The head is relatively smaller and narrower in the males, with only 10 (smallest number) and 13 or 14 (between rear edges) cephalic scales between the supraoculars (compared to 12 and 16 in the female, and fewer paired chin shields (6 or 7 compared to 8 in the females). There are 23 scale rows at mid-body and 15 scale rows anterior to the vent in CAS 213587, but 25/17 in the other two specimens.

The hemipenis is everted on both sides of the male paratype CAS 219783 and is similar to other species of *Trimeresurus*, being elongated and bifurcated for about 75–80% of the total length and lacking basal spines. The post-fork region is calcyed, and the edges of the calyces are pointed and longer near the fork. The insertion of the hemipenis retractor muscle is at 75% of the length of the tail measured from the vent.

**Comparisons.** *Trimeresurus ayeerwadyensis* sp. nov. is most closely related to *T. purpureomaculatus* and *T. erythrurus* and is also morphologically most similar to those species. They can be distinguished from other mainland *Trimeresurus* species (sensu Malhotra and Thorpe 2004a) in having 17 dorsal scale rows just anterior to the vent, compared to 15, and generally more than 21 scale rows at mid-body. *Trimeresurus purpureomaculatus* can be distinguished from *T. ayeerwadyensis* sp. nov. by a combination of a more triangular rostral scale and a larger number of cephalic scales between the supraoculars (averaging 15.5 at the widest point versus 13.15). *Trimeresurus erythrurus* is less easily distinguished but tends to be smaller (especially for females) and has less heavily and sharply keeled scales on the body and temporal region.

Dorsal color pattern is highly variable ranging from light or olive-green with no distinct blotches, similar to *T. erythrurus*, to olive-green with dark, irregular blotches, similar to *T. purpureomaculatus*. The ventrolateral side of head is yellowish, and there is a white dorsoventral stripe present in both sexes. The iris color varies from deep red to golden.

**Distribution.** *Trimeresurus ayeyarwadyensis* sp. nov. occurs at Hlawga Park in the Yangon Region and Pyapon and Myaungmya districts in the Ayeyarwady Region. The northern and western limits of its distribution likely lie somewhere in between the Myaungmya and Patheingyi districts in the Ayeyarwady Region. Southward, it could occur in mangroves in Mon State.

**Natural history.** In the Pyapon and Myaungmya districts in the Ayeyarwady Region, snakes were found in mangrove forests, whereas at Hlawga Park in the Yangon Region, snakes were found in forested habitats around a lake that is not connected to any mangrove system. In that regard, *T. ayeyarwadyensis* sp. nov. is more similar to *T. erythrurus* as opposed to *T. purpureomaculatus*, which is a strict mangrove-associated species.

**Etymology.** The specific epithet “*ayeyarwadyensis*” refers to the Ayeyarwady River (= Irrawaddy River), which is the largest and one of the most important rivers in Myanmar. The river forms an expansive delta that is bounded by the Patheingyi River to the west and the Yangon River to the east. These rivers and their associated basins also mark the westernmost and easternmost distribution boundaries of *T. ayeyarwadyensis* sp. nov.

## Discussion

In terms of color pattern, *Trimeresurus ayeyarwadyensis* sp. nov. shares characteristics with both *T. purpureomaculatus* and *T. erythrurus*. For example, specimen CAS 213410 from Yangon is bright green with no dorsal markings (Fig. 4A) and is virtually identical to *T. erythrurus* (Fig. 4B). A photograph of an unvouchered live specimen from Yangon corroborates this observation (Fig. 5). Contrastingly, CAS 219764 from Pyapon District in the Ayeyarwady Region has a dark or olive-green base dorsal coloration with distinct dark blotches (Fig. 4C) reminiscent of *T. purpureomaculatus*—albeit *T. purpureomaculatus* does not have a green base dorsal coloration (Chan et al. 2022). On the other hand, CAS 212245 from Myaungmya District in the Ayeyarwady Region appears to be an intermediate of the other two specimens in having a dark or olive-green base dorsal coloration with no distinct blotches (Fig. 4D). Despite these variations, all three specimens were unambiguously shown to represent a distinct, monophyletic lineage that is not conspecific with either *T. purpureomaculatus* or *T. erythrurus* (Chan et al. 2023). The distinct evolutionary trajectory of the new species coupled with the lack of morphological differentiation makes *T. ayeyarwadyensis* sp. nov. an archetypal example of a true cryptic species.

Chan et al. (2023) showed that both *T. erythrurus* and *T. ayeyarwadyensis* sp. nov. co-occur in the Ayeyarwady Region. A population in the Patheingyi District was shown to be highly admixed between *T. erythrurus* and *T. ayeyarwadyensis* sp. nov. but was clearly conspecific with *T. erythrurus*, which is reflected in the morphological similarity presented in this paper. A mere ~50 km away in the Myaungmya District, a relatively pure population of *T. ayeyarwadyensis* sp. nov. occurs instead of *T. erythrurus* (Chan et al. 2023), indicating that there is a narrow contact zone around this area that serves as a natural distribution barrier for both *T. erythrurus* and *T. ayeyarwadyensis* sp. nov. (*T. erythrurus* has not been documented south of Patheingyi and *T. ayeyarwadyensis* sp. nov. has not been documented north or west of Myaungmya). No live photographs are available of snakes from the Patheingyi District, but examination of preserved speci-



**Figure 4.** *Trimeresurus* species in Myanmar **A** *T. ayeyarwadyensis* sp. nov. (CAS 213410) from Hlawga Wildlife Park, Yangon Region (photo by CAS-Myanmar Herpetology Survey team, CAS-MHS) **B** *T. erythrurus* (CAS 235958) from Phalum District, Chin State (photo by Hla tun) **C** *T. ayeyarwadyensis* sp. nov. (CAS 219764) from Meinmahla Kyun Wildlife Sanctuary, Pyapon District, Ayeyarwady Region (photo by Hla tun) **D** *T. ayeyarwadyensis* sp. nov. (CAS 212245) from Mwe Hauk Village, Ayeyarwady Region (photo by Dong Lin).



**Figure 5.** Photographs of an unvouchered, live specimen of *Trimeresurus ayeyarwadyensis* sp. nov. from the Yangon Region, Myanmar (Photos by Wolfgang Wüster).

mens shows a lack of distinct dorsal markings. Towards the south, there is a distribution gap at Mon State where neither *T. ayeyarwadyensis* sp. nov. nor *T. purpureomaculatus* has been documented. However, this could be due to the

lack of sampling in that area. *Trimeresurus purpureomaculatus* has been documented as far north as the Dawei District in the Tanintharyi Region and it is plausible that another contact zone exists between *T. ayeerwadyensis* sp. nov. and *T. purpureomaculatus* in the intervening area, possibly within Mon State. Interestingly, the mtDNA placed populations from the Tanintharyi Region in a clade with the new species from Yangon and Areyarwady (Chan et al. 2022), whereas the genomic DNA placed the Tanintharyi populations in a clade with *T. purpureomaculatus* (Chan et al. 2023), thus reflecting putative hybridization or possible mtDNA introgression in this group. The demographic history and phylogeographic patterns of *T. erythrurus*, *T. ayeerwadyensis* sp. nov., and *T. purpureomaculatus* represent a compelling model to study speciation dynamics at contact zones and allude to the possibility of similar phenomena occurring in other groups of Asian pit-vipers. Our results provide a useful roadmap to guide future studies and highlight the need for targeted sampling in the Ayeyarwady Region and Mon State.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

KOC conceived, designed, executed, wrote, and supervised the study; DG and AM helped with data collection/analysis, writing, and provided intellectual input; SA provided financial and intellectual support; AS, ITL, ISL, SR, and CC helped with data collection and provided intellectual input.

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## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Raw morphological data of specimens examined in this study

Authors: Kin Onn Chan, Shahrul Anuar, Ananthanarayanan Sankar, Ingg Thong Law, Ing Sind Law, Rasu Shivaram, Ching Christian, Daniel G. Mulcahy, Anita Malhotra

Data type: xlsx

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Link: <https://doi.org/10.3897/zookeys.1186.110422.suppl1>

# A review of the anthidiine bees (Apoidea, Megachilidae) in Thailand

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## Abstract

Bees of the tribe Anthidiini (Apoidea: Megachilidae) are notable pollinators consisting of resin bees, wool-carder bees, and cleptoparasitic bees. Twelve anthidiine species were historically reported in Thailand, though the taxonomic information of the group was needed revising. In this study, 165 (97♀, 68♂) anthidiine bee specimens deposited at the Chulalongkorn University Natural History Museum, Thailand, were examined with material obtained from various museum collections. Specimens were principally collected in Thailand with some from Laos and Myanmar. Here, at least eight genera and 15 species of anthidiine bees are recognized: *Anthidiellum* (5), *Bathanthidium* (1), *Eoanthidium* (1), *Euaspis* (4), *Pachyanthidium* (1), *Pseudoanthidium* (1), *Stelis* (1), and *Trachusa* (1). *Dianthidium chinensis* Wu, 1962, *Eoanthidium chinensis* (Wu, 1962), *Eoanthidium semicarinarum* Pasteels, 1972, and *Eoanthidium punjabensis* Gupta & Sharma, 1953 are relegated as junior synonyms of *Eoanthidium* (*Hemidiellum*) *riparium* (Cockerell, 1929), **stat. nov.** Both *Anthidiellum* (*Pycnanthidium*) *latipes* (Bingham, 1897) from Phang Nga and *Euaspis* aff. *wegneri* Baker, 1995 from Chumphon were identified as new records for Thailand. *Trachusa* aff. *vietnamensis* Flaminio & Quaranta, 2021 from Phitsanulok is a new record for the genus found in Thailand, whereas *Pseudoanthidium* (*Pseudoanthidium*) *orientale* (Bingham, 1897) is a new record for Laos. Annotated comments are provided for some taxa and identification keys for the Thai anthidiine bees is provided.

**Key words:** Pollinator, resin bees, Southeast Asia, taxonomy, wool carder bees



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## Introduction

Megachilid bees in tribe Anthidiini are robust, usually with yellow maculation and sparse pubescence on the body. The diagnostic characters for the Anthidiini include a short pterostigma (length less than twice of its width), the absence of a median spine on the metanotum, and in many species also by an absence of long hairs on the hind tibial surface (Michener 2007; Gonzalez et al. 2012). Anthidiine bees are cosmopolitan, comprising more than 900 described species worldwide (Ascher and Pickering 2022) that exhibit various nesting strategies within their solitary lifestyles.

The tribe Anthidiini is classified into three groups, based on their nesting material usages: resin users, plant fiber users, and cleptoparasitic species (Fabre 1891; Michener 2007). Because of the variations in morphology, numerous classification systems were hypothesized (see Pasteels 1969; Warncke 1980; Michener 2007; Urban and Moure 2012). Recently, Litman et al. (2016) suggested that many anthidiine genera are paraphyletic based on the results of a molecular phylogeny, the authors classified the anthidiine bees into five monophyletic clades: *Anthidium* group, *Anthodioctes* group, *Dianthidium* group, *Trachusa* group, and *Stelis* group.

Anthidiine bees have been scarcely collected in Thailand, except for the cleptoparasitic *Euaspis polynesia* Vachal, 1903 since its preferred host, *Megachile* (*Callomegachile*) *disjuncta* (Fabricius, 1781), is common. Only 12 species of Anthidiini have been previously recorded in Thailand (Ascher and Pickering 2022), belonging to six genera: *Anthidiellum* (5 species), *Bathanthidium* (1 sp.), *Euaspis* (3 spp.), *Pachyanthidium* (1 sp.), *Pseudoanthidium* (1 sp.), and *Stelis* (1 sp.) (Friese 1925; Cockerell 1929; Dover 1929; Pasteels 1980; Baker 1995; Engel 2009; Tadauchi and Tasen 2009; Niu et al. 2019; Nalinrachatakan et al. 2021b). Nalinrachatakan et al. (2021b) described two new species, one of *Anthidiellum* (*Ranthidiellum*) and another of *Stelis* (*Malanthidium*), as well as documented their nesting biology.

There are persistent taxonomic difficulties for Thai anthidiines which need revision since many species were only recorded once. For example, the rare endemic resin bee genus *Anthidiellum* subgenus *Ranthidiellum*, of which four species were recorded, two were only known from females (see Pagden 1934; Pasteels 1969, 1972; Ascher et al. 2016). *Stelis siamensis* Friese, 1925 was described solely from one male specimen and later synonymized under *Bathanthidium binghami* (Friese, 1901) by Niu et al. (2019). Furthermore, a single female of *Dianthidium riparium* Cockerell, 1929 was synonymized under *Anthidiellum* (*Pycnanthidium*) by Soh et al. (2016) since *Dianthidium* is known to be a New World genus. Hence, this study aims to summarize the current status of the anthidiine bees in Thailand by combining museum specimen data with a citizen science database.

## Material and methods

One hundred and sixty-five anthidiine specimens (97♀, 68♂) were examined in this study. Most of the specimens were collected after 2003 and deposited at the Chulalongkorn University Natural History Museum (**CUNHM**; 71♀, 54♂). Others were on loan from the Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University (**CMU**; 1♀), Department of Entomology, Faculty of Agriculture, Kasetsart University (**KKIC**; 3♀), and from the Princess Maha Chakri Sirindhorn Natural History Museum, Faculty of Science, Prince of Songkla University (**PMCS**; 1♀). Specimens in Kumar et al. (2017) and deposited at the Department of Entomology, University of Agricultural Sciences (**UAS**; 10♀, 5♂), Bangalore, India, were also examined. Access to the type specimens and other materials was kindly provided by the Natural History Museum, London (**NHMUK**; 5♀), Naturalis Biodiversity Center: Leiden, Netherlands (**NBC**; 1♀, 2♂), the Royal Belgian Institute of Natural Sciences (**RBINS**; 1♂), Snow Entomological Museum Collection, Lawrence, Kansas, USA (**SEMC**; 5♀, 4♂),

Natural History Museum, Berlin, Germany (**ZMB**; 1♂), and the Zoological Survey of India (**ZSI**; 1♂).

Specimens were photographed with two photographic systems. The first system used the Canon 7D Mark II digital camera attached to a Zeiss Stemi 508 stereomicroscope, with a T2-T2 1.6× SLR long-distance microscope lens, controlled via Canon EOS Utility software. The second system used the identical digital camera but was mounted into the Cognisys Stackshot Macro Rail Package system and attached with Canon MP-E 65 mm f/2.8 1-5× macro lens. These sets of photographs were calibrated using AXIOVISION SE64 Rel. 4.9.1 software, for the measurement of the morphological characters of the specimens. All images taken were then post-processed using Adobe Photoshop CC 2018 and Adobe Lightroom CC 2018 software. Other software, including Adobe Illustrator CC 2018, ImageJ, Google Earth Pro, and QGIS (3.16.0) were also used to produce the illustrations, examining small and often overlooked characters, and ascertaining the localities of the samples through mapping.

Male bee specimens were dissected for their genitalia and terminalia examination: i.e., using 3M KOH to clear out muscular artifacts and later preserved in glycerin (adapted from Gonzalez et al. 2012 and Nalinrachatakan et al. 2021b). All terminology used follows Michener and Griswold (1994), Michener et al. (1994), Michener (2007), Engel (2009), and Kasperek (2017). The abbreviations T1, T2, T3, ..., S1, S2, S3, ... and F1, F2, F3, ... are referred to in sequential order of tergum, sternum, and antennal flagellomere, respectively.

In addition to records obtained through specimen examinations, the five Thai Anthidiini taxa were consulted in iNaturalist (2023) to expand spatiotemporal coverage for these bees. All images were identified by one or more of the authors. Due to the inherent uncertainty of identification of species-level taxa likely new to science from images, we do not treat these occurrences in full here, but do comment on the image-based records where pertinent under entries for the set of species could be confirmed by specimens. The records can be accessed through the URL “<https://www.inaturalist.org/observations/>” followed by its corresponding observation identification number provided in material section.

## Results

### Taxonomic account

#### ***Anthidiellum* Cockerell, 1904**

*Anthidium* (*Anthidiellum*) Cockerell, 1904: 3. Type species: *Trachusa strigata* Panzer, 1805, by original designation.

**Note.** *Anthidiellum* is a small-robust genus that has an arcuate subantennal suture (Fig. 2C), carinated omaulus (see Fig. 2A), open scutoscutellar suture (see Fig. 2B), and presence of a propodeal fovea behind the spiracle. Two distinct subgenera were recognized in Thailand: *Pycnanthidium* Krombein, 1951, with a generally black-yellow integument and a prominent frontal carina on T1; and *Ranthidiellum* Pasteels, 1969, which is slightly larger in comparison, with a general black to reddish brown integument (Fig. 4A, B), and with a general appearance similar to a stingless bee.

***Anthidiellum (Pycnanthidium) smithii* (Ritsema, 1874)**

Fig. 1

*Anthidium smithii* Ritsema, 1874: 111. (♂) Male holotype from Ambarawan, Java (NBC, not examined).

*Anthidium minutissimum* Bingham, 1903: 6. (♂) Male holotype from “Biserat, Jalor, Siam” [= Yala province, Thailand] (NHMUK, images examined).

*Anthidium javanicum* Friese, 1909: 257. (♂) Two syntypes from Buitenzorg [= Bogor, Java], collected by Schmiedeknecht.

*Anthidiellum smithii smithii* (Ritsema): Pasteels 1972: 89–93, figs 31–39; Soh et al. 2016: 51–54, figs 1A, 2.

**Material examined.** 11 (3♀, 8♂). **MYANMAR** (new record): 1♀, Dawei city, Dawei Hospital (13°59.117'N, 98°7.479'E, alt. 4 m), 3 May 2018, N. Warrit et al. (CUNHM: BSRU-AA-6909); **THAILAND**: 4♂, Chumpon, Lang Suan District, Ban Suan Phueng, (10°01'N, 99°03'E, alt. 10 m), 15 Jul. 2003, N. Warrit (CUNHM: BSRU-AA-1245–1248); 1♀, Khon Kaen (new record), Phu Wiang District, 26 May 2016, N. Warrit et al. (CUNHM: BSRU-AA-4482); 1♂, Surat Thani, Koh Samui District, 17 Jul 2003, N. Warrit (CUNHM: BSRU-AA-1244); 1♀, 3♂, Ubon Ratchathani (new record), Phu Chong Na Yoi National Park, Trail to National Park Protection unit PorJor5 (14°33'4.35"N, 105°25'36.80"E, alt. 216 m), on *Colona auriculata* (Desf.) Craib. [Malvaceae], 28 Sep. 2020, N. Warrit et al. (CUNHM: BSRU-AB-1367–1370).

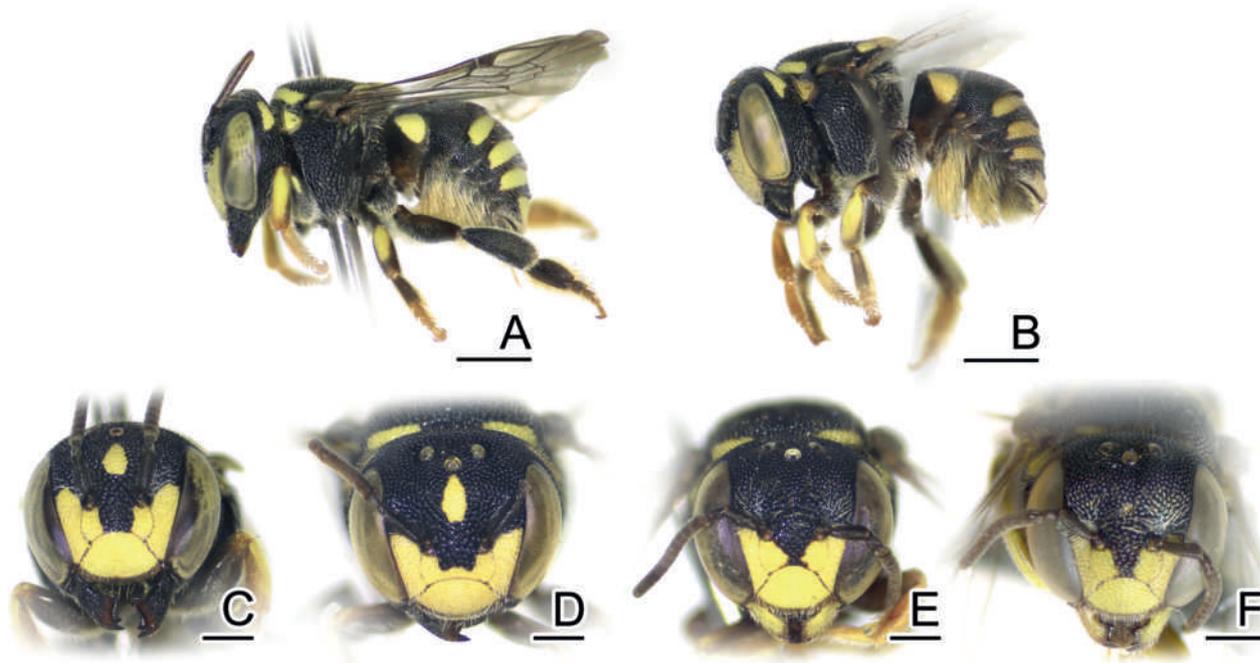
**Distribution.** INDONESIA (Bangka, East Kalimantan, Java, Sumba, Maluku Island), LAOS (Houaphanh), MALAYSIA (Negeri Sembilan, Penang), MYANMAR (Dawei, new record), PHILIPPINES (Palawan), SINGAPORE, THAILAND (Chaiyaphum, Chiang Mai: Tadauchi and Tasen 2009, Chonburi, Chumpon, Khon Kaen, Nan, Surat Thani, Ubon Ratchathani (new record), Yala).

This species can be rarely found in the Southeast Asian region. A similar species, *Anthidiellum (Pycnanthidium) carinatum* (Wu, 1962), is known from China (Hainan, Yunnan) and India (Tripura) (see Niu et al. 2016; Sardar et al. 2022).

**Diagnosis.** Within subgenus *Pycnanthidium*, this species has a small black body (3.9–5.0 mm) with yellow maculations on all tagmata. It differs from other congeners by its metasomal coloration, i.e., T1 with yellow markings laterally, T2 entirely black, T3–T6 with broad yellow bands, mostly interrupted medially on T3; axilla yellow; broad yellow marginal band on scutellum, medially interrupted; outer surface of the hind tibia and hind basitarsus with longitudinal carinae; black apical comb of S5 in male interrupted medially resembles small notch; gonostylus bifid. However, this species is similar to *A. carinatum* (Wu, 1962) from China, although Niu et al. (2016) suggested subtle differences, primarily related to punctures size and color pattern.

**Floral association.** *Bidens pilosa* L. (Asteraceae), *Muntingia calabura* L. (Muntingiaceae) (Soh et al. 2016); *Microcos tomentosa* Sm. (Malvaceae) (Pasteels 1972); an individual from Ubon Ratchathani was found collecting pollen on the flower of *Colona auriculata* (Desf.) Craib. (Malvaceae).

**Remarks.** *Anthidiellum smithii* was originally reported in Thailand from Yala province as *A. minutissimum* Bingham, 1903. More than a hundred years later, Tadauchi and Tasen (2009) reported it from Chaiyaphum and Chiang Mai



**Figure 1.** *Anthidiellum smithii* (Ritsema, 1874) **A, B** lateral habitus of females from Myanmar (BSRU-AA-6909) and Khon Kaen (BSRU-AA-4482), Thailand, respectively **C–F** faces of Myanmar female, Khon Kaen female, and Chumporn males (BSRU-AA-1245, 1247). Scale bars: 1 mm (**A, B**); 0.5 mm (**C–F**).

provinces. Variations in color pattern of *A. smithii* are widely recognized, as noted by Pasteels (1972) and Soh et al. (2016).

In this study, four males collected at the same time and place in Chumpon Province display varying color patterns. Yellow teardrop markings on the frons are small in two specimens (Fig. 1E, F) (BSRU-AA-1246, BSRU-AA-1247 though the antero-lateral scutal bands are different in size), whereas teardrop marking is expanded in specimens BSRU-AA-1248, and absent in BSRU-AA-1245. All specimens examined have yellow supraclypeal area that is medially interrupted; however, a female from Khon Kaen (BSRU-AA-4482) (Fig. 1D) and one male from Ubon Ratchathani (BSRU-AB-1370) have a yellow marking on its basal area while others do not. Color variation in this species thus can occur in sympatric populations and may be a continuous trait. More discussion on yellow maculation variations is elaborated by Soh et al. (2016).

### ***Anthidiellum (Pycnanthidium) latipes* (Bingham, 1897)**

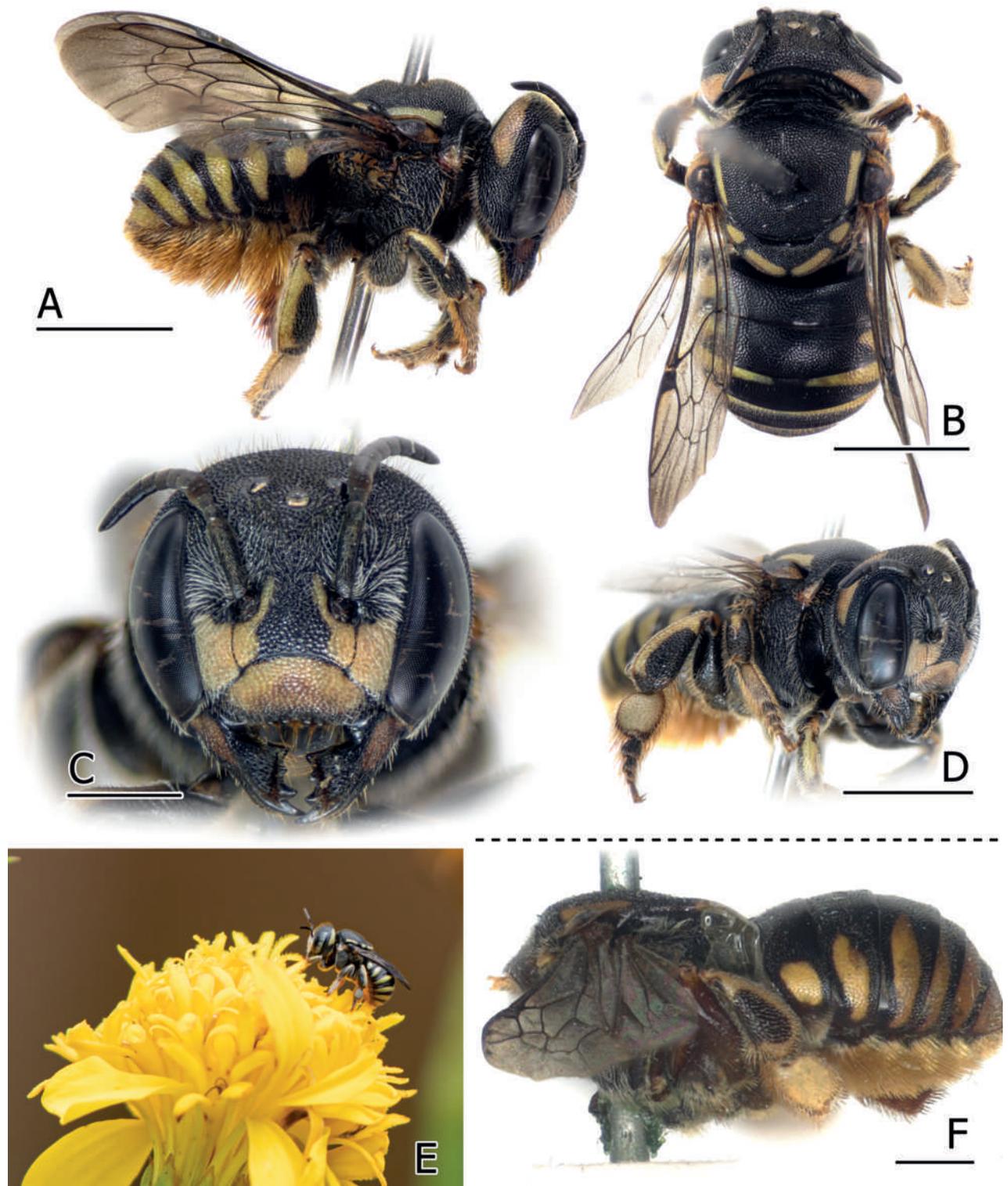
Fig. 2

*Anthidium latipes* Bingham, 1897: 495 (♀). Holotype from “Rangoon” [= Yangon], Myanmar (NHMUK, examined).

*Paraanthidium latipes* Bingham: Wu 1962: 162–163, fig. 16 (♀, ♂ nov.); Wu et al. 1988: 61 (♀, ♂).

*Trachusa (Paraanthidium) latipes* (Bingham, 1897): Wu 2006: 180 (♀, ♂).

*Anthidiellum (Pycnanthidium) latipes* (Bingham, 1897): Niu et al. 2016: 337, 341–343 (♀, ♂).



**Figure 2.** Female of *Anthidiellum latipes* (Bingham, 1897) from Phang Nga, Thailand (BSRU-AB-0162) (A–E), and the female holotype of *A. latipes* from Yangon, Myanmar (NHMUK 014026066: picture modified from NHMUK data portal) (F) A lateral habitus B dorsal habitus C face D lateral angle showing the omaulus and hind legs E female wandering on the marigold flower (*Tagetes erecta* L.), photographed by Andaman Kaosung F lateral habitus. Scale bars: 2 mm (A, B, D); 1 mm (C, F).

**Material examined.** 3 (3♀). **MYANMAR:** 1♀ holotype, Rangoon [= Yangon, Myanmar], 1–87 Bingham coll., *Anthidium latipes* ♀ Bingham Type B.M. TYPE HYM. 17a.1873, Col. C.T. Bingham. 96–30. (NHMUK 014026066); **THAILAND:** 2♀,

Phang Nga, Kapong District, Tha Na Subdistrict (8°41'38.14"N, 98°24'28.87"E, alt. 29.4 m), 30 Apr. 2020, A. Kaosung (CUNHM, BSRU-AB-0162, 0163).

**Records from iNaturalist (2023).** MYANMAR: Yangon, Yangon District, (16°47'12.3"N, 96°08'38.1"E) observed by 'chimik' on 25 Apr. 2022. (observation id: 113229246). THAILAND: Chiang Mai, Mueang District, Suthep-Pui (18°49'00.5"N, 98°55'26.8"E, accuracy 240 m) observed by 'jackychiangmai' on 9 Apr. 2023 (observation id: 154207614), and on 13 Apr. 2023 (observation id: 154700134 and 154702585).

**Distribution.** CHINA (Yunnan), MYANMAR (Yangon), THAILAND (Chiang Mai (new record from iNaturalist 2023), Phang Nga (new record)).

**Diagnosis.** *Anthidiellum latipes* can be assigned to a group of Asian *Pycnanthidium* which includes *A. butarsis* Griswold, 2001, *A. ramakrishnae* (Cockerell, 1919), *A. rasorium* (Smith, 1875), *A. coronum* (Wu, 2004), and *A. cornu* Tran & Engel, 2023. The group contains medium-sized bees without carina on their hindlegs (Griswold 2001; Niu et al. 2016; see also Tran et al. 2023).

**Floral association.** Marigold (*Tagetes erecta* L., Asteraceae, see Fig. 2E) and also yardlong bean (*Vigna unguiculata* (L.), Fabaceae).

**Remarks.** The knowledge on the Asian *Pycnanthidium* is relatively scant due to the limited material and the damages in type specimens such as in *Anthidiellum ramakrishnae* (Griswold, 2001), and with the materials of *A. coronum* (discussed below). The female holotype of *A. latipes* from Myanmar is also not in good condition, the head and most of the legs were missing while the mesosoma and metasoma were glued together.

There is a possibility that *A. coronum* (Wu, 2004) is a junior synonym of *A. latipes*, as the color patterns on the supraclypeal area (Fig. 2C), hindlegs (Fig. 2D), and the shape of enlarged hind basitarsus, with ~ 1.3 length/width ratio, are more or less similar. In addition, the Thai specimens and a specimen recorded from Myanmar (through iNaturalist) share similar maculation on the supraclypeal area as seen in *A. coronum*, including the presence of mesad process running along the upper rim of the antennal socket.

### ***Anthidiellum (Ranthidiellum) apicepilosum* (Dover, 1929)**

Fig. 3

*Dianthidium apicepilosum* Dover, 1929: 55 (♀). Holotype from "Khao Ram, Siam" [= Nakhon Si Thammarat, Thailand] (NHMUK, examined); photograph available at <https://data.nhm.ac.uk/object/17eba820-1a7d-4175-a9f8-f367b04dbf94/1632960000000>.

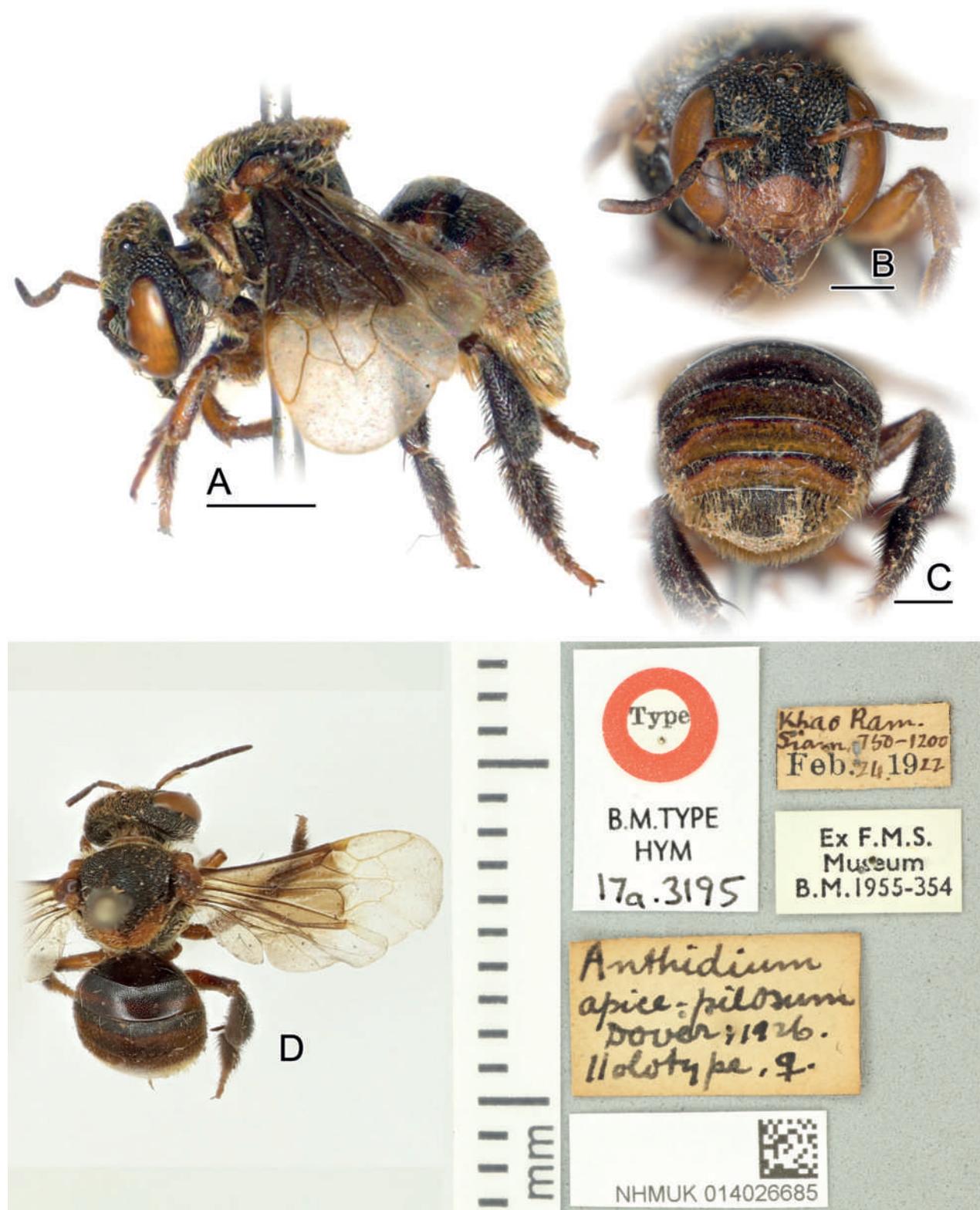
*Dianthidium apicepilosum* Dover: Pagden 1934: 490–492 (♂ nov.).

*Anthidiellum (Ranthidiellum) apicepilosum* (Dover): Pasteels 1969: 48–49.

*Anthidiellum (Ranthidiellum [sic!]) apicepilosum* (Dover): Pasteels 1972 (redescription): 103–106, unjustified emendation of *Ranthidiellum* Pasteels, 1969.

**Material examined.** 1♀. Khao Ram, Siam [= THAILAND: Nakhon Si Thammarat: Ronpibun, Khao Ramrome], 750–1200 [possibly altitude], 24 Feb. 1922, *Anthidium apicepilosum* Dover, 1926 (holotype NHMUK: 014026685).

**Distribution.** MALAYSIA (Negeri Sembilan, Penang, Selangor), THAILAND (Nakhon Si Thammarat). The species is rarely found, hence the records are based



**Figure 3.** Female holotype of *Anthidiellum apicepilosum* (Dover, 1929) (NHMUK: 014026685) **A** lateral habitus **B** face **C** rear view of metasoma showing T6 **D** dorsal view and labels, modified from NHMUK data portal. Scale bars: 2 mm (**A**); 1 mm (**B**, **C**).

on the original designation (Dover 1929) and the additional report of their nest (Pagden 1934)

**Diagnosis.** *Anthidiellum apicepilosum* has a black body with distinct brownish coloration disrupted. Since only the female is known, the most comparable

characters include the following: clypeus, scape, lower paraocular area, tegula, axilla, and the margin of scutellum brownish; scutum black; wing base conspicuously dark brown, clearly contrasting to apical hyaline parts on 1<sup>st</sup> submarginal cells; T1–T5 with reddish to brownish ferruginous apical band that becomes wider on the rear segments; T6 black; the rear of the metasoma covered with yellowish hairs; foreleg light brownish, generally brighter than in midleg and hindleg, which are almost black on their tibia and basitarsus. According to Pagden (1934) and Pasteels (1972), the male is superficially similar to the female but has lighter ferruginous leg parts especially on midleg and hindleg; S5 with a marginal black comb (> 60 teeth), gonoforceps bifid as in other *Ranthidiellum* species.

**Remarks.** This is the first species of *Ranthidiellum* that has been documented for its nesting biology (Pagden 1934). In morphology, the species is very close to *Anthidiellum rufomaculatum* (Cameron, 1902) with a minor difference in that the female holotype of “*Protoanthidium rufomaculatum*” [= *Anthidiellum rufomaculatum*, female from Kuching, Sarawak, Malaysia] has more minute paraocular marks, more slender marginal bands on its axilla and scutellum, and a reddish black translucent band present basally on the black integument of T1–T5. However, to confirm the status, there is no certain evidence that the males described by Pagden (1934) are the exact *Anthidiellum apicepilosum*, even though the locality of Bakit Kutu, Selangor, is adjacent to the Negeri Sembilan, where the female paratype was caught (further discussion in Nalinrachatakan et al. 2021b).

### ***Anthidiellum (Ranthidiellum) ignotum* Engel, 2009**

Fig. 4A

*Anthidiellum ignotum* Engel, 2009: 30–34, figs 1–3. (♀) Holotype from Sakaerat Environmental Research Area, Nakhon Ratchasima Province, Thailand (SEMC, not examined).

*Anthidiellum ignotum* Engel: Soh et al. 2016: 55 (♀); Nalinrachatakan et al. 2021b: 164–167, figs 2, 4 (right), 5 (right) (♀, ♂ nov.).

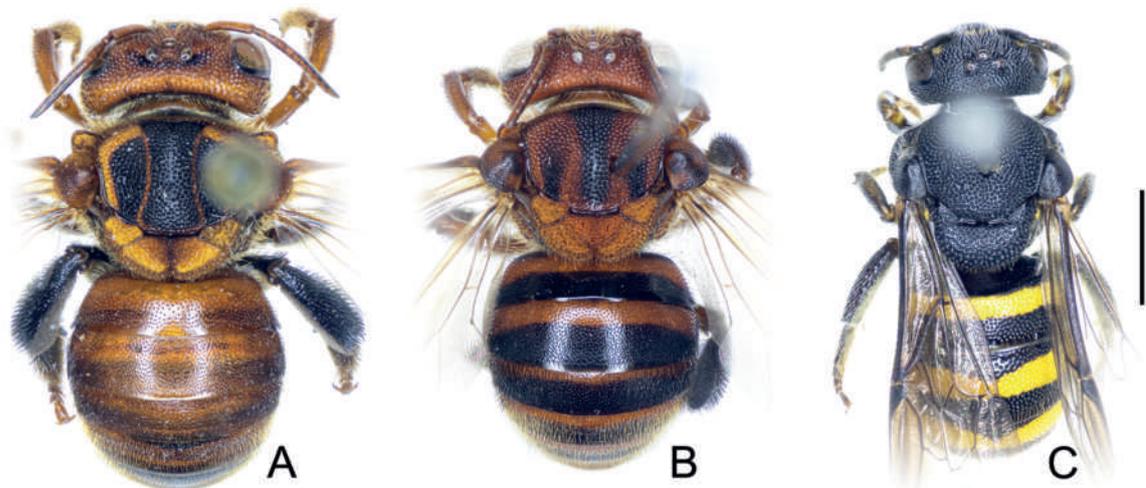
**Material examined.** (6♀, 1♂). Same specimens as in Nalinrachatakan et al. (2021b).

**Record from iNaturalist (2023).** THAILAND: Chiang Mai, Mueang District, Suthep Subdistrict (18°49'0.47"N, 98°55'26.81"E) uploaded by ‘jackychiangmai’ on 27 Oct. 2022 (observation id: 140223648).

**Distribution.** THAILAND (Chiang Mai, Nakhon Ratchasima, Phayao). The species is rare and appears to be endemic.

**Floral association.** Plant family Amaranthaceae (possibly *Achyranthes aspera* L., commonly known as devil’s horsewhip), shown in the iNaturalist observation noted above. Also, the bee must mobilize plant resin as do other *Ranthidiellum* species (Pagden 1932; Pasteels 1972; Nalinrachatakan et al. 2021b).

**Remarks.** *Anthidiellum ignotum* has distinct sexual dimorphism in which the male particularly had its base coloration brighter, with a noticeable black facial mark. More details on the presence of *Ranthidiellum* species in Thailand and its variation were discussed in Nalinrachatakan et al. (2021b).



**Figure 4.** Comparing specimens of *Anthidiellum* (*Ranthidiellum*) recently collected from Thailand, and its cleptoparasite **A** female *Anthidiellum ignotum* Engel, 2009 (BSRU-AA-1249) **B** female paratype of *Anthidiellum phuchongense* Nalinrachatakan & Warrit, 2021 (BSRU-AB-0159) **C** male paratype of cleptoparasite *Stelis flavofuscinular* Nalinrachatakan & Warrit, 2021 (BSRU-AB-0156). Scale bar: 2 mm.

***Anthidiellum* (*Ranthidiellum*) *phuchongense* Nalinrachatakan & Warrit, 2021**

Fig. 4B

*Anthidiellum* (*Ranthidiellum*) *phuchongensis* Nalinrachatakan & Warrit in Nalinrachatakan et al. 2021b: 167–171, see figs 3, 4 (left), 5 (left). (♀, ♂) Male holotype and female paratypes from Ubon Ratchathani, Thailand.

**Material examined.** (5♀, 1♂). Same specimens as in Nalinrachatakan et al. (2021b). Holotype was transferred to NHMUK in April 2023..

**Distribution.** THAILAND (Ubon Ratchathani). From a survey in other adjacent national parks in Ubon Ratchathani, there is evidence that *Ranthidiellum* is present through an abandoned nest with collapsed structures (i.e., resin became opaque, whitish, and the entrance apically fractured).

**Floral association.** Mobilizing the resin of plants in the family Dipterocarpaceae, possibly *Dipterocarpus obtusifolius* Teijsm. ex Miq., which is broadly distributed along their nesting habitats.

**Bee cleptoparasites.** *Stelis flavofuscinular* Nalinrachatakan & Warrit, 2021.

**Remarks.** The species-group name *phuchongensis* is changed to *phuchongense* following a mandatory change for gender agreement under ICZN article 34.2. The species was discovered to build its nest in a dipterocarp forest in Phu Chong Na Yoy National Park, in a preexisting hole near water stream. Their nesting structures are unique, with a distinct downwardly curved, resinous, translucent tube. Further details of its nesting biology and morphology variations were discussed in Nalinrachatakan et al. (2021b).

***Bathanthidium* Mavromovstakis, 1953**

*Bathanthidium* Mavromoustakis, 1953: 837. Type species: *Dianthidium bifoveolatum* Alfken, 1937, by original designation.

**Note.** *Bathanthidium* is an Asiatic genus consisting of small to medium-sized species that are mostly found in China. They come with almost black body and distinct yellow maculation (see Fig. 5); face without juxta-antennal carina (Fig. 5E), preoccipital ridge and omaulus not carinate; if omaular carina present, then it does not continue to the venter; presence of propodeal fovea behind propodeal spiracle. The genus was revised by Niu et al. (2019).

***Bathanthidium (Manthidium) binghami* (Friese, 1901)**

Fig. 5

*Anthidium fraternum* Bingham, 1897 (nec Pérez 1895): 495 (♀). Holotype from Tenasserim, Myanmar, image also examined in NHMUK under <https://data.nhm.ac.uk/media/02e0fc5c-8359-4414-89a1-7d0a53aed3>.

*Anthidium binghami* Friese, 1901: 224, replacement name for *Anthidium fraternum* Bingham, 1897.

*Manthidium binghami* (Friese, 1901): Pasteels 1969: 43.

*Stelis siamensis* Friese, 1925: 40 (♂). Holotype from “Siam bei Hinlap” [= Nan province, Thailand] (ZMB, examined).

*Paraanthidium concavum* Wu, 1962: 164 (♂). Holotype from China, Yunnan, Xishuangbanna (IZCAS: Institute of Zoology, Chinese Academy of Sciences, images examined).

*Trachusa (Paraanthidium) concavum* (Wu, 1962): Wu 2006: 174, ♂ (key), 184, ♂ (redescription), fig. 100a–e.

*Bathanthidium (Manthidium) binghami* (Friese, 1901): Rasmussen and Ascher 2008: 30; Niu et al. 2019: 106, fig. 8A–H; Sardar et al. 2022: 78, fig. 6.

**Material examined.** (2♂). **INDIA:** 1♂, West Bengal, Buxa Tiger Reserve, 22 miles, East Damanpur (26°37.067'N, 89°33.633'E), 27 Mar. 2019, A. Rameshkumar (ZSI) as in Sadar et al. (2022). **THAILAND:** 1♂, Siam [= Thailand], Hinlap [= Nan province, “Hinlap” must refer to the area of “Baan Hinlap”, or “Huai Hinlap reservoir” as currently named (not in Chaiyaphum province) in Pua district, Sila lang Subdistrict], Januar [= January], H. Fruhstorfer, *Stelis siamensis*, ♂, 1904, Friese det., Type (ZMB).

**Records from iNaturalist (2023).** THAILAND: Chiang Mai Province, Chiang Dao District, (19°24'44.3"N, 98°55'17.3"E) observed by ‘charlielglasser’ on 23 Mar 2023 (observation id: 160344574 and 160340826).

**Distribution.** CHINA (Yunnan), INDIA (Sikkim, West Bengal), MYANMAR (Tenasserim), THAILAND (Chiang Mai (new record from iNaturalist 2023), Nan), LAOS (Luang Prabang).

**Diagnosis.** *Bathanthidium binghami* has a robust, small to medium-sized body with black integument disrupted by striking yellow markings. The species is distinctly separated from its congeners by the combination of the follows: yellow on its mandible, clypeus, and paraocular area that do not exceed beyond the antennal socket plane; narrow yellow stripe laterally on T2–T5, while tending to abut together on the rear segment; yellow stripe on T6 and also T7 in male; rounded omaulus; T6 (also in the smaller male T7) sub-truncate, with distinct median elevation that extends its apical margin (Fig. 5C, F; Niu et al. 2019: fig. 8E).



**Figure 5.** Type specimen of *Stelis siamensis* Friese, 1904, male, from Nan province, Thailand, which was recently synonymized with *Bathanthidium* (*Manthidium*) *binghami* (Friese, 1901) **A** lateral view **B** dorsal view **C** posterior angle of metasoma **D** original label **E** face **F** dorsal of metasoma. Scale bars: 1 mm.

**Remarks.** *Bathanthidium* was revised by Niu et al. (2019), where *Stelis siamensis* Friese, 1925, historically collected from Nan, Thailand, was synonymized under *B. binghami*. Niu et al. (2019) also thoroughly provided pictures of the female holotype of "*Paraanthidium concavum*" in comparison to other species. Sadar et al. (2022) pointed out the problematic documentation of its

distribution (erroneously recorded for India) while confirming that *B. binghami* was found in India. The species displays the unique sub-truncate apical terga in both sexes.

Through personal communication with Mr. Charles H. Glasser, who provided the iNaturalist records, we know that the bee inhabits farmland cultivated by the indigenous people of Lisu tribe.

### ***Eoanthidium* Popov, 1950**

*Dianthidium* (*Eoanthidium*) Popov, 1950: 316. Type species: *Anthidium insulare* Morawitz, 1873, by original designation.

*Eoanthidium* (*Eoanthidiellum*) Pasteels, 1969: 51. Type species: *Anthidium elongatum* Friese, 1897 = *Anthidium clypeare* Morawitz, 1873, by original designation.

**Note.** An old-world genus which is mostly discernable from other genera by its more slender, striking black-yellow body, with a distinct juxta-antennal carina (Fig. 6B), rounded preoccipital ridge, carinated omaulus (see Fig. 6A, C), and rounded scutellum (Fig. 6B). Male sterna lack apical comb, penis valve enlarged, and pointed apically. The genus comprises four subgenera with 21 species in the Afrotropical, Palaearctic and Indo-Malayan regions (Kasperek and Griswold 2021). This work focuses on the subgenus *Hemidiellum* which has only one species to date. The bee subgenus *Hemidiellum* has a relatively small body length (~ 6–7 mm; Fig. 6) compared to its congeners, with the combination of characters as follow: omaular carina complete; distinct juxta-antennal carina; subantennal suture almost straight; upper margin of keirotrichiate area of hind tibia curved, not carinated; T4–T6 laterally with small tubercles in both sexes; arolia present. In addition to the type species, Gupta (1993) previously assigned *Eo. punjabense* Gupta & Sharma, 1993 to the subgenus *Hemidiellum* (see also Kasperek and Griswold 2021).

### ***Eoanthidium* (*Hemidiellum*) *riparium* (Cockerell, 1929), comb. nov.**

Figs 6–8

*Dianthidium riparium* Cockerell, 1929: 204 (♀). Holotype from Nan, Thailand (NHMUK, examined).

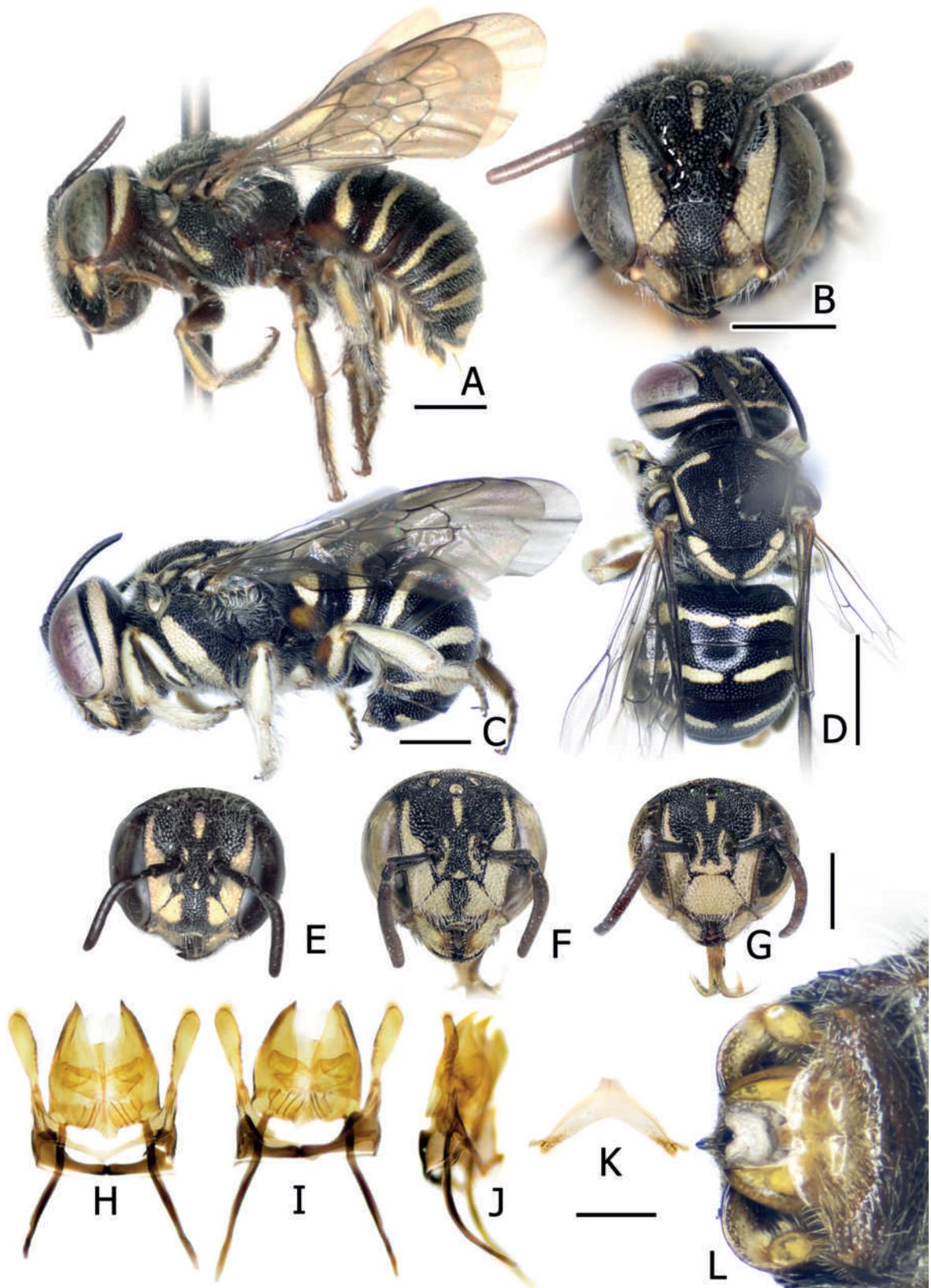
*Dianthidium chinensis* Wu, 1962: 167–168, figs 22–26 (♂) (syn. nov.). Type from Yunnan, Xishuangbanna, 9 Apr 1955.

*Eoanthidium* (*Hemidiellum*) *semicarinatum* Pasteels, 1972: 112–116 (♀, ♂) (syn. nov.). Female holotype and male paratypes from Pondicherry State, Karikal, India (NBC, examined).

*Eoanthidium* (*Hemidiellum*) *punjabensis* Gupta & Sharma in Gupta 1993: 37–39, fig. figs 65–77, 79 (♂) (syn. nov.). Male holotype from Pathankot, Punjab, 4 Jun 1991.

*Eoanthidium* (*Hemidiellum*) *punjabense* Gupta & Sharma in Gupta 1993: 37–39, figs 65–77, 79, mandatory change for gender agreement.

*Eoanthidium* (*Eoanthidium* s. str.) *chinensis* (Wu, 1962): Wu, 2006: 134, fig. 66 (syn. nov.).



**Figure 6.** *Eoanthidium (Hemidiellum) riparium* (Cockerell, 1929). Female holotype of *Dianthidium riparium* Cockerell, 1929 (syn.) from Thailand (A, B). Male from Thailand (BSRU-AB-4360) (C, D). Female from Laos (BMNH-ENT-2017-196 (ACQ)) (E), Female from Laos (BSRU-AA-1224) (F), male from Laos (BSRU-AA-1236) (G–K), and male from Thailand (BSRU-AB-4358) (L) H–J male genitalia in dorsal, ventral, and lateral view K male S8 L apical sterna of male in ventral view. Scale bars: 2 mm (D); 1 mm (A–C, E–G); 0.5 mm (H–L).

**Material examined.** 39 (16♀, 23♂). **INDIA:** Karnataka: Bangalore, GKVK, 1♀, 2 Apr. 1982, Ghorepade, 1♂, 15 Apr. 2013, Girish, (UAS); Mysore, 1♀, 19 Apr. 2009, 2♀, 16 Apr. 2009, 1♂, 5 Apr. 2009, Dhanyavathi. (UAS); 1♂, Mandya, 1 May 2014, Veereshkumar (UAS; same specimens as Kumar et al. 2017); 1♂, Coimbatore, 3 Mar. 1950, P. Susai Nathan [*Eoanthidium semicarinatum* Past. D.B. Baker det. 1982 / D.&M. Baker collection KUNHM#2004-en-004 / SEMC0975139] (SEMC25); 1♂, Madras State Coimbatore (alt. 1,400 m) Apr. 1962, P. Susai Nathan [*E. semicarinatum* det. Pasteels 1969 / PARATYPE / R.M.N.H.B. 24.136] (RBINS113); 1♂, Hisar, 15 May 1986, A. Rahman [D.&M. Baker collection KUNHM#2004-en-004 / SEMC0975140] (SEMC28); 1♀, Karnataka Malak-samudra Tank, 2 Mar. 1984, K. Ghopada [B21 / Ghorpade collection Bangalore / *E. (Hemidiellum) semicarinatum* det. C.G. Michener / SEMC1321747 KUNHM-ENT] (SEMC41); Pondicherry State Karikal, Mar. 1962, P. Susai Nathan, 1♀ [*E. semicarinatum* n. sp. Pasteels det. 1969 / HOLOTYPE / RMNH.INS 943212] (NBC001), 1♂ [*E. semicarinatum* n. sp. J.Pasteels det. 1969 / ALLO-TYPE / PARATYPE / RMNHS.INS 943188] (NBC033), 1♂ [*E. semicarinatum* n. sp. J.Pasteels det. 1969 / PARATYPE / RMNHS.INS 943189] (NBC034); **LAOS** (new record): Champasak, Si Phan Don, 3♀, 11♂, Don Det, 20 Jan. 2015, N. Warrit et al. (CUNHM: BSRU-AA-1220–1222, 1224–1226, 1228–1230, 1232–1234, 1236–1237); 1♀, KHONG ISLAND [= Don Khong], 25 Oct. 2008, D.W. Baldock, E. Popov *Hemidiellum* Pasteels *riparium* (Cckll.) det. Risch, 2008 (NHMUK: BMNH-ENT-2017-196 (ACQ)); **MYANMAR** (new record): 1♀, Dawei city (13°50.933'N, 98°9.647'E, alt. 15 m), 3 May. 2018, N. Warrit et al. (CUNHM: BSRU-AA-6896); **PAKISTAN:** Punjab, Lahore, 2 May 1979, P.H.B. Baker, 1♂, [*E. semicarinatum* Past. D.B. Baker det. 1982 / D.&M. Baker collection KUNHM#2004-en-004 / SEMC0975141] (SEMC26), 1♂, [D.&M. Baker collection KUNHM#2004-en-004 / SEMC0975140] (SEMC27), 4♀, [D.&M. Baker collection KUNHM#2004-en-004 / SEMC0975143–0975146] (SEMC29–32); **THAILAND:** 1♀, type, nan. Siam Jan. 7. (Cockerell) [= Nan province, 7 Jan, year is not indicated on the label, but Cockerell's work was published in 1929], *Dianthidium riparium* TYPE: Ckll., B.M. TYPE HYM. 17?? [?? = may be "01" but is difficult to read] 1939, Brit. Mus. 1933-567 (NHMUK 014026126); 1♂, Chiang Mai, Chiang Dao District, Chiang Dao Wildlife Sanctuary (19°24'53.2506"N, 98°54'53.2218"E, alt. 541 m) specimen from TIGER project T-5776, 19/25 Feb 2008, Songkran & Apichart (CUNHM: BSRU-AB-4358); 1♂, Lampang, Mueang Pan District, Chae Son National Park (18°49'44.2488"N, 99°28'15.1026"E, alt. 509 m), specimen from TIGER project T-5413, 7/14 Apr 2008, Boonruen & Acharaporn (CUNHM: BSRU-AB-4360).

**Distribution.** CHINA (Yunnan, new record), INDIA (Haryana: Hisar, Karnataka: Bangalore, Koppala, Mysore, Mandya, Tamil Nadu: Karikal, Coimbatore, Punjab: Pathankot), LAOS (Champasak, new record), MYANMAR (Dawei, new record), PAKISTAN (Punjab: Lahore), THAILAND (Chiang Mai (new record), Lampang (new record), Nan (new record)).

**Diagnosis.** The species exhibits pale yellow maculation, remarkably on supraclypeal area (which is reduced medially into a unique shape or absent (Fig. 6), as shown for Laotian, Myanmarese, Thai holotype of *Dianthidium riparium* Cockerell, 1929 and Chinese *D. chinensis* Wu, 1962), two paramedian yellow stripes on the scutum, and wide yellow bands on all terga which is often a little disrupted at the median on T1–T5 and also T6 in males. In males, T7 with a broadly rounded lateral lobe and a small median notch with cutting

end. Genitalia as in Fig. 6H–J, gonostylus in ventral with inner swollen base, apodeme of penis valves extremely extended.

**Floral associations.** The record of Chinese element (Wu 1962) mentioned “Eupatoreae sp.” (today recognized as Eupatorieae, Asteraceae) from China. Noteworthy, the group includes the globally invasive “Tropical whiteweed” (*Ageratum conyzoides* L.), that also widely distributed in South China and locally used as a biocontrol plant to enhance the productivity of farmland (Huang et al. 2011). The study from India by Gorain et al. (2012) reported the visitation of *Eoanthidium punjabense* on “ghaf” tree (*Prosopis cineraria* (L.) Druce (Fabaceae)).

**Remarks.** Although the specimens from China, Laos, Myanmar, and Thailand are different in their coloration compared to the type bearing the name *Eoanthidium* (*Hemidiellum*) *semicarinatum*, some characters and male genitalia are unique among the genus, and obviously comparable (see also the figures in Wu 1962; Pasteels 1972; Kumar et al. 2017). The female of “*Dianthidium riparium*” was described by Cockerell (1929), but he did not refer to its juxta-antennal carina, the main character of the genus *Eoanthidium*, and *Eoanthidium* was designated later (Popov 1950). Likewise, the description and the illustrations of *Eoanthidium* (*Eoanthidium*) *chinensis* (Wu, 1962) (Wu 1962: figs 23–26; 2006: fig. 66) and photographs of further material held in IZCAS provided by Ze-Qing Niu are adequate to synonymize this taxon with *Eo. riparium*.

When compared with “*Eo. semicarinatum*” specimens from India and Pakistan, it is evident that the individuals from Southeast Asia and China (Yunnan) are larger and darker, and tend to come with a reduction in pale yellow facial maculation in supraclypeal area, frons, and on mesepisternum, scutum, and scutellum (Fig. 7). Most of the paramedian band on the scutum is obscure, narrow, and not connected to the anterolateral mark, while the yellow mark on female hindlegs is not fully extended as in Indian and Pakistani specimens, thus, making the apical part of tibia, basitarsus, and the most of tarsi black. The pattern in most of the Laotian and Myanmarese specimens have a distinct reduction of the supraclypeal mark in the middle. A specimen from Khong Island (Fig. 6E) shows more reduction, evidenced by the disruption in the middle of the stripe below the antennal socket, coupled with an additional apical disruption noticed on the clypeus. For the female holotype of *Dianthidium riparium* from Nan, Thailand, the maculation is more reduced, clearly absent in its supraclypeal area (Fig. 6B), and thus has a strong disruption on the clypeus.

Individuals from the eastern part of the distribution (China, Laos, Myanmar, and Thailand) have a black background color of the integument with yellow markings (Fig. 8). The same is true for the populations in southern India. However, a male from northern India (Hisar) has a reddish brown background color of meso- and metasoma; the ground color of the head is black (face) and reddish brown (vertex). Individuals from Pakistan take an intermediate position, characterized by a scutum with a black background and the abdominal terga with a reddish brown background color (Fig. 8). No sexual differences were noted in the distribution of this pattern, i.e., both sexes are paler in Pakistan (4 females, 2 males) and north India (1 male), while the eastern populations are dark in both sexes.

Additionally, specimens from India and Pakistan have a much larger paramedian mark on the scutum, often extending to connect with the anterolateral mark, and generally they display more extensive maculations. The female almost has a fully yellow hindleg, sometimes with the black left on the tarsi and

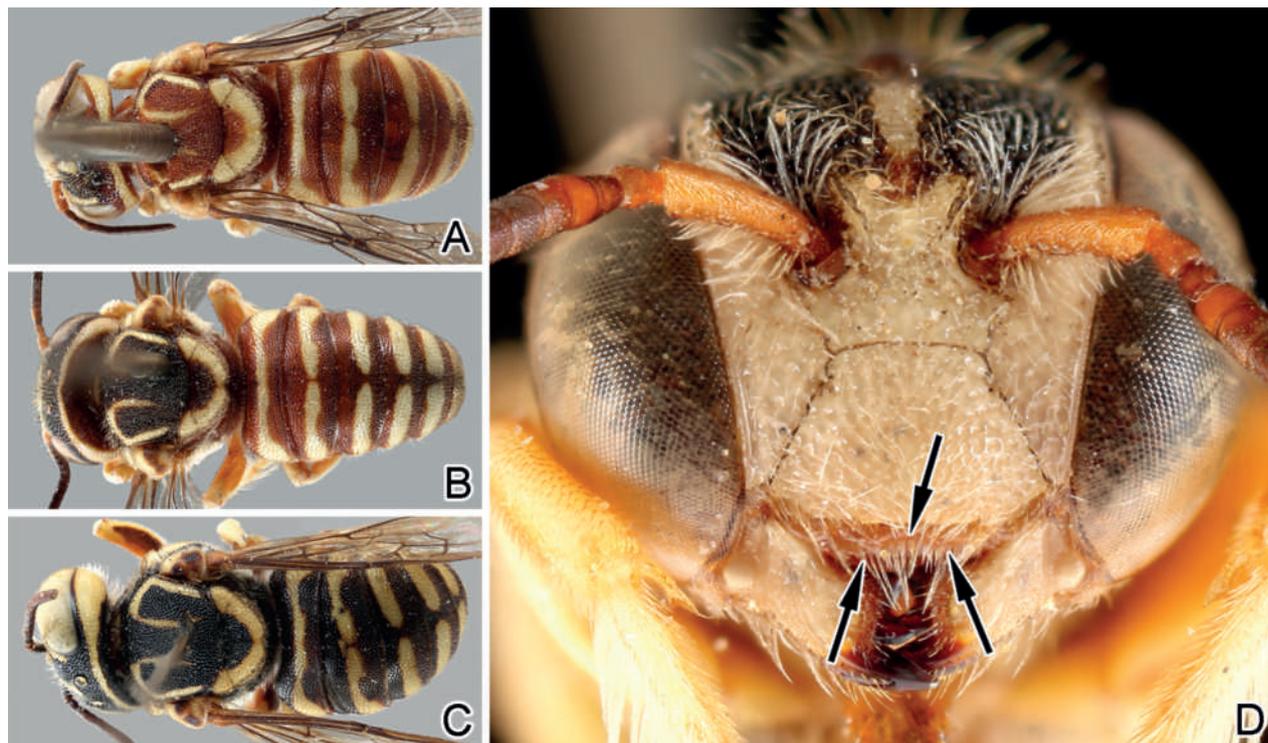


Figure 7. Illustration of *Eoanthidium (Hemidiellum) riparium* (Cockerell, 1929) facial maculation mapped according to their geographic locations (blue boxes indicate Indian-Pakistani morphs, reddish boxes indicate Indochina morphs; a morph marked with asterisk is illustrated based on Wu 1962: fig. 22).

parts of basitarsus. Such individuals with richer yellow maculation are typical for Pakistan. Some females from southern India (including those shown by Kumar et al. 2017: figs 7, 8) have a greater reduction in the yellow clypeal mark, resulting in a complete black stripe in the median area, whereas other specimens come with fully yellow without any black disruption.

For some West Palaearctic *Eoanthidium* and *Rhodanthidium* species, Kasparek (2019b, 2020, 2021) reported regional variations in the color pattern and discussed the possibility that the “darker” forms might be a result of adaptation to solar radiation. While in some cases, color variation follows geographical clines, there seems to be reproductive isolation between pale and dark forms in other cases. We observed a clear geographical pattern of color variants in *Eo. riparium*, but also intermediate forms in Pakistan (see Fig. 8), indicating that there is no reproductive isolation.

In addition, *Eoanthidium punjabense* Gupta & Sharma, 1993 is established here as a new synonym of *Eo. riparium*. Gupta (1993) noted that these two species are distinguished by the form of the apical margin of the clypeus, the shape of genitalia, color pattern of the integument and body size. While Gupta (1993) solely relied on Pasteels’ (1972) description, the larger material exam-



**Figure 8.** Male of *Eoanthidium riparium* (Cockerell, 1929) from different regions **A–C** dorsal view of individuals from South India (Karnataka), Pakistan (Panjab), and North India (Hisar, Haryana) respectively **D** face of a male *Eoanthidium riparium* from Pakistan (SEMC27). Note the change in the ground color from black (**A**) across reddish brown on terga and black on scutum (**B**) to entirely reddish brown (**C**). Also, note the shape of the reddish apical margin of the clypeus (black arrows) which is similar to the drawing of Gupta (1993: fig. 65) for *Eo. punjabense*.

ined by us as well as the examination of Pasteels' type material enabled a better understanding of the range of variation. The reddish apical margin of the clypeus is crenulated and somewhat irregularly formed. Our material includes one male with two protrusions (Fig. 8D), very similar to those shown by Gupta (1993: fig. 65). With respect to differences in genital morphology, Gupta (1993) may have been misguided by the incomplete drawings by Pasteels (1972). Body size of *Eo. punjabense* was found to be within the variability range of *Eo. riparium*.

### ***Euaspis* Gerstaecker, 1858**

*Euaspis* Gerstaecker, 1858: 460. Type species: *Thynnus abdominalis* Fabricius, 1793, by original designation.

*Dilobopeltis* Fairmaire, 1858: 266. Type species: *Dilobopeltis fuscipennis* Fairmaire, 1858 = *Thynnus abdominalis* Fabricius, 1793, by original designation.

*Parevaspis* Ritsema, 1874: 71. Type species: *Parevaspis basalis* Ritsema, 1874, by designation of Sandhouse 1943: 585.

**Note.** As a cleptoparasitic bee, *Euaspis* has a distinct median longitudinal carina (Fig. 9B), juxta-antennal carina, and often comes with reddish metasoma. Baker (1995) revised the Asiatic species and Tran et al. (2016) noted and discussed three species from Vietnam. Additional material is still crucially required to prove and justify some problematic species in this genus.

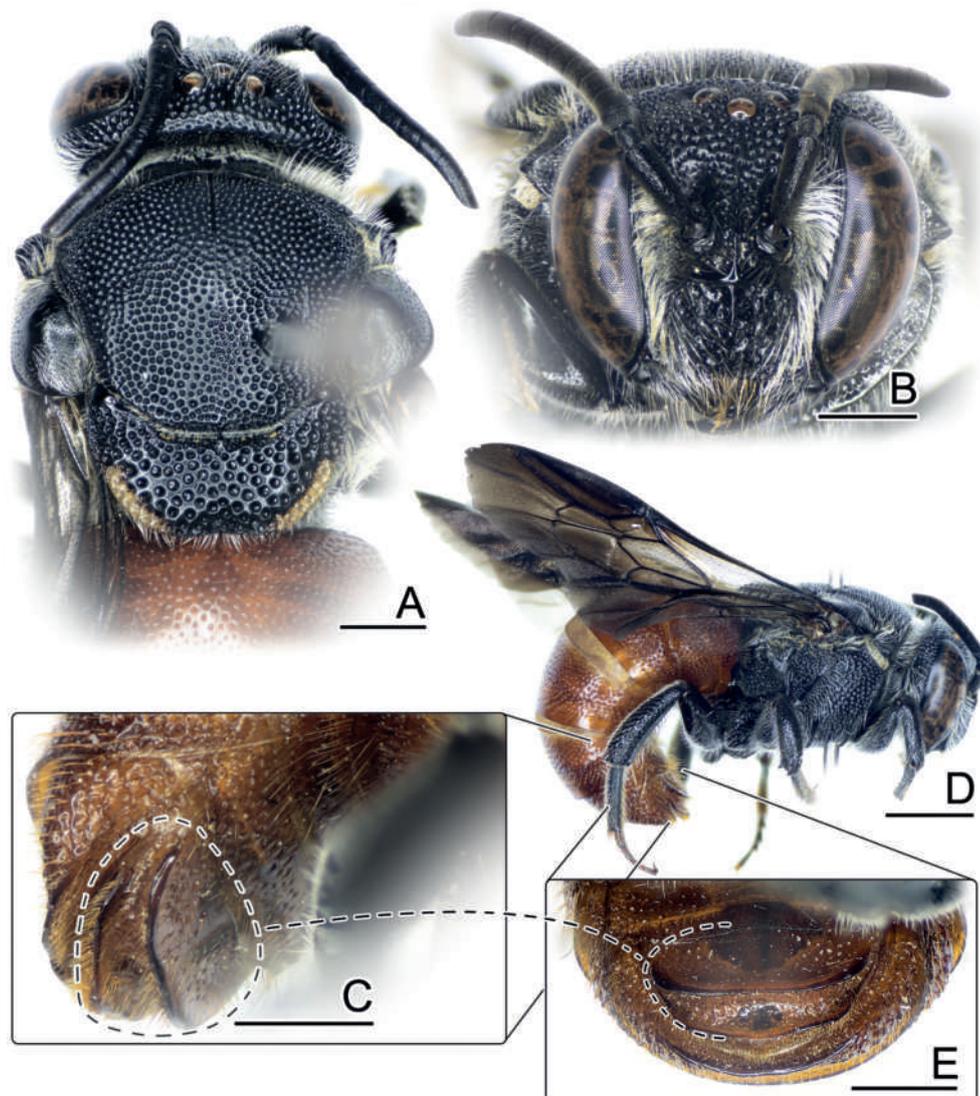
***Euaspis aequicarinata* Pasteels, 1980**

Fig. 9

*Euaspis aequicarinata* Pasteels, 1980: 78 (♀, ♂). Female holotype from Kalabankan, Sabah, Malaysia (image in NHMUK examined under <https://data.nhm.ac.uk/media/80bdf262-c729-42ef-bf3a-3180eb81ceb2>).

*Euaspis aequicarinata* Pasteels: Baker 1995: 289, 290, fig. 13; Soh et al. 2016: 57; Tran et al. 2016: 516, fig. 13.

**Material examined.** (1♀, 2♂). **THAILAND:** 1♀, Chiang Mai (new record), Chom Thong District, Ban Luang Subdistrict, Doi Inthanon National Park, Ban Mae Klang Luang. (18°32'17.9"N, 98°32'49.6"E, alt. 1,057 m), 30 Aug. 2021, on *Co-leus scutellarioides* (L.) Benth. [Lamiaceae], T. Srimaneeyanon et al. (CUNHM: BSRU-AB-4120); 2♂, Phayao (new record), Mueang District, Maeka Subdistrict, Phayao University (19°1'31.45"N, 99°53'24.17"E, alt. 558 m), 1 Jun 2012, W. Suwannarak et al. (CUNHM: BSRU-AA-4445, 4462).



**Figure 9.** Females of *Euaspis aequicarinata* Pasteels, 1980 (BSRU-AB-4120) **A** mesosoma including the scutellum **B** face **C, E** **S6** **D** lateral habitus. Scale bars: 2 mm (**D**); 1 mm (**A–C, E**).

**Distribution.** CHINA (Yunnan), INDONESIA (Java), LAOS (Vientiane), MALAYSIA (Negeri Sembilan, Borneo: Sabah, Sarawak), THAILAND (Chiang Mai (new record), Nakhon Ratchasima, Phayao (new record), Surat Thani)), VIETNAM (Kon Tum, Hoa Binh).

**Diagnosis.** Typically for *Euaspis*, *Eu. aequicarinata* has a black body with a reddish metasoma, and a median carina and a juxta-antennal carina are present on its face. This is the only species that has a distinct longitudinal carina on the clypeus, while the sculptures are confluent. Pale yellow patches are found on the lateral margin of scutellum and posterior margin of axilla (absent on axilla for female in this study, in contrast to the monochrome pictures in Baker (1995: fig. 30); compared to *Eu. strandi*, the band is more restrict and more yellowish. Female S6 obtuse, with distinct elevated basal platform (Fig. 10C, E). Male S5 with median subcircular hyaline area and median tooth, genitalia with an apical lamina which is longer than 2× its width.

**Floral associations.** *Coleus scutellarioides* (L.) Benth. (Lamiaceae).

**Remarks.** As mentioned in Soh et al. (2016), the status of male *Eu. aequicarinata* is not confirmed since the male allotype was designated from Surat Thani, Thailand, while originally, the female holotype is from Sabah (in Borneo), Malaysia. For this reason, there is currently no proof that the male is associated with the holotype regardless of the fact that they are similar in clypeal form and mesosomal maculation. Also, Pasteels (1980) did not mention any male genitalia or associated structures. Baker (1995) subsequently rectified the erroneous type locality in his key for Oriental *Euaspis* species, noting that *Eu. aequicarinata* should have an apical lamina longer than twice its width.

### ***Euaspis polynesia* Vachal, 1904**

Fig. 10A–D

*Stelis abdominalis* Smith, 1858 (nec Fabricius 1793): 7. (♂) Holotype from Celebes [= Sulawesi] (OUMNH: Oxford University Museum, not examined).

*Euaspis polyesia* Vachal, 1903a: 97. (♀ nov., ♂), incorrectly labeled (Baker 1995), replacement name of *Stelis abdominalis* Smith, 1858.

*Euaspis polynesia* Vachal, 1903b: 173, justified emendation.

*Euaspis smithii* Friese, 1904: 137, unnecessary replacement name.

*Parevapis impressus* Vierick, 1924: 745. (♀, ♂) Male holotype and female allotype from Surigao, Mindanao (USNM: United States National Museum, not examined).

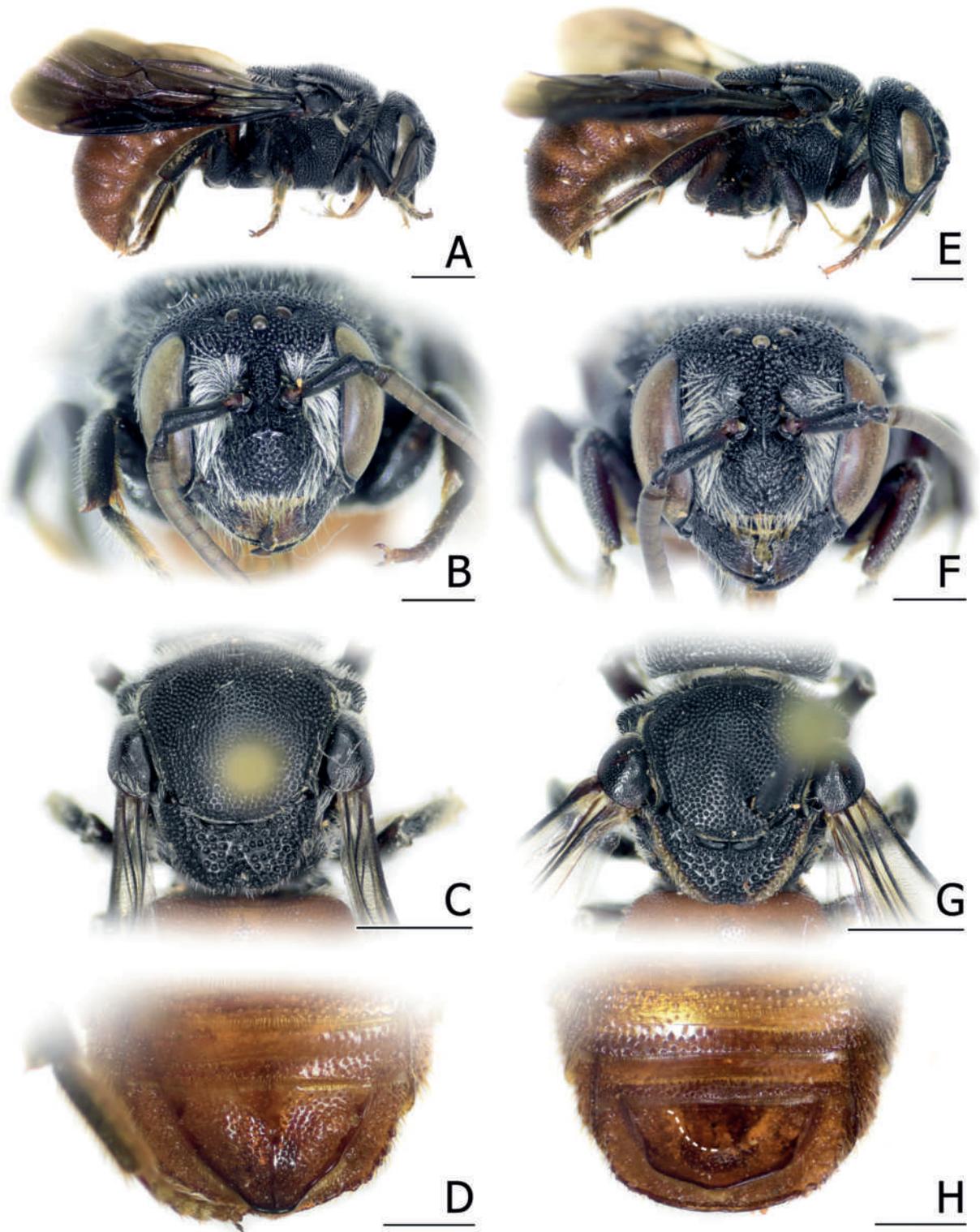
*Euaspis basalis chinensis* Cockerell, 1930: 50. (♀, ♂). Female type and male cotype from Foochow, China (NHMUK, not examined).

*Euaspis (Parevapis) polynesia* Vachal: Popov 1933: 377.

*Euaspis (Parevapis) polyesia* Vachal: Pasteels 1980: 76–89, incorrectly labeled.

*Euaspis polynesia* Vachal: Baker 1995: 286–289; Soh et al. 2016: 55–56, figs 1, 6; Tran et al. 2016: 517–518, figs 7–10; Ghosh et al. 2023: 193–196, fig. 1.

**Material examined.** 43 (20♀, 23♂). **THAILAND:** 1♀, Chainat (new record) [with obscured label] (KKIC); 1♀, Chanthaburi, Makam District, 25 May 2015, N. Chattanabun (CUNHM: BSRU-AA-4458); Chiang Mai, Chom Thong District, Ban Luang Subdistrict, Doi Inthanon National Park, Ban Mae Klang Luang, Tourist Station, 1♀, (18°32'2.8"N, 98°32'55"E, alt. 1015 m), 16 Jun. 2019, N. Warrit



**Figure 10.** Females of *Euaspsis polynesia* Vachal, 1904 (BSRU-AA-4453) (A–D) and *Euaspsis strandi* Meyer, 1922 (BSRU-AA-4470) (E–H) A, E lateral habitus B, F face C, G mesosoma including the scutellum D, H S6, with a white dash line indicating boundary of the median elevated area in the left. Scale bars: 2 mm (A, C, E, G); 1 mm (B, D, F, H).

et al., on *Cuphea hyssopifolia* K. [Lythraceae] (CUNHM: BSRU-AA-7927); 1♀, (18°32'28.4"N, 98°32'57.2"E, alt. 1020 m), 26 Oct. 2020, T. Srimaneeyanon et al. (CUNHM: BSRU-AB-1372), Chiang Mai, Chom Thong District, Ban Luang Sub-district, Doi Inthanon National Park, Ban Mae Klang Luang. 2♂, (18°32'12.17"N,

98°32'48.99"E, alt. 1,056 m), 17 Feb. 2021, Srimeeeyanon et al. (CUNHM: BSRU-AB-2810, 2843), 1♀ 2♂, (18°32'29.7"N, 98°32'1.2"E, alt. 1,033 m), 01:00–05:00PM, 7 May 2021, T. Srimeeeyanon et al. (CUNHM: ♀ BSRU-AB-3459, ♂ BSRU-AB-3548, 3550), 1♀ 2♂, (18°32'29.6"N, 98°33'01.4"E, alt. 1,012 m), 30 Aug. 2021, T. Srimeeeyanon et al. (CUNHM: ♀ BSRU-AB-4153, ♂ BSRU-AB-4154, 4155). Chiang Mai, Chom Thong District, Ban Luang Subdistrict, Doi Inthanon National Park, Mae Klang Waterfall, 1♀, (18°29'40.70"N, 98°40'01.95"E, alt. 330 m), 18 Feb. 2021, T. Srimeeeyanon et al. (CUNHM: BSRU-AB-3056), 1♀, (18°29'33.2"N, 98°40'13.1"E, alt. 319 m), 8 May 2021, T. Srimeeeyanon et al. (CUNHM: BSRU-AB-3320). 1♀, Chiang Mai, Mae Rim District, Pong Yaeng Subdistrict, Queen Sirikit Botanic Garden, 21 Aug. 2016, Aerial net, N. Chatthanabun (CUNHM: BSRU-AB-1782); Kanchanaburi (new record), 1♀, Tha Sao Dist., Hell-fire pass interpretive centre (14°21'4.1472"N, 98°57'23.5476"E, alt. 240 m), 17 Dec. 2021, S. Deowanish et al. (CUNHM: BSRU-AB-5493). 1♀, 1♂, Sai Yok District, Wang Krachae Subdistrict (14°11'6.5724"N, 99°3'6.9258"E, alt. 102.3 m), 24 Jun. 2016, N. Warrit et al. (CUNHM: BSRU-AA-4480, 4483); 1♂, Mukdahan (new record), Mueang District (16°34'11.4630"N, 104°43'47.1426"E, alt. 139 m), 18 Jan. 2017, N. Warrit et al. (CUNHM: BSRU-AA-4903); Nakhon Pathom (new record), 1♀, Kamphaeng Saen District, 8 Jul. 2003, Subat (KKIC). 1♀, 21 Nov 2002, Pornwat (KKIC). 2♂, (13°44'58.3908"N, 99°52'33.1242"E, alt. 14 m), 10 Jul. 2015, N. Warrit et al. (CUNHM: BSRU-AA4466, 4467). 2♀, KU Kamphaengsaen Campus, Insect Park (14°02'18.1500"N, 99°58'56.5016"E, alt. 3 m), 29 Jul. 2015, N. Warrit et al. (CUNHM: BSRU-AA-4476, 4477); 6♂, Phayao (new record), Mueang District, Maeka Subdistrict, Phayao University (19°1'31.45"N, 99°53'24.17"E, alt. 558 m), 1 Jun. 2012, W. Suwannarak et al. (CUNHM: BSRU-AA-4446, 4448, 4450, 4463, 4464, 4465); 1♀, Phetchabun (new record), Lomsak District, Bungkla Subdistrict (18°15'N, 103°58'E, alt. 162 m), 18 Oct. 2009, K. Attasopa & P. Phukphume (CUNHM: BSRU-AA-4442); 1♀, Phetchaburi (new record), Kang Kra Chan District, 18 Apr. 2012, C. Rungsri (CUNHM: BSRU-AA-4443); Ratchaburi (new record), 1♀, Jom Bung District, 26 May 2012, N. Warrit & W. Suwannarak (CUNHM: BSRU-AA-4453). 2♂, Ratchaburi, Suan Phueng District, Pasutara resort (13°31'5.9226"N, 99°20'51.6366"E, alt. 104.71 m), 2 Aug. 2019, P. Senawong et al. (CUNHM: BSRU-AB-0764, 0765); Saraburi (new record), 1♂, Kaeng Khoi District, Chula-Saraburi (14°31'3"N, 101°1'41"E, alt. 43 m), 15 Aug. 2015, N. Warrit et al. (CUNHM: BSRU-AA-4481). 1♀, (14°31'23.4300"N, 101°1'43.5216"E, alt. 52.89 m), 13 Oct. 2018, N. Warrit et al. (CUNHM: BSRU-AB-0154); 2♂, Trang, Na Yong District (7°33'8.0892"N, 99°46'33.6072"E, alt. 24 m), 11 Jun. 2015, N. Warrit et al. (CUNHM: BSRU-AA-4457, 4459); Ubonratchathani (new record), 1♂, Khueng Nai District, Ko Ae Subdistrict, Ubon Rachathani Rajabhat Univ. Faculty of Agriculture, 30 Aug. 2020, P. Traiyasut et. al. (CUNHM: BSRU-AB-1704), 1♂, Na Chaluai District, Phu Chong Na Yoi National Park (14°26'4.98"N, 105°15'31.04"E, alt. 269 m), 23 Jan. 2015, N. Warrit et. al. (CUNHM: BSRU-AA-4460), 1♀, Na Chaluai District, Phu Chong Na Yoi National Park, Pa Lan Pa Chad (14°26'5.36"N, 105°15'39.92"E, alt. 280 m), 27–29 Sep. 2020, P. Traiyasut et. al. (CUNHM: BSRU-AB-1704).

**Distribution.** CHINA (Anhui, Fujian, Gansu, Guangdong, Hebei, Hunan, Jiangsu, Jiangxi, Shangdong, Xizang, Yunnan, Zhejiang), HONG KONG, INDONESIA (Bali, Bangka Island, Engano Island, Java, Maluku Islands [Ambon, Buru, Kai islands], Sebesi Island, Sumatra, Sulawesi), INDIA (Arunachal Pradesh), JAPAN (Okina-wa Prefecture), LAOS (Xiengkhouang), MALAYSIA (Kedah, Kelantan, Melaka,

Penang, Perak, Selangor), MYANMAR (Shan State, Tenasserim, Yangon), NEPAL (Kathmandu), PHILIPPINES (Luzon, Mindanao), SINGAPORE, TAIWAN (Pingtung), THAILAND (Chiang Mai, Chainat (new record), Kanchanaburi (new record), Loei, Mukdahan (new record), Nakhon Pathom (new record), Pattani, Phayao (new record), Phetchabun (new record), Phetchaburi (new record), Ratchaburi (new record), Saraburi (new record), Satun, Songkhla (new record), Surat Thani, Trang (new record), Ubon Ratchathani (new record)), VIETNAM (Bak Kan, Dak Lak, Dak Nong, Dien Bien, Hoa Binh, Phu Tho, Son La, Thanh Hoa, Vinh Phuc).

Most of the previous records were documented by Baker (1995) and Pasteels (1980). The species is widely distributed in Eastern Asia, especially in South-east Asia, where Soh et al. (2016), Tran et al. (2016), and Ghosh et al. (2023) reported additional distribution records.

**Diagnosis.** This *Euaspis* species has an entirely reddish metasoma, while the prosoma and mesosoma are all black; face with longitudinal carina and a median longitudinal ridge; clypeus with uniform punctation; punctures on the scutellum looser and coarser than on the scutum; scutellum large, strongly produced posteriorly, apicomediaally with a depressed area; female S6 acute, with a median carina, without a distinct basal area (Fig. 10D); male S6 without emargination at the margin; male genitalia with the apical lamina with a length less than twice its width.

**Floral associations.** A female collected from Chiang Mai was wandering on the inflorescences of “Tropical whiteweed” *Ageratum conyzoides* L. (Asteraceae), “Black-Jack” *Bidens pilosa* (L.) Benth. (Asteraceae), and “Mexican heather” *Cuphea hyssopifolia* K. (Lythraceae). For Singapore, Soh et al. (2016) reported that *Eu. polynesia* visits the flowers of *Averrhoa carambola* L. (Oxalidaceae), *Grammatophyllum speciosum* Blume (Orchidaceae), *Muntingia calabura* L. (Muntingiaceae), and *Premna serratifolia* L. (Lamiaceae). They also mention *Cordia cylindristachya* (Ruiz & Pav.) Roem. & Schult. (Boraginaceae) and *Antigonon leptopus* Hook. & Arn. (Polygonaceae), of which the latter genus was also given by Baker (1995). The recent study from India by Ghosh et al. (2023) found *Eu. polynesia* nectaring on *Fagopyrum esculentum* Moench (Polygonaceae).

**Host-parasite relationship.** Bingham (1897) noted a single male of *Eu. polynesia* accessing the nest of *Megachile disjuncta* (Fabricius, 1781). Since the female generally takes on the parasitizing task, this must not be the direct act of the invasion for parasitisation, but the reason remains unknown.

**Remarks.** *Euaspis polynesia* is the most common anthidiine bees in Thailand, exhibiting a size range, with the females ranging 9.0–13.1 mm and the males 6.2–12.1 mm. As a cleptoparasitic bee, its occurrence seems to follow the distribution of its hosts, especially *Megachile disjuncta* (see Baker 1995), which is very common and widely distributed in Thailand. Thus *M. disjuncta* is a megachilid species with most individuals curated at the CUNHM (i.e., 102 specimens curated, from 905 Megachilidae specimens). All 11 *Euaspis* localities in the CUNHM database (Nalinrachatakan et al. 2021a) have been associated with bees from the genus *Megachile* (at least 7 subgenera were identified). *Aethomegachile* (8 localities) and *Callomegachile* (8) contributed the most, e.g., *M. (Ca.) umbripenis* (6), *M. (Ca.) disjuncta* (5), and *M. (A.) laticeps* (6). Other notable species belonged to *M. (Creightonella) fraterna* (5), and another cleptoparasitic bee genus *Coelioxys* was also found in eight of 11 occasions. This information shows that both *Euaspis* and *Coelioxys* may have a wide range of their candidate host, and there is the possibility of overlap or of having an evolutionary pressure on each

other that cannot be ignored, as both genera were reported to come with different brood-parasitizing strategies (Litman et al. 2016; Litman 2019).

A probable new species of *Euaspis* from Singapore (Soh et al. 2016) is superficially similar to *Eu. polynesia*, but mostly differs in the terminalia, apical terga coloration, and with a pale mark on the scutellum margin, while their genitalia are superficially similar. Further studies and molecular evidence are required to resolve this taxonomic conundrum.

### ***Euaspis strandi* Meyer, 1922**

Fig. 10E–H

*Euaspis* (*Parevaspis*) *strand*i Meyer, 1922: 236, 239 (♀, ♂, syntypes, male selected as lectotype by Baker 1995). Type locality erroneously noted as “Sikkim”, and Baker (1995) corrected it to be Mindanao, Philippines (ZMB, not examined).

*Parevaspis bakeri* Vierick, 1924: 745 (♂). Holotype from Kolambugan, Mindanao, Philippines (USNM: United States National Museum, not examined).

*Euaspis strandi* (Meyer): Baker 1995: 291, 293.

**Material examined.** (2♀). **THAILAND:** Phayao (new record), Mueang District, Maeka Subdistrict, Phayao University (19°1'31.45"N, 99°53'24.17"E, alt. 558 m), 1 Jun. 2012, W. Suwannarak et al. (CUNHM: BSRU-AA-4444, 4470).

**Distribution.** CHINA (Yunnan, “Kinpin”: Wu 1962: 168 as *Parevaspis bakeri*), THAILAND (Nakhon Ratchasima, Phayao: new record), PHILIPPINES (Mindanao).

**Diagnosis.** *Euaspis strandi* has a reddish metasoma, whereas the rest of the body is black, with a remarkable pale yellow stripe on the mesonotum (i.e., axilla and scutellum with pale yellow marginal band); clypeus with coarse, somewhat irregular punctures (Fig. 10F); punctures on scutellum looser and coarser than on scutum; scutellum large, produced posteriorly with a small shallow median emargination; female apical margin of S6 obtuse, with an enlarged basal platform which contributes ~ 1/2 of the sternal length (Fig. 10H); male was purposed by Viereck (1924) as without mesosomal yellow stripe, apical lamina of gonoforceps with a length of more than twice its width.

**Floral associations.** *Sindora siamensis* Teijsm. ex Miq. (Fabaceae) is associated with the female collected from Nakhon Ratchasima, Thailand (Baker 1995).

**Remarks.** In Thailand, *Eu. strandi* was reported from Sakaerat, Nakhon Ratchasima province in 1995 (Baker 1995). Two females examined from Phayao province were quite large (11.4 mm and 11.5 mm) compared with *Eu. polynesia*, which varies considerably in size.

The female individual was not observed in this study. Previously, two male specimens had been designated, the first one by Meyer (1922) as syntype together with a female specimen, and the second by Viereck (1924) as *Parevaspis strandi*. Both specimens were redescribed and discussed by Baker (1995), and the locality of Meyer’s syntype was corrected and the male was selected to be a lectotype. Therefore, the validity of the female identity is still ambiguous, also mentioned in Baker (1995): syntypes were mislabeled, collected without any notes to confirm that they come from the same locality, and are doubtfully paired since the notable character does not match, i.e., an absence of the marginal mark on scutellum and axilla, which is noticeable in the female.

***Euaspis* aff. *wegneri* Baker, 1995**

Fig. 11

*Euaspis wegneri* Baker, 1995: 290, figs 24, 31 (♀). Holotype from "BATJAN" [= Bacan, north Maluku, Indonesia] (NBC, not examined).

**Material examined.** 1♀. **THAILAND:** Chumporn (new record?), Sawi District, Na Sak Subdistrict (10°10'10.7"N, 98°56'50.5"E), 1 Jun. 2021, Suntaree Kanchananiyom. (PMCS: SK-BSRU-0068 [association number with CUNHM]).

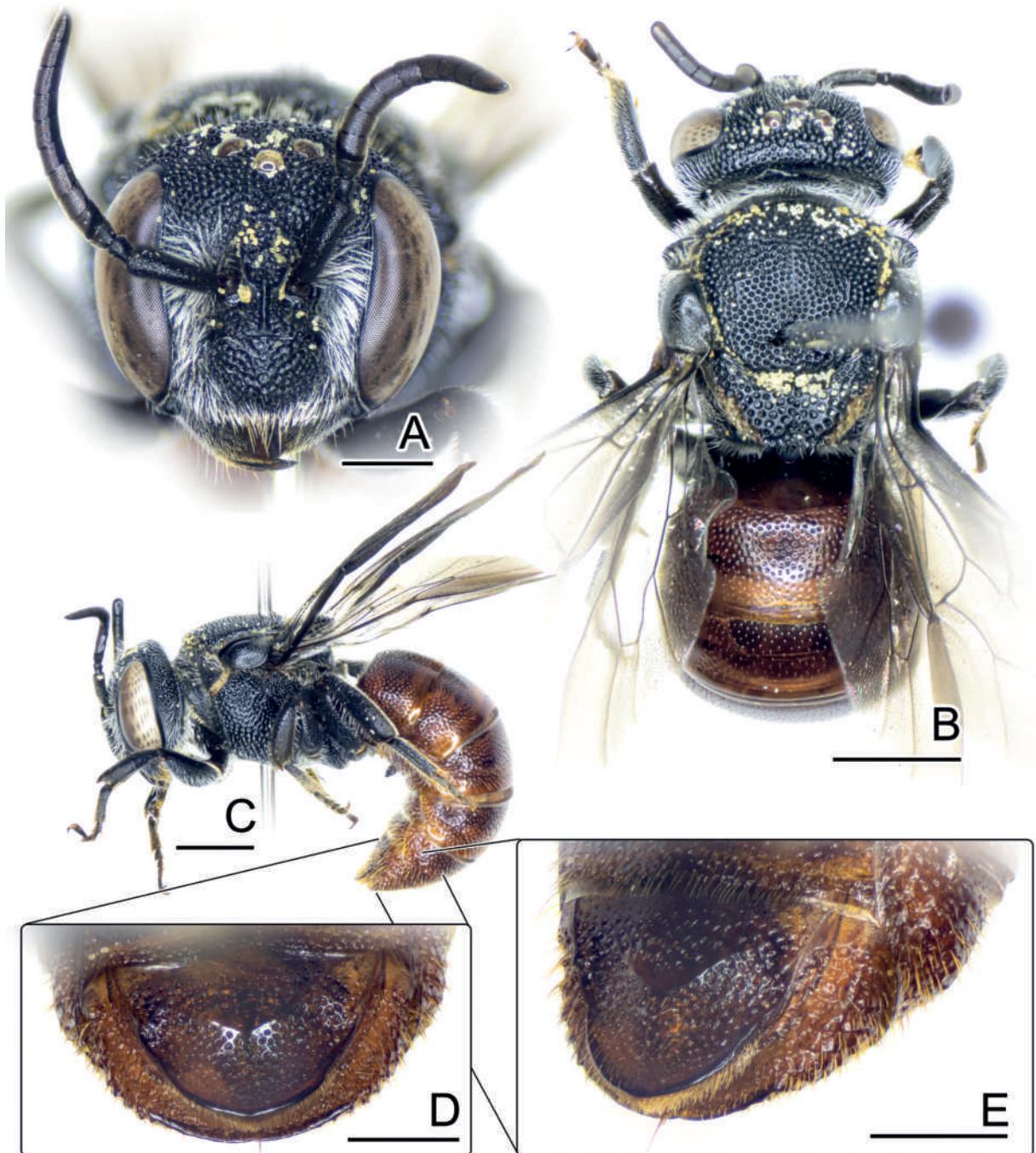


Figure 11. Female of *Euaspis* aff. *wegneri* Baker, 1995 **A** face **B** dorsal habitus **C** lateral habitus **D, E** S6. Scale bars: 2 mm (**B, C**); 1 mm (**D, E**); 0.5 mm (**A**).

**Distribution.** INDONESIA (Bacan province in north Maluku [= Batjan (in Dutch) in Baker (1995)], THAILAND (Chumporn, new record?).

**Diagnosis.** This female *Euaspis* aff. *wegneri* has a typical black body and reddish metasoma, with a pale yellow stripe on the mesonotum (Fig. 11B) resembling *Eu. strandi* (pale yellow marginal band on scutellum, more minute on axilla). Clypeus moderate in size, somewhat confused punctures, while the punctures on the scutellum are coarser than on scutum. Female S6 (Fig. 11D, E) subacute with obscured basal platform contributing almost half of the length of S6 while the apical half of S6 forming faint median carina, lateral margin with small, obscured, blunt teeth. In contrast, yellow maculation in mesosoma is absent in the holotype of *Eu. wegneri* (see Baker 1995: figs 24, 28, 31) and has more fine and dense punctures on the scutum and scutellum, while S6 seems to be more acute.

**Floral associations.** Unknown.

**Remarks.** *Euaspis wegneri* has been described on the basis of a single female and has never been reported after that. The holotype of *Eu. wegneri* represented with monochrome digitization in Baker (1995: figs 24, 28, 31, for S6, face, and mesonotum, respectively) arguably has a black mesosoma, with more fine and dense punctures on scutellum compared to scutum. Here, we decided to put this Thai specimen as *Eu. aff. wegneri* since the other characters are comparable especially on S6 which is distinctive for each *Euaspis* species. Other characters can be considered variations, although S6 of the holotype seems to be more acute. With the possibly new species noted in Soh et al. (2016) (also noted in the *Eu. polynesia* section above), we consider that examination of more specimens coupled with molecular analyses are needed in order to resolve the true identity of these enigmatic specimens.

### ***Pachyanthidium* Friese, 1905**

*Anthidium* (*Pachyanthidium*) Friese, 1905: 66–75. Type species: *Anthidium bicolor* Lepeletier, 1841 designated by Cockerell 1920: 298.

*Pachyanthidium* Friese: Cockerell 1930: 45.

**Note.** This genus can be easily distinguished by its explicit robust body, closed scutoscutellar suture (Fig. 12C), and lamellated preoccipital carina, omaular carina, and scutellum (see Fig. 12C, E). Eardley and Griswold (2017) revised 16 Afrotropical species of this genus from 18 described species, the two remaining species are in the subgenus *Trichanthidium*, including a one-time discovered *Pachyanthidium himalayense* (Gupta & Sharma, 1993) and *Pachyanthidium lachrymosum* (Smith, 1879) discovered from India and Chaityaphum, Thailand (Bingham 1897; ITIS 2008; Tadauchi and Tasen 2009; Kumar et al. 2017).

### ***Pachyanthidium* (*Trichanthidium*) *lachrymosum* (Smith, 1879)**

Fig. 12

*Anthidium lachrymosum* Smith, 1879: 463 (♀, ♂, syntype). from Bombay [Mumbai, Maharashtra, India] (NHMUK reg. number NHMUK014026059, examined).

*Anthidium lachrymosum* Smith: Bingham, 1897: 492.

*Anthidium serapiforme* Friese, 1914: 322 (♂). Holotype from Perak [Perak, Malaysia] (ZMB, not examined).

*Pachyanthidium lachrymosum* (Smith): ITIS 2008: <http://www.itis.gov>.

*Pachyanthidium lachrymosum* (Smith): Kumar et al. 2017: 452, 457–459, figs 15, 16.

**Material examined.** (24♀, 3♂). **INDIA:** 1♀, Bombay Dist. [= Mumbai, Maharashtra], B.M. TYPE HYM.17a 1866, (syntype) (NHMUK 014026059); Karnataka: Mysore, 1♀, 17 Apr. 2009, Dhanyavathi; “Yerbahalli” [must be Yerehalli, Mysore], 1♀, 29 Jul. 2014, Revanasidda (UAS); Bangalore, GKVK, 1♀, 29 Jul. 2014, Veereshkumar, 1♀, 25 Jun. 2014, Sunil, 1♀, 18 Nov. 2014, Zameer, 2♂, 29 Apr. 2010, Arathi (UAS); Tamil Nadu: 1♀, Coimbatore, 5 Sep. 1950, P S Nathan (UAS); **THAILAND:** 1♂, Chiang Mai (new record), Chom Thong District, Ban Luang Subdistrict, Doi Inthanon National Park, Ban Mae Klang Luang (18°32'29.7"N, 98°32'01.2"E, alt. 1,033 m), 7 May 2021, on *Bidens pilosa* (L.) [Asteraceae], T. Srimeeyanon et al. (CUNHM: BSRU-AB-3551); 2♀, Kamphaeng Phet: Pang Sila Thong District, 7 Aug. 2015, N. Warrit et al. (CUNHM: BSRU-AA-4478, 4479); 6♀, Khon Kaen, Phu Wiang District, 26 May 2016, N. Warrit et al. (CUNHM: BSRU-AA-4484–4489); 1♀, Phayao, Mueang District, Phayao University (19°1'41.9334"N, 99°52'59.9730"E, alt. 493.41 m), 8 Oct. 2019, N. Warrit et al. (CUNHM); 8♀, Phetchabun, Namnao District, 19 Jun. 2017, N. Warrit et al. (CUNHM: BSRU-AA-4649–4656).

**Distribution.** INDIA (Karnataka (Bangalore, Mysore), Malabar (as per Bingham (1897), must refer to “Malabar coast” on southwest India), Maharashtra (Mumbai), Tamil Nadu (Coimbatore), MALAYSIA (Perak), MYANMAR (Tenasserim), THAILAND (Chaiyaphum, Chiang Mai (new record), Kamphaeng Phet (new record), Khon Kaen (new record), Phayao (new record), Phetchabun (new record)).

The records from Smith (1879) based on both sexes were noted to come from “Bombay district”, whereas Bingham (1897) who later revise Smith’s work, additionally mentioned “Malabar” and “Tenasserim” without any further information, also without any note if the additional material was examined. Most of the records from India are already listed by Kumar et al. (2017). Friese (1914) reported a male from Malaysia. In Thailand, Tadauchi and Tasen (2009) reported the species from Phu Khiao Wildlife Sanctuary in Chaiyaphum province.

**Diagnosis.** *Pachyanthidium lachrymosum* can be distinguished from other congeneric species by its black body with a white lateral band of short white hairs on the metasoma; lamellate parts are often translucent reddish brown to black; eyes with sparse short hairs; mandibles with four teeth; arolia absent; male similar to females but mostly differs in the presence of the arolia, three mandibular teeth, lateral spines on T3–T6, and a tridentate T7 (Fig. 12F, G), also noted in Bingham (1897) and Kumar et al. (2017). *Pachyanthidium lachrymosum* also exhibits a robust, lamellate preoccipital ridge and an omaular carina, which are typical for *Pachyanthidium*.

**Floral associations.** *Bidens pilosa* (L.) (Asteraceae) (this study), *Leucas aspera* (Willd.) Link (Lamiaceae) (Kumar et al. 2017).

**Remarks.** The other four species of *Pachyanthidium* (*Trichanthidium*) were revised by Eardley and Griswold (2017). *Pa. lachrymosum* is the only species that does not have any integument maculation and has not been reported from

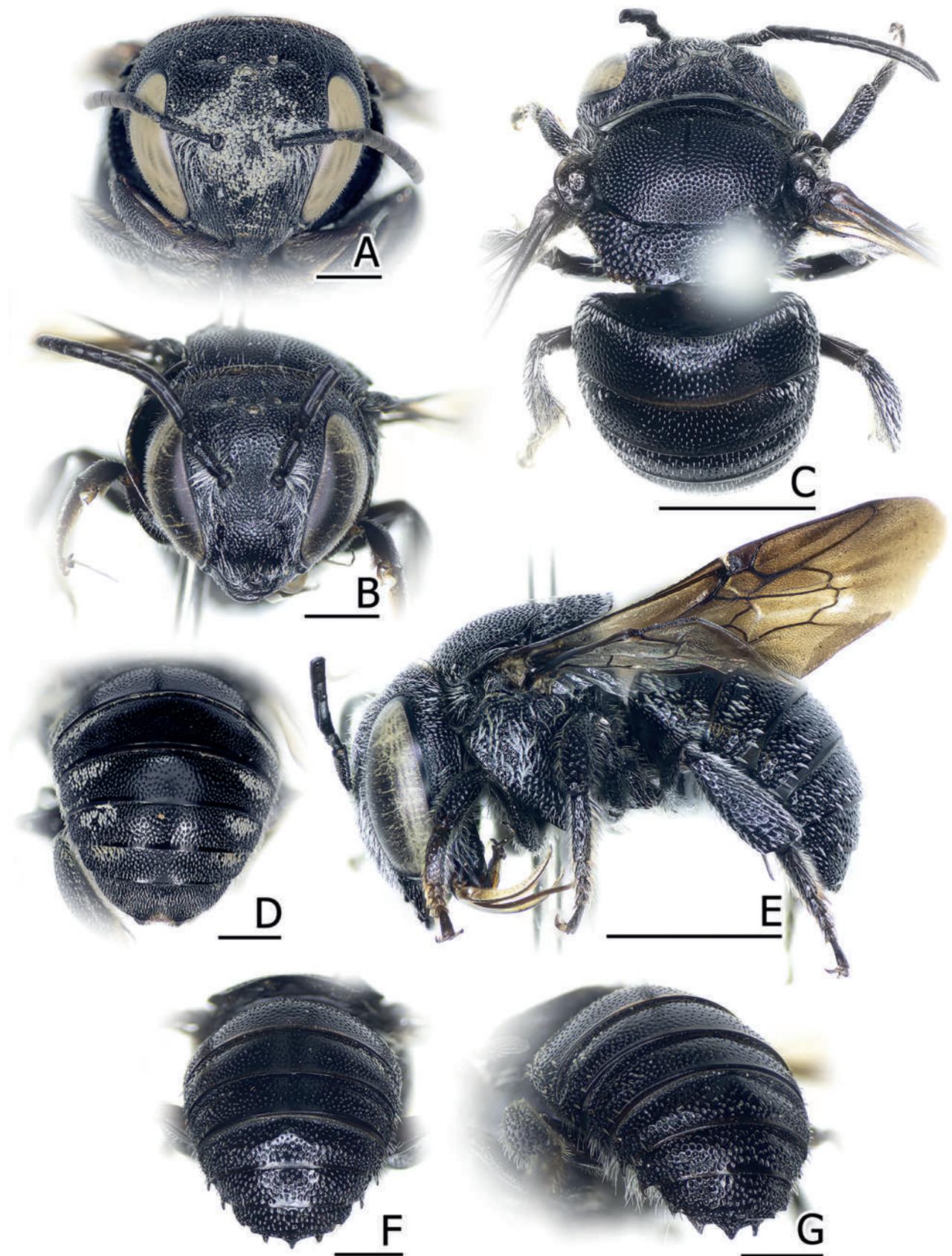


Figure 12. *Pachyanthidium (Trichanthidium) lachryosum* (Smith, 1879) **A** female face **B** male face **C** male dorsal habitus **D** female metasoma **E** male lateral habitus **F**, **G** male metasoma. Scale bars: 2 mm (C, E); 1 mm (A, B, D, F, G).

the Afrotropical region. When compared with Indian specimens from both Kumar et al. (2017) and Smith (1879), we notice that the white hair patch on the metasoma appears to be more clumped and dense in Indian specimens, while in the Thai specimens the hairs seem looser and the patch more extended. The patch on the scutum is absent in some Thai individuals and white body patches are absent in some Indian specimens.

All specimens in CUNHM are females. Promisingly, two individuals are full of pollen trapped by the facial pubescence (Fig. 12A); thus, with their broad facial area, *Pa. lachrymosum* may also can gather pollen by rubbing it with its face (see also Portman et al. 2019; Kasperek et al. 2022).

Kumar et al. (2017) provides a note on Indian *Pa. lachrymosum* flight period (April, June, July, and November) and floral visitation (see above). Our specimens from Thailand were collected in May, June, and August.

### ***Pseudoanthidium* Friese, 1898**

*Anthidium* (*Pseudoanthidium*) Friese, 1898: 101. Type species: *Anthidium alpinum* Morawitz, 1873, designated by Sandhouse, 1943: 593. See Kasperek and Ebmer 2023.

*Paranthidiellum* Michener, 1948: 25. Type species: *Anthidium cribratum* Morawitz, 1875, by original designation.

*Pseudoanthidium* (*Paraanthidiellum*) Pasteels, 1969: 79, unnecessary emendation of *Paranthidiellum* Michener.

*Pseudoanthidium* (*Carinellum*) Pasteels, 1969a: 80. Type species: *Anthidium ochrognathum* Alfken, 1932, by original designation.

*Trachusa* (*Orientotrachusa*) Gupta, 1993: 50. Type species: *Anthidium orientale* Bingham, 1897, by original designation.

*Pseudoanthidium* Friese: Pasteels 1969: 76–77.

**Note.** *Pseudoanthidium* commonly has a tentorial pit placed below the connection of the subantennal suture and the epistomal suture (Michener 2007). The female mandible has more than four teeth, and the terga are without an apically depressed area (see Fig. 13E; Litman et al. 2016). As a very broad, ill-defined complex group, the South East Asian fauna is represented by only one subgenus, *Pseudoanthidium* s. str.

### ***Pseudoanthidium* (*Pseudoanthidium*) *orientale* (Bingham, 1897)**

Figs 13, 14A, 15

*Anthidium orientale* Bingham, 1897: 496 (♀). Holotype from Tenasserim, Myanmar, image examined in NHMUK under <https://data.nhm.ac.uk/media/b2906d76-cd81-4776-9133-9558ce51baca>.

*Anthidium kryzhanovskii* Wu, 1962: 167 (♀). Holotype from Jinping Xian, Yunnan, China (IZCAS: Institute of Zoology, Chinese Academy of Sciences, not examined).

*Pseudoanthidium* (*Paraanthidiellum*) *orientale* (Bingham): Pasteels 1969: 79, 80.  
*Trachusa* (*Orientotrachusa*) *orientale* (Bingham, 1897): Gupta 1993: 55, 56, 58,  
figs 141–159 (♂ nov.).

*Anthidium* (s. str.) *kryzhanovskii* Wu, 1962: Wu 2006: 157 (♀, ♂).

*Pseudoanthidium* (*Pseudoanthidium*) *orientale* (Bingham): ITIS 2008: <http://www.itis.gov>.

*Pseudoanthidium* (*Pseudoanthidium*) *orientale* (Bingham): Niu et al. 2021: 139–142.

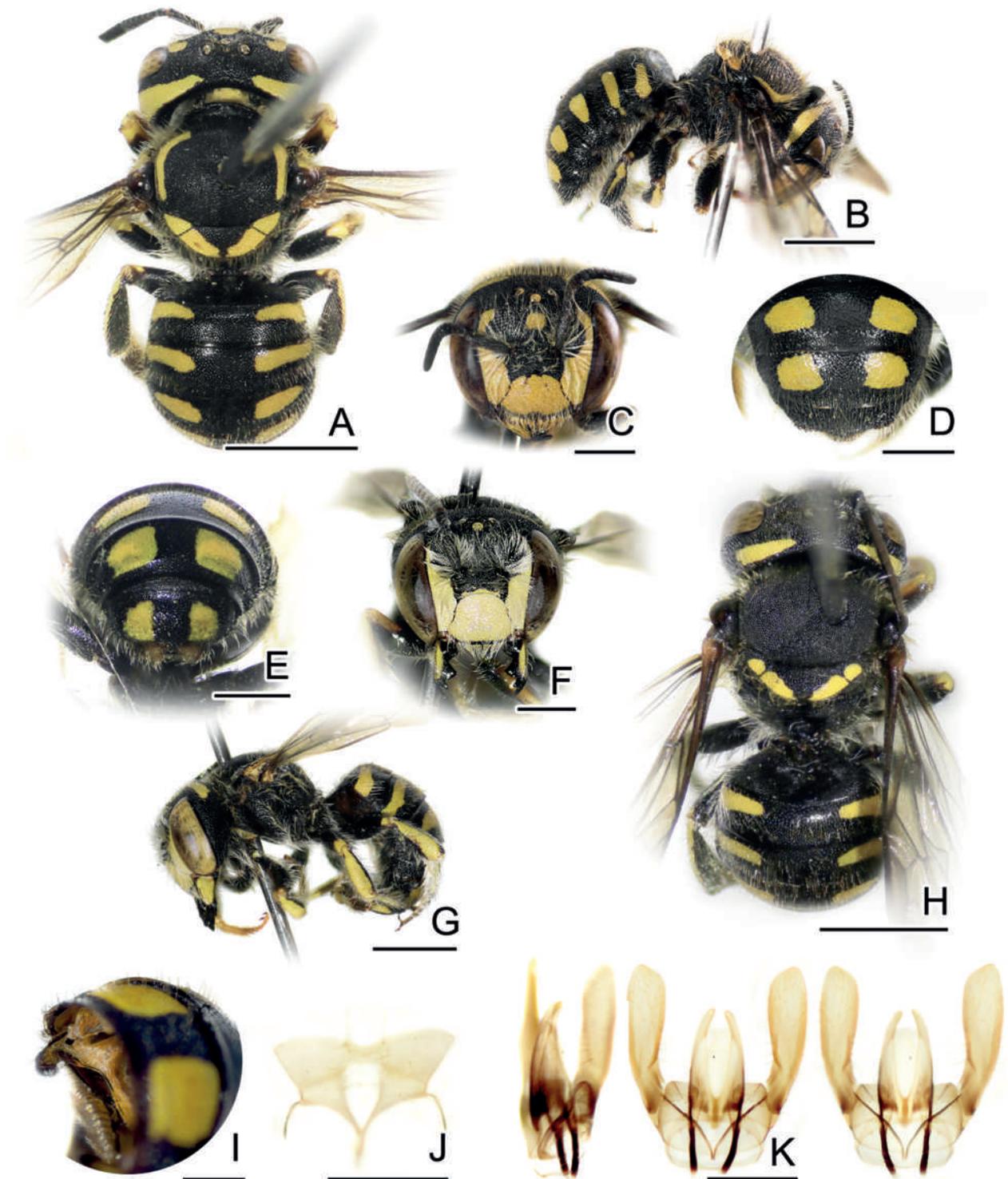
**Material examined.** (10♀, 3♂). **LAOS** (new record): 3♀, Champasak, Si Phan Don, Don Det, 20 Jan. 2015, N. Warrit et al., (CUNHM: BSRU-AA-1223, 1227, 1231); 1♀, Pakse, Bolaven plateau, Phu Suam Water Fall (15°16'44"N, 105°53'23"E), 19 Jan. 2015, N. Warrit et al., (CUNHM: BSRU-AA-1235); **THAILAND**: Chiang Mai, 1♀, Mae Chaem District, Baan Na Jon (18°42'11.3970"N, 98°16'59.3754"E, alt. 874.93 m), 9 Dec. 2015, N. Warrit et al. (CUNHM: BSRU-AA-1243); 1♀, Chiang Mai, Chom Thong District, Doi Inthanon National Park, (18°32'12.39"N, 98°31'14.44"E, alt. 1,267 m), 16 Feb. 2021, Srimaneyanon et al. (CUNHM: BSRU-AB-2970); 1♀, Kamphaeng Phet, Mueang District, (16°28'18"N, 99°29'43"E, alt. 10 m), 4 Dec. 2015, C. Thanosing (CUNHM: BSRU-AA-1252); 1♂, Lampang, Mueang Pan District, Chae Son National Park (18°50'15.0498"N, 99°28'19.5594"E, alt. 451 m), TIGER project T-2922, Malaise trap, 8/14 Dec 2007, Boonruen & Acharaporn (CUNHM: BSRU-AB-4361); 2♀, 2♂, Mae Hong Son, Pang Tong, Under Royal Forest Park 2/ Pang Ung (19°29'58.3008"N, 97°54'42.1014"E, alt. 1,164 m), 10 Dec. 2015, N. Warrit et al. (CUNHM: ♀ BSRU-AA-1239, 1240, ♂ BSRU-AA-1241, 1242); 1♀, Phayao, Mueang District, Mae-ka Subdistrict, Phayao University (CUNHM: BSRU-AA-1238, Phayao University).

**Records from iNaturalist (2023).** THAILAND: Chiang Mai, San Sai District, San Sai Noi Subdistrict (18°49'08.6"N, 99°01'15.1"E) uploaded by 'jackychiangmai' on 14 Jan. 2022 (observation id:104911660); Chiang Rai, Chiang Saen Lake, Viang Yonok Hotel (20°15'42.5"N, 100°02'59.5"E), uploaded by 'pam-pilombino' on 27 Jan 2020.

**Distribution.** CAMBODIA (Mondulkiri: Ascher et al. 2016), CHINA (Yunnan), INDIA (Alwar, Poona, Solan, Tolawas, Udaipur), LAOS (Champasak (new record), MYANMAR (Tenasserim), THAILAND (Chiang Mai, Chiang Rai (new record from iNaturalist 2023), Kamphaeng Phet (new record), Lampang (new record), Lamphun (new record from iNaturalist 2023), Mae Hong Son (new record), Phayao (new record)). For the Indian records from Gupta (1993), see Remarks section below.

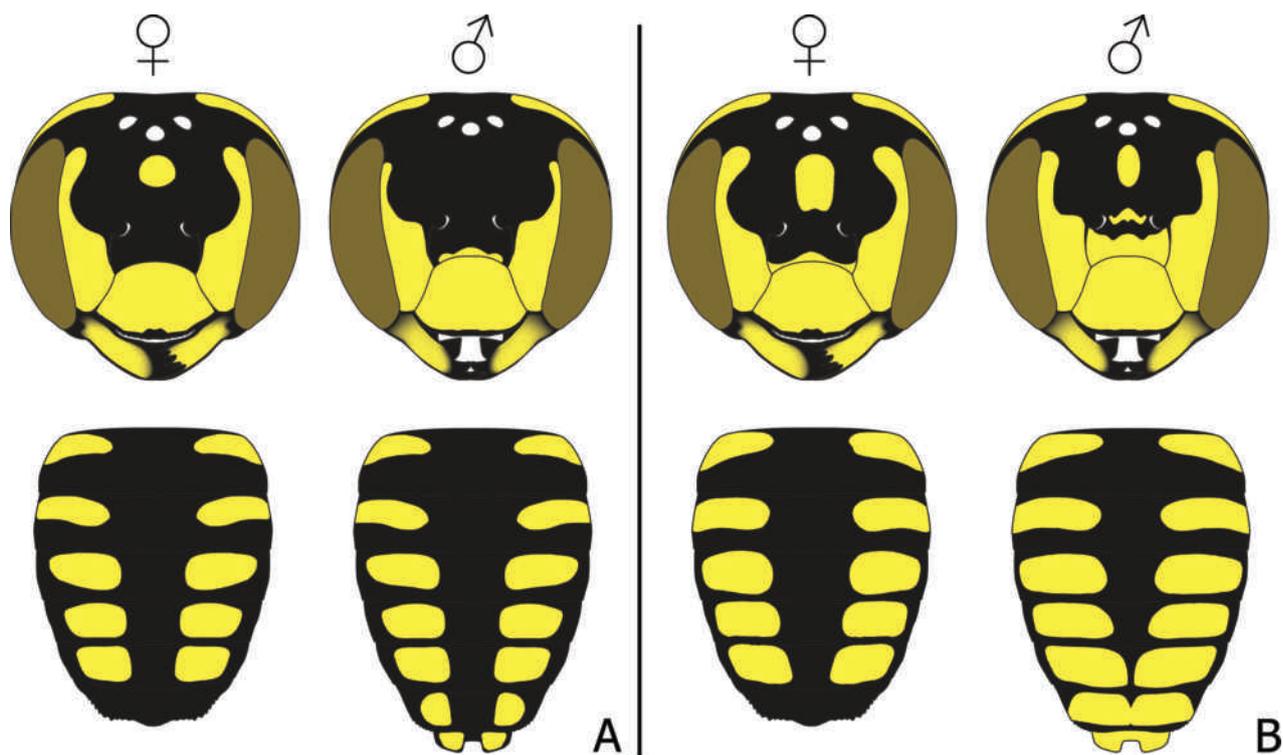
**Diagnosis.** *Pseudoanthidium orientale* is a medium-sized bee (6–8 mm) and usually has a black integument with yellow maculations in all tagmata. It has a remarkably pale yellow mark on the paraocular area reaching close to the top of eyes, female mandibles with five or six teeth, rounded scutellum with broad marginal maculation which is medially disrupted, tibia and tarsi yellow except black on the venter, female terga with yellow paramedian maculation on T1–T5 in female, which is more laterally extended on T1 and T2 and nearly rectangular in T3–T5. The male looks superficially similar to the female but has a different dentition of the mandible (i.e., distinctly tridentate, broader especially the inner tooth), and maculation on T6 and T7. Male genitalia broad.

**Floral associations.** Plants with hairy surfaces (see iNaturalist observation from Lamphun) must be the resources for the nesting material. Also, from iNat-



**Figure 13.** *Pseudoanthidium orientale* (Bingham, 1897) [showing Mae Hong Son female (BSRU-AA-1240) (A–D), and male (BSRU-AA-1241) (E–K)] A, H dorsal habitus B, G lateral habitus C, F face D female T6 E male T7 I male S5 J S8 K male genitalia in lateral (left), dorsal (middle) and ventral (right). Scale bars: 2 mm (A, B, G, H); 1 mm (C–F); 0.5 mm (I–K).

uralist image from Chiang Mai, the photographs clearly show the bee wandering on the inflorescence of *Antigonon leptopus* Hook. & Arn. (Polygonaceae), which is a hairy plant, although there is no direct evidence to this claim. Niu et al. (2021) also noted the floral associations for *Ps. orientale* including *Blumea* sp. (Asteraceae), *Eupatoreae* sp. (Asteraceae), *Helianthus annuus* L. (Asteraceae),

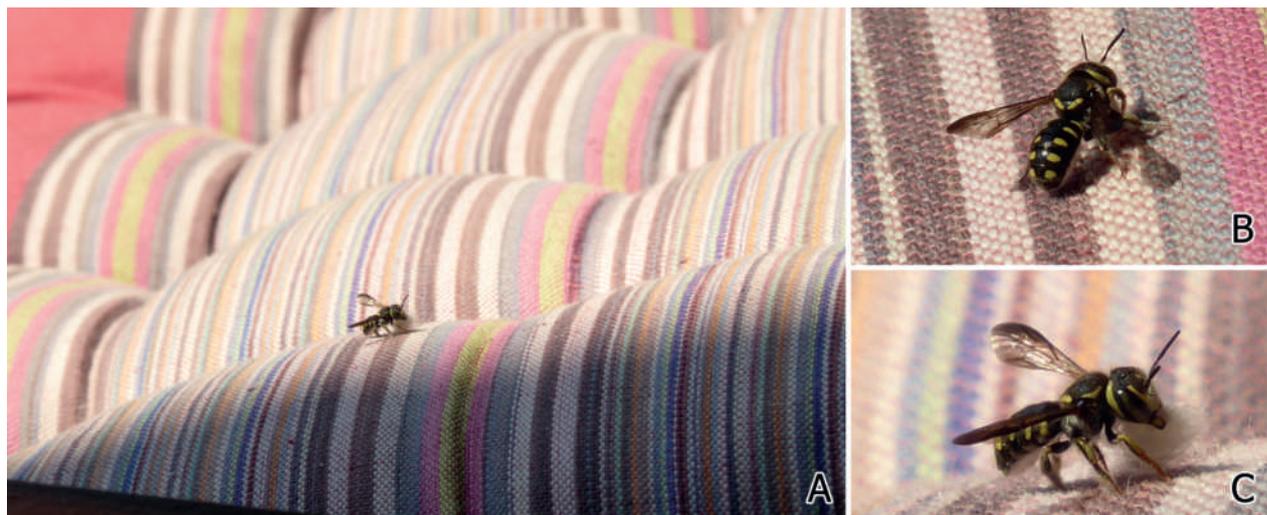


**Figure 14.** Facial and metasomal illustrations of **A** *Pseudoanthidium orientale* (Bingham, 1897) and **B** *Ps. rotundiventre* (Pasteels, 1980).

*Tephrosia purpurea* (L.) Pers. (Fabaceae; originally noted as *Tephrosia hamiltoni*), and *Tridax* sp. (Asteraceae; originally noted as “*Tridex*”).

**Remarks.** This species was described by Bingham (1897) as *Anthidium orientale*, based on a female from Tenasserim, Myanmar. Pasteels (1969) then assigned it to *Pseudoanthidium* (*Paraanthidiellum*) Michener, 1948. Gupta (1993) provided both female and male descriptions and illustrations from India, which is designated as *Trachusa* (*Orientotrachusa*) *orientale*. Later, the species was listed as *Ps.* (*Pseudoanthidium*) in the World Bees Checklist Project (ITIS 2008). A female *Ps. orientale* was reported from a monochrome image from Chiang Mai, Thailand (Tadauchi and Tasen 2009), and a male was reported from Bousra, Mondulkiri, Cambodia (Ascher et al. 2016) with photographs. However, the CUNHM male specimens studied here are apparently different from the male descriptions and photographs from Cambodia in lacking the antero-lateral maculation on the scutum. Gupta’s (1993) male description from India also stated that “tergum first with a complete band, T2 and T3 interrupted medially, T4–T7 entirely yellow” which is incongruent to the CUNHM specimens studied here, and also to the photographs of the male from Cambodia; therefore, the Indian specimens need further study.

*Pseudoanthidium orientale* is superficially similar to *Ps. rotundiventre* (Pasteels, 1987) from Sri Lanka and India. Kumar et al. (2017) mentioned that these two species were probably referred to the same species. As most of their distinct morphology is similar, the difference is based on color (Fig. 14). First, *Ps. rotundiventre* has a more extended maculation on the face, the female has an entirely yellow clypeus, and a W-shape maculation on the supraclypeal area.



**Figure 15.** *Pseudoanthidium orientale* wandering around on a cushion with a fiber ball (A–C for cropped close-up). Original photographs taken by Pamela Piombino.

Seven Thai females of *Ps. orientale* come without supraclypeal mark and show reduction of the clypeal color as a black stigma, while the remaining two from Laos have an entirely yellow clypeus and the supraclypeal mark is only a triangle in the median part. Second, it is remarkable that *Ps. rotundiventre* comes with more extended maculation on the male terga: the median interruption of the band tends to decrease from base to apex on the metasoma. Specifically, T5 and T6 are nearly uninterrupted and T7 is entirely yellow. The *Ps. orientale* males show much more consistency in the median interruption of the tergal maculation, making it appear similar to the female. Hence, Gupta's *Ps. orientale* male description from India is more congruent to *Ps. rotundiventre*.

As the male genitalia of *Ps. orientale* illustrated in Gupta (1993: fig. 159) is inadequate to compare with microphotographs of the male genitalia of *Ps. rotundiventre* (see Kumar et al. 2017: fig. 20C), we used a microphotograph of Thai *Ps. orientale* genitalia which was prepared and preserved in glycerin. Overall, the genitalia seem to be identical, but the photograph of the genitalia of *Ps. rotundiventre* is not clear, so the shape and sclerotization of the apodeme of penis valve is hard to compare with that of *Ps. orientale* (cleared one in Fig. 13K). However, the respective characters do not seem to have much of value in sexual differentiation. From the evidence, it seems that color variation in *Pseudoanthidium orientale* is variable, and potentially similar to *Ps. rotundiventre*. At the same time, *Ps. flaviventre* Cameron, 1897, belonging to the same subgenus, displays much more obvious differences in male genitalia and other external body parts (also see Kumar et al. 2017). Because of the similarities, Kumar et al. (2017) suggested that *Ps. rotundiventre* is a junior synonym of *Ps. orientale*, but the types need to be examined before making a final decision.

Finally, there is an observation of wool-collecting behavior of a female of *Ps. orientale* from Chiang Saen Lake, Chiang Rai, Thailand, reported and observed by Ms. Pamela Piombino [user: 'pam-piombino'] on 27 January 2020, and published on iNaturalis.org (iNaturalist 2023). Based on personal communication, the bees landed on a cushion with a mouthful of fiber-ball, wandered

around for a few minutes, and eventually flew off without any “active” fiber-collecting behavior. We are not sure if this fiber was collected precisely from the cushion or elsewhere, but this is the first time that fiber-collecting behavior has been observed in this species.

### **Stelis Panzer, 1806**

*Trachusa* Jurine, 1801: 164 (nec Panzer 1804). Type species: *Apis aterrima* Panzer, 1798, by designation of Morice and Durrant 1915: 426. Suppressed by Commission Opinion 135, 1939 (Direction 4).

*Stelis* Panzer, 1806: 246. Type species: *Apis aterrima* Panzer, 1798 (nec Christ 1791) = *Apis punctulatissima* Kirby, 1802, monobasic.

*Gyrodroma* Klug in Illiger 1807: 198; Klug 1807: 225. Type species: *Apis aterrima* Panzer, 1798 (not Christ 1791) = *Apis punctulatissima* Kirby, 1802, designated by Sandhouse 1943: 555. [Sandhouse incorrectly considered *Gyrodroma* to be monobasic; two species were listed by Klug in Illiger 1807, which has page priority over Klug 1807].

*Gymnus* Spinola, 1808: 9. Type species: *Apis aterrima* Panzer, 1798 (nec Christ 1791) = *Apis punctulatissima* Kirby, 1802, monobasic.

*Ceraplastes* Gistel, 1848: x [10], unjustified replacement for *Stelis* Panzer, 1806. Type species: *Apis aterrima* Panzer, 1798 (nec Christ 1791) = *Apis punctulatissima* Kirby, 1802, autobasic.

*Chelynia* Provancher, 1888: 322. Type species: *Chelynia labiata* Provancher, 1888, monobasic [see Provancher 1889].

*Melanostelis* Ashmead, 1898: 283. Type species: *Melanostelis betheli* Ashmead, 1898 = *Stelis rubi* Cockerell, 1898, by original designation.

*Stelidium* Robertson, 1902: 323. Type species: *Stelidium trypetinum* Robertson, 1902, monobasic [see Michener 1997].

*Microstelis* Robertson, 1903: 170, 175. Type species: *Stelis lateralis* Cresson, 1864, by original designation.

*Stelis (Pavostelis)* Sladen, 1916: 313. Type species: *Stelis montana* Cresson, 1864, monobasic.

*Stelis (Stelidina)* Timberlake, 1941: 131. Type species: *Stelis hemirhoda* Linsley, 1939, by original designation.

*Stelis (Stelidiella)* Timberlake, 1941: 133. *Lapsus* for *Stelidina* Timberlake, 1941.

*Stelis (Leucostelis)* Noskiewicz, 1961: 126, 132. Type species: *Gyrodroma ornatura* Klug, 1807, by original designation.

**Note.** Most of the cleptoparasitic bees of the Anthidiini are attributed to the genus *Stelis* due to the very diverse morphs. The recent works by Michener and Griswold (1994), Michener (2000, 2007), and Kasperek (2015) for species in Europe, North Africa, and the Middle East provide comprehensive information for *Stelis*. Female *Stelis* notably lack scopa and juxta-antennal carina, while the carinae on prosoma and mesosoma can be absent or weakly present. In males, T7 is round, weakly bilobed, or trilobed. The only subgenus discovered in Thailand is *Malanthidium* (see Nalinrachatakan 2021b), only known by males and can be recognized by its distinct postero-lateral hook on its axilla.

***Stelis (Malanthidium) flavofuscinular* Nalinrachatakan & Warrit, 2021**

Fig. 4C

*Stelis (Malanthidium) flavofuscinular* Nalinrachatakan & Warrit in Nalinrachatakan et al. 2021b: 172–175, see figs 6, 7. (♂) Holotype and paratype from Phu Chong Na Yoy National Park, Ubon Ratchathani, Thailand (CUNHM).

**Material examined.** (2♂). Same specimens as in Nalinrachatakan et al. (2021b). Holotype transferred to NHMUK in April 2023.

**Distribution.** THAILAND (Ubon Ratchathani: Phu Chong Na Yoy National Park).

Since *Stelis* is a cleptoparasitic bee, its distribution must be in accordance with its host. Noteworthy, the other known species of the subgenus *Malanthidium*, *S. macaccensis* (Friese, 1914) is known from Malaysia; thus, *Malanthidium* is the only subgenus of *Stelis* present in South East Asian region.

**Bee host.** *Anthidiellum phuchongense* Nalinrachatakan & Warrit, 2021.

**Floral association.** Unknown.

**Remarks.** With only two males known, some differences between both specimens and their biology were mentioned and discussed in Nalinrachatakan et al. (2021b).

***Trachusa* Panzer, 1864**

*Trachusa* Panzer, 1804: 14–15. Type species: *Trachusa serratulae* Panzer, 1804 = *Apis byssina* Panzer, 1798, by designation of Sandhouse 1943: 605.

*Diphysis* Lepeletier, 1841: 307. Type species: *Diphysis pyrenaica* Lepeletier, 1841 = *Apis byssina* Panzer, 1798, monobasic.

*Megachileoides* Radoszkowski, 1874: 132. Type species: *Trachusa serratulae* Panzer, 1804 = *Apis byssina* Panzer, 1798, by designation of Michener 1995: 375.

*Megachiloides* Saussure, 1890: 35, incorrect spelling of *Megachileoides* Radoszkowski, 1874; see Michener 1995.

**Note.** A medium to large, robust, round-edged species, genus *Trachusa* appears to be sister to the remainder of the tribe Anthidiini (Litman et al. 2016). Recently, Kasperek (2017, 2019a) reviewed the old-world *Trachusa* and described new Malaysian species, respectively.

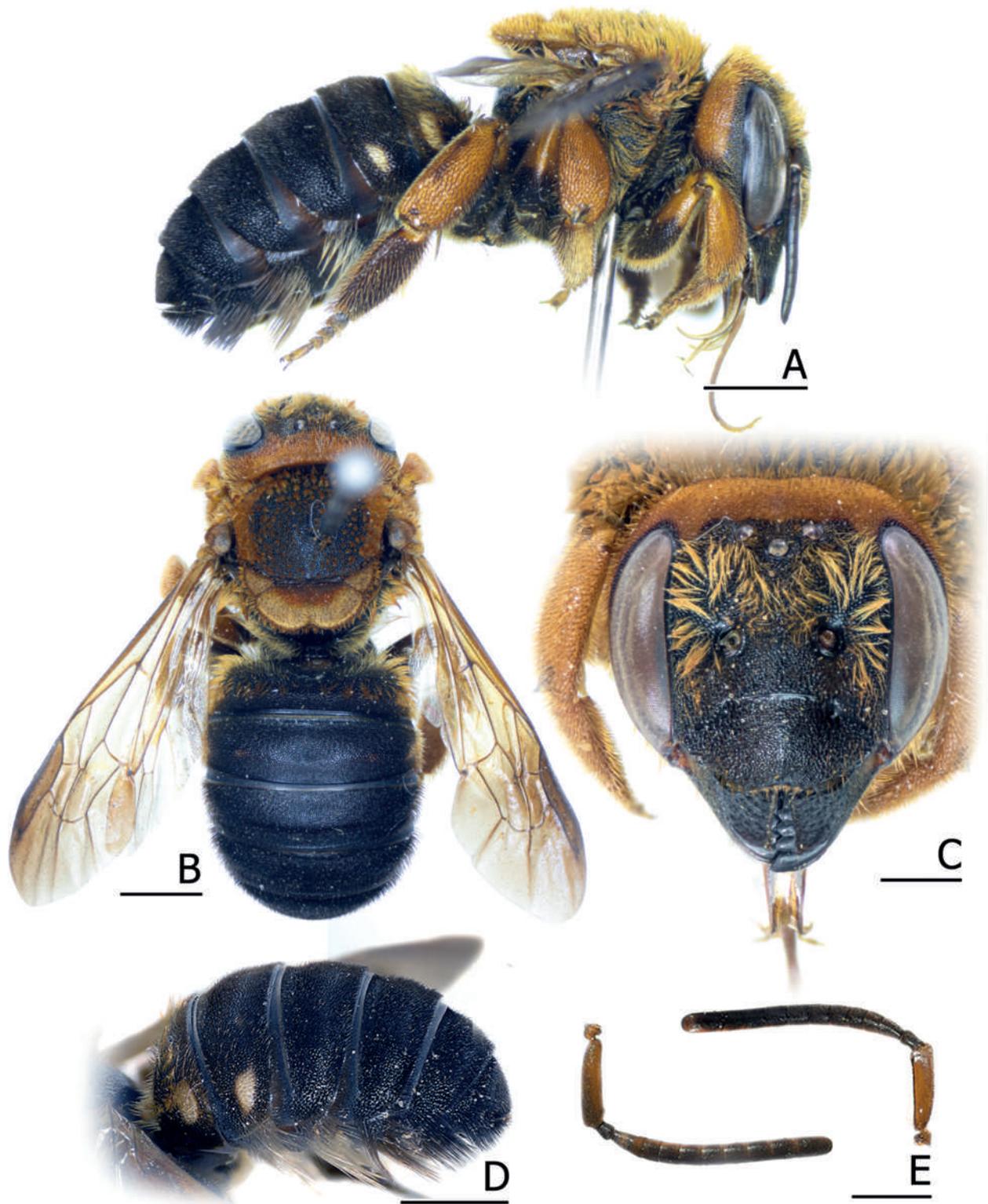
***Trachusa aff. vietnamensis* Flaminio & Quaranta, 2021**

Figs 16, 17

*Trachusa vietnamensis* Flaminio & Quaranta in Flaminio et al. 2021: 307–310, fig. 1 (♀). Holotype from Quang Nam, Vietnam (CREA: Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (Bologna, Italy), examined).

**Material examined.** (5♀). **THAILAND:** Phitsanulok, Nakhon Thai District, 27 May 2014, N. Warrit et al. (CUNHM: BSRU-AA-4471–4475).

**Distribution.** THAILAND (Phitsanulok) and VIETNAM (Quang Nam).



**Figure 16.** Female *Trachusa* aff. *vietnamensis* BSRU-AA-4473 (A), 4744 (B–E) A lateral habitus B dorsal habitus C face D lateral of metasoma E antennae. Scale bars: 2 mm (A, B, D); 1 mm (C, E).

**Diagnosis.** The species is very close to, or maybe identical to *Trachusa vietnamensis*. Only the female is known: body large, robust, and black. Bands with yellowish, orangish, or light-brown coloration on the vertex, preoccipital area,

anterolateral of scutum, and scutellum, while mesosoma covered in orangish pubescence. The Thai specimens are distinguished from the Vietnamese material by a unique elongate metasoma making it more chalicodomiform, and more limited maculation on the metasoma (fully striped on T1 and T2 of *T. vietnamensis*, small pale marks on the side of T1 and T2 and minute or absent on T3 and T4 for Thai specimens). The species is also close to *T. ovata*, from which it differs by the combination of five mandibular teeth, clypeus black with ill-defined shiny median longitudinal line, conspicuous rounded light-brown scutellum which seems darker basally, and head with orangish to light-brown maculations running continuously from the vertex to genal area.

**Description. Female:** Body length 13.4, 13.3, 13.2, 13.0, 13.3 mm, head width 4.3, 4.3, 4.2, 4.3, 4.1 mm, intertegular distance 3.6, 3.8, 3.8, 3.7, 3.5 mm, respectively. Wingspan 25.0, 25.6, 25.0, 24.7, 24.9 mm.

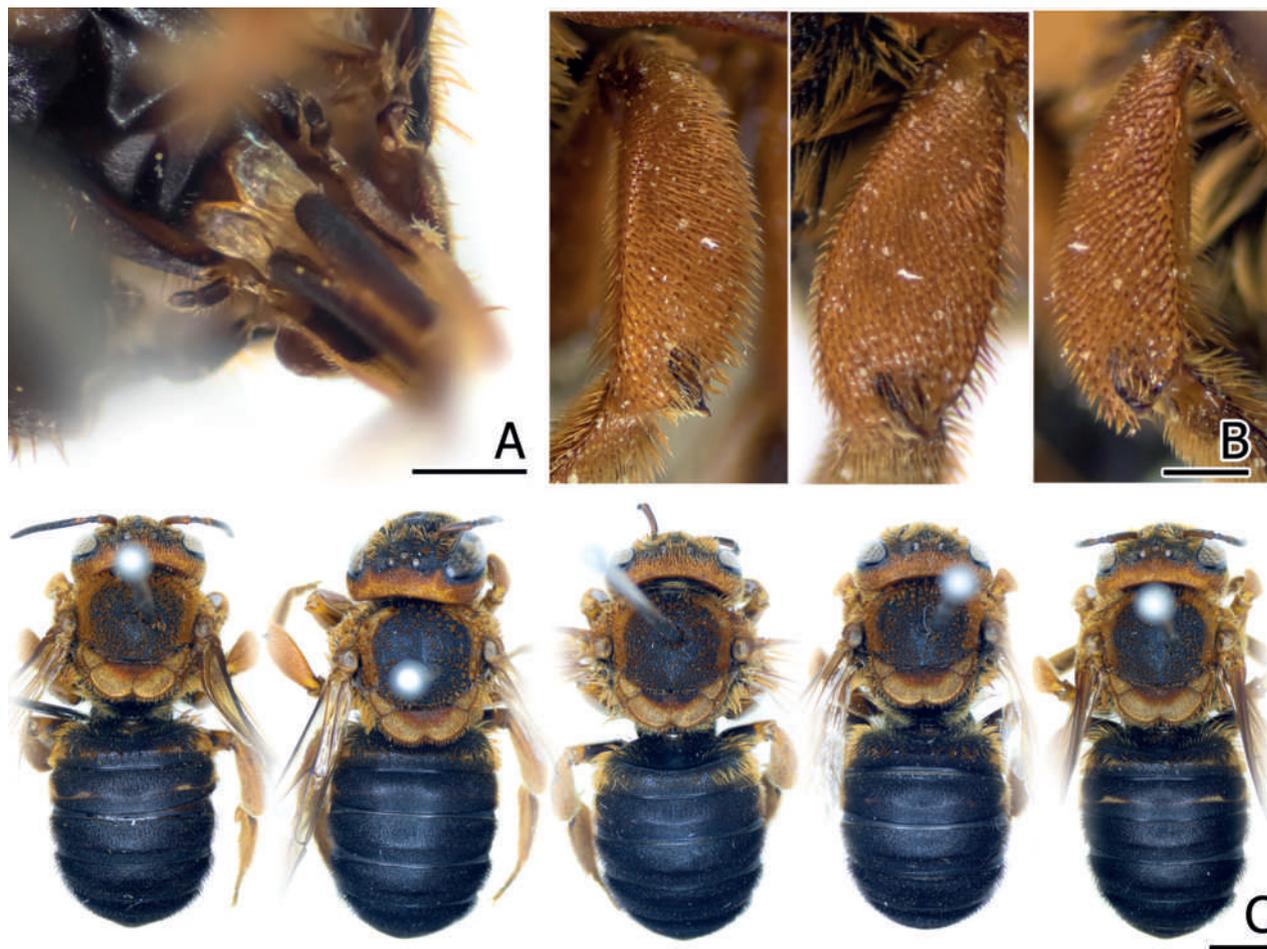
Head largely black, with light-brown band on vertex running continuously to genal area, lighter on occipital ridge, but not abutting margins of eyes and ocelli. Clypeus (see Fig. 16C) black, slightly convex but shallow depression apically with ill-defined shiny median longitudinal line; middle and apical area with smaller and denser punctures, apical margin slightly crenulate thus looking somewhat emarginate at middle. Surfaces apically covered with sparse short yellow hair that is dense and long. Supraclypeal area black, slightly convex, with shiny median longitudinal line. Mandible broad with apex ~ 1.5× wider than base. Mandibles somewhat dull, black with light-brownish mark on basal area, inner surfaces with hairs. Four mandibular teeth, apical tooth larger than inner. Labrum black, with yellow bristles on its apex. Maxillary palpus three-segmented, black but dark brown on rounded basal segment. Subantennal suture slightly arcuate outwards. Antenna generally brown, scape brown but darker on front, pedicel dark brown to black, flagellum lighter in color on medial front, F1 and F2 brighter than rest. Ocelloccipital distance not more than 1.5× interocellar distance. Head covered with fulvous hair, denser and longer on frons, paraocular area, and lower part of genal area.

Mesosoma black, covered with fulvous hair except on pronotum with exposed shiny black median area with coarse punctures. Pronotal lobe strongly carinated, light brown. Mesepisternum black. Omaulus carinated, extending to ventral part of thorax. Scutum laterally carinate, punctures uniform, dense, with light-brown color on anterolateral band, not abutting together in middle. Axilla rounded laterally, entirely light brown. Tegula fulvous with dark patch lining medio-posterior. Scutellum broad, apically round with median emargination, light brown, darker on median triangular basal area.

Wing subhyaline, fuscate, forewing darker at apical margin and marginal cell. Pterostigma brown. Veins dark brown to black; 2<sup>nd</sup> recurrent vein abutted to 2<sup>nd</sup> submarginal crossvein distally.

Legs covered with short fulvous hairs. Coxae, trochanters, and basal parts of femora dark reddish brown to black; legs otherwise light brown except dark brown on inner surfaces of basitarsus and tarsi, slightly subtle on outer surfaces of hind basitarsus and hind tarsal segments. Apical tarsal segments with apical dark spot. Claw with inner tooth, light brown, apically black. Arolium present, dark brown to black.

Metasoma black. Discs of all terga swollen, with fine dense punctures. Terga covered with short black hairs, lighter to fulvous hairs on T1–T3 lateral surface.



**Figure 17.** Female *Trachusa* aff. *vietnamensis* (BSRU-AA-4475) **A** ventral habitus of mouthparts (with removed mandible) **B** mid tibia **C** all specimens in dorsal view, showing variation (BSRU-AA-4471–4475, respectively from left to right). Scale bar: 2 mm (**C**); 0.5 mm (**A**, **B**).

es, longer fulvous hairs covering frontal carina of T1. T1 and T2 with small pale lateral patches (Figs 16A, B, D, 17C) with part of obscured thin line extending to median. In some paratypes, these patches can be more or less expressed, tending to form an almost continuous thin but obscure band on disc, and also obscurely found on T3 and T4 in one specimen (BSRU-AA-4471). Scopa pale yellow on S2 and S3, gradually darker on S4, and becoming black on S5 and S6. S1–S6 reddish brown to black, darker apically. T6 with barely visible small median emargination.

**Remarks.** *Trachusa* species have been reported from upper and lower Indochina but with limited materials (Soh et al. 2016; Kasperek 2017, 2019a; Flaminio et al. 2021). It is plausible that the lack of previous records in Thailand, Burma, and Cambodia may be due to limited collecting, in addition to the general rareness of *Trachusa* bees. This is the first record of *Trachusa* in Thailand: all specimens are neither complete nor in perfect condition.

Since the species is very close to *T. vietnamensis* from Vietnam, here we propose that the Thai specimens belong to the same species. The differences in tergal bands on the metasoma may be considered as variation; however, the Thai specimens exhibit a more elongate metasoma. To confirm that both spe-



**Figure 18.** Groups of *Trachusa* (*Paraanthidium*) sp. primarily identified as *longicornis* group sensu Kasperek (2017), gather with other bee species. Photograph: Jacky Cudon.

cies are indeed the same, DNA barcoding would be useful since the barcode of *T. vietnamensis* was provided by Flaminio et al. (2021).

Subgeneric placement of *Trachusa vietnamensis* is still uncertain. Kasperek (2017, 2019a) assigned three species groups in *Paraanthidium* with their characteristics: *interrupta* group (female with bright yellow maculations on black, wasp-like), *longicornis* group (Indomalayan species with female having dull yellow maculation except on the mesosoma), *ovata* group (female completely without maculation), and the remaining *Trachusa xylocopiformis* (Mavromoustakis, 1954), for which only the male is known Fukien, China, is large and black except for yellow on lower part of the face.

*Trachusa vietnamensis* seems to not be congruent with any of these groups, but is closely related to the *ovata* group by its face, especially in its clypeal shape, and the reduced maculation on the metasoma. Also, the superficially color pattern and almost parallel-sided body form are not congruent with the robust-megachiliform that occurs in all described females of *Paraanthidium*; from this, it more resembles the subgenus *Orthanthidium* from mainland China and Taiwan for which two fairly different species are known: *Trachusa formosana* (Friese, 1917) and *T. cornopes* Wu, 2004. *Orthanthidium* was designated

by Mavromoustakis (1953) prominently for its parallel-sided axilla, truncated scutellum, and small spine on the tibial apex. As *Orthanthidium* is still problematic in its status (Kasperek 2017), future work is still needed.

The astonishing record of another *Trachusa* species that is completely different from the aforementioned *T. aff. vietnamensis* has been retrieved from the citizen science database platform iNaturalist (iNaturalist 2023) from Thailand: Chiang Mai, Mueang District, Suthep Subdistrict, Doi Suthep-Pui (18°49'00.5"N, 98°55'26.8"E), observed repeatedly by “jackychiangmai” on 16 Apr, 30 Apr, and 27 May 2022 (observation id: 111730798, 114167310, 119204705 respectively).

Since the identification is restricted to the available photographs, we cannot identify the bee definitively. These observations show multiple *Trachusa* bees (25+) grouping on a semi-limestone concrete surface (Fig. 18) along with other bees, including *Ceratina* (*Ceratinidia*) spp., *Chelostoma aureocinctum* (Bingham, 1897), possibly *Hylaeus* sp., and a Halictinae bee (possibly *Pachyhalictus* or *Lasioglossum*). This *Trachusa* species exhibits a large robust body with a round scutellum, while the yellow band on T4–T6 and the yellowish brown patch apically on the leg can also be noticed. Most bees that can be speculated for their sex are usually female based on their pollen-loaded metasomal scopa. Based on the available information, we classify these *Trachusa* bees to the subgenus *Paraanthidium*, primarily within *longicornis* group that contains four described species at present: *T. longicornis* (Friese, 1902), *T. maai* (Mavromoustakis, 1953), *T. mui* (Mavromoustakis, 1937), and *T. rufobalteata* (Cameron, 1902) (see discussion above and Kasperek 2017). Collections and information are needed in order to confirm its identity.

### Keys to the species of Anthidiine bees in Thailand

Two keys are provided below, one for females and one for males. The keys are modified from Baker (1995), Michener (2007), Engel (2009), Niu et al. (2019), and Flaminio et al. (2021). They both exclude morphospecies known only from citizen science records.

#### Key to females of anthidiine bees in Thailand

Excluding *Stelis flavofuscinular* as the female is unknown but must presumably be identifiable to genus due to the absence of metasomal scopa and juxta-antennal carina.

- 1 Mandible with  $\geq 4$  teeth. Terga without depressed apical zone (genus *Pseudoanthidium*); body black with distinct yellow maculation, especially lateral yellow patch on all terga except T6 ..... ***Pseudoanthidium orientale***
- Mandible teeth  $< 4$  teeth. Terga with apical zone either depressed or not depressed. Terga without yellow maculation but, if present, the pattern will differ from above ..... **2**
- 2 Face with both longitudinal median carina and juxta-antennal carina (Figs 9B, 10B, F). Metasomal scopa absent (genus *Euaspid*) ..... **3**
- Face without carinae as described above. Metasomal scopa present ..... **6**

- 3 S6 acute with median carina and lateral tooth (Fig. 10D). Scutellum extended with medial shallow depression, black without pale maculation on the margin..... ***Euaspis polynesia***
- S6 broad, obtuse, or subacute, with a basal platform. Scutellum black with pale maculations on the margin..... **4**
- 4 S6 with distinct basal platform (Fig. 9C, E). Clypeal punctures irregular, with a strong distinct median carina (Fig. 9B)..... ***Euaspis aequicarinata***
- Basal platform of S6 not distinct but can be noticed at median area. Clypeus without median carina while punctation somewhat irregular ..... **5**
- 5 S6 apical margin obtuse, basal platform arise as a bulge on the median area (Fig. 10H)..... ***Euaspis strandi***
- S6 apical margin subacute, basal platform smaller (Fig. 11D, E). In *Euaspis wegneri* sensu Baker (1995: fig. 31), punctures on scutum and especially on scutellum finer and denser (larger in Thai specimens, see Fig. 10B).....  
..... ***Euaspis aff. wegneri***
- 6 Face with a pair of juxta-antennal carinae but without longitudinal median carina (Fig. 6B, E–G). (genus *Eoanthidium*); subantennal suture straight...  
..... ***Eoanthidium riparium***
- Face without any distinct carina. Subantennal suture arcuate ..... **7**
- 7 Large species (length > 11 mm). Cu-V of hindwing usually  $\geq$  half of 2<sup>nd</sup> M+Cu. (genus *Trachusa*)..... ***Trachusa aff. vietnamensis***
- Smaller (< 11 mm long). Cu-V of hindwing < half of 2<sup>nd</sup> M+Cu..... **8**
- 8 Omaular carina not extending down to the venter of thorax (genus *Bathanthidium*); Paraocular area black. T6 with median raised platform (similar to Fig. 5C)..... ***Bathanthidium binghami***
- Omaulus with a distinct carina, extended to the venter of thorax. T6 without raised platform..... **9**
- 9 Preoccipital ridge and omaulus lamellate (Fig. 12E) (genus *Pachyanthidium*). Body black. Metasomal terga with basolateral white hair patches. Arolia absent..... ***Pachyanthidium lachrymosum***
- Omaulus carinated but not lamellated (genus *Anthidiellum*). Body black with yellow maculations, or reddish to fulvous. Metasoma without clumping white hair patches; arolia present ..... **10**
- 10 Body black with distinct yellow maculations scattered in most parts. The apex of mandible little wider than its base. T1 with obvious anterior carina which separates frontal and dorsal surfaces ..... **11**
- Body somewhat orangish to fulvous, or black. If black, without distinct yellow maculations on metasoma. Apex of mandible  $\sim 1.5\times$  wider than its base. T1 without distinct carina ..... **12**
- 11 Small species (length  $\sim 4\text{--}5$  mm). Hind tibia and basitarsus simple without any distinct swollen parts. T1 with lateral yellow patches. T2 black while T3–T6 with yellow transverse band which is often medially disrupted on T3 (see Fig. 1)..... ***Anthidiellum smithii***
- Moderate species (length  $\sim 7$  mm). Hind tibia and basitarsus distinctly enlarged. Yellow marks present on each tergum, medially disrupted on T1–T3, and becoming full stripes on T4–T6 (see Fig. 2) .....  
..... ***Anthidiellum aff. latipes***

- 12 Head extensively black, brownish on clypeus and lower part of paraocular area. Scutum black. Metasoma dark brown to black, with metallic red infused especially on T2 and T3..... ***Anthidiellum apicepilosum***
- Head orange or fuscous, without extensive black maculation; if present, only on frons. Scutum reddish or fulvous, with extensive black marks. Metasoma reddish or fulvous, sometimes with black marks ..... **13**
- 13 Body largely ferruginous. T6 black, covered with golden-white hairs. T1–T5 sometimes with scattered black maculations (Fig. 4A).....  
..... ***Anthidiellum ignotum***
- Body appears reddish to orange. T6 orange while T1–T5 have a black apical band (Fig. 4B) ..... ***Anthidiellum phuchongense***

### Key to males of anthidiine bees in Thailand

Excluding the males of *Anthidiellum* aff. *latipes*, *Euaspsis* aff. *wegneri*, and *Trachusa* aff. *vietnamensis*, as they are unknown. Also note that the status of male *Euaspsis aequicarinata* and *Eu. strandi* is still ambiguous.

- 1 Arolia absent. Preoccipital ridge and omaulus not carinate. Terga without depressed apical zone (genus *Pseudoanthidium*). Body black with yellow maculation. S3 with apical extended lobe, lined with a series of yellow hair fringes (Fig. 13I) ..... ***Pseudoanthidium orientale***
- Arolia present. Preoccipital ridge smooth or carinated, omaulus carinated, or at least in the dorsal part. Body black with yellow maculation, or different. S3 without extended apical lobe ..... **2**
- 2 Face with both longitudinal median carina and juxta-antennal carinae (as Figs 9B, 10B, F) (genus *Euaspsis*) ..... **3**
- Face without combination of carinae as in above ..... **5**
- 3 Scutellum extended with distinct medial shallow depression, black. Apical lamina of gonoforceps with length < 2× its width ..... ***Euaspsis polynesia***
- Scutellum apically with small median notch, black, with or without pale maculation on the margin. Apical lamina of gonoforceps length > 2× its width..... **4**
- 4 Clypeal punctation irregular, with a strong median carina. Scutellum black with pale maculation on the margin.....  
..... ***Euaspsis aequicarinata sensu Pasteels (1980)***
- Clypeus without median carina while the punctures only somewhat irregular. Scutellum black without pale maculation on the margin .....  
..... ***Euaspsis strandi sensu Baker (1995)***
- 5 Face with a pair of juxta-antennal carinae but without longitudinal median carina (Fig. 6B, E–G). (genus *Eoanthidium*); Subantennal suture strait.....  
..... ***Eoanthidium riparium***
- Face without distinct carina. Subantennal suture arcuate ..... **6**
- 6 Front and middle tibia with two apical spines (genus *Stelis*). Body elongate. Axilla with yellow posterolateral hook ..... ***Stelis flavofuscicular***
- Front and middle tibia with one apical spine. Body robust, not elongate. Axilla without posterolateral hook..... **7**

- 7 Omaular carina incomplete, not extending down to the venter of thorax (genus *Bathanthidium*); Paraocular area black. T6 and T7 with median raised platform (Fig. 5C)..... ***Bathanthidium binghami***
- Omaulus distinctly with complete carina. Terga without median raised platform ..... **8**
- 8 Preoccipital ridge and omaulus carinate or lamellate (Fig. 12E) (genus *Pachyanthidium*); Body black. Metasomal terga with basolateral white hair patches ..... ***Pachyanthidium lachrymosum***
- Omaulus carinate but not lamellate (genus *Anthidiellum*). Body black with yellow maculations, or reddish to fulvous. Metasoma without basolateral white hair patches ..... **9**
- 9 Small species (< 6 mm). Body black with yellow maculation. T1 with obvious anterior carina and lateral yellow maculation. T2 black while T3–T7 with yellow transverse band often medially disrupted on T3..... ***Anthidiellum smithii***
- Larger species (usually > 6 mm). Body somewhat orangish to fulvous. T1 without frontal carina ..... **10**
- 10 Scutum black. Metanotum black to dark ferruginous, brighter in T5–T7....  
..... ***Anthidiellum apicepilosum***
- Scutum and metanotum reddish or fulvous, with black marks infused.... **11**
- 11 Body integument largely ferruginous. Face with extensive black marks. S4 gradulus incomplete. Apical lamina of gonoforceps without inner apical angulation ..... ***Anthidiellum ignotum***
- Body integument appears orangish. Facial black mark restricted to the frons. S4 gradulus complete. Apical lamina of gonoforceps with inner apical angulation ..... ***Anthidiellum phuchongense***

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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# Larval polyphagy of *Cataspilates marceloi* (Lepidoptera, Geometridae), a Neotropical geometrid moth with flightless females

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## Abstract

Surveys in the arid shrubland of the central Andes revealed larval polyphagy for *Cataspilates marceloi* Vargas, 2022 (Lepidoptera, Geometridae, Ennominae, Boarmiini), a geometrid moth with flightless females. This discovery suggests that, as well as in the Holarctic fauna, larval polyphagy would have been important for the evolution of flightlessness among Neotropical geometrid moths of the tribe Boarmiini.

**Key words:** Andes, Asteraceae, brachyptery, polyphagous caterpillar, Solanaceae

## Introduction

The image that usually comes to mind when thinking about Lepidoptera is one of a winged insect. However, wing reduction has evolved independently in many lineages of this insect order (Heppner 1991; Sattler 1991). Cases of wing reduction in the highly diverse moth family Geometridae are restricted to females, and involve species of different subfamilies (Sattler 1991). A molecular phylogenetic study of Holarctic geometrid moths of the subfamily Ennominae revealed seven independent transitions to wing reduction, four of which occurred in the tribe Boarmiini (Wahlberg et al. 2010). Divergence between the oldest clade in this tribe with flightless females and its winged sister group was dated to about 37 Ma, and only 7 Ma for the youngest transition (Murillo-Ramos et al. 2021).

Wing reduction remained overlooked in the Neotropical fauna of Boarmiini until the recent discovery of brachypterous females of *Cataspilates marceloi* Vargas, 2022 on the arid western slope of the central Andes of northern Chile. Its genitalia morphology suggests closeness to *Glena* Hulst, 1896, *Glenoides* McDunnough, 1922 and some species currently included in *Physocleora* Warren, 1897, all of which have winged females (McDunnough 1920; Pitkin 2002). Furthermore, an analysis based on mitochondrial DNA sequences clustered *C. marceloi* with “*Physocleora*” sp. (BOLD Process ID GEOCO032-20, Sample ID LMR\_Geo035) (Vargas 2022). Recent phylogenetic studies revealed polyphyly for *Physocleora* and clustered its species near *Glena*, *Glenoides* and other genera distantly related to the Holarctic lineages of Boarmiini with flightless females (Brehm et al. 2019; Murillo-Ramos et al. 2021). Although further studies are needed to understand the phylogenetic relationships of *C. marceloi*, the



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currently available data suggest that transition to wing reduction in this Neotropical geometrid moth would have been independent of those previously recognized in the Holarctic Boarmiini.

In addition to robust phylogenies, knowledge of natural history of geometrid moths provides important insights to improve the understanding of their evolutionary patterns. Based on the integration of these two aspects for the tribe Operophterini of the subfamily Larentiinae, Snäll et al. (2007) proposed that some groups are predisposed to the evolution of wing reduction due to certain previously evolved permissive life history traits, a hypothesis consistent with results of subsequent studies dealing with Holarctic Ennominae (Wahlberg et al. 2010). A broad host plant range is an especially relevant permissive trait (Snäll et al. 2007; Wahlberg et al. 2010). Larval polyphagy is associated with the four independent transitions to wing reduction in the Holarctic Boarmiini (Wahlberg et al. 2010). In contrast, a single host plant (*Adesmia spinosissima* Meyen, Fabaceae) has been documented for the South American *C. marceloi*, hindering the understanding of this case of flightlessness. The aim of this contribution is to provide new host records that reveal larval polyphagy for this little-known geometrid moth.

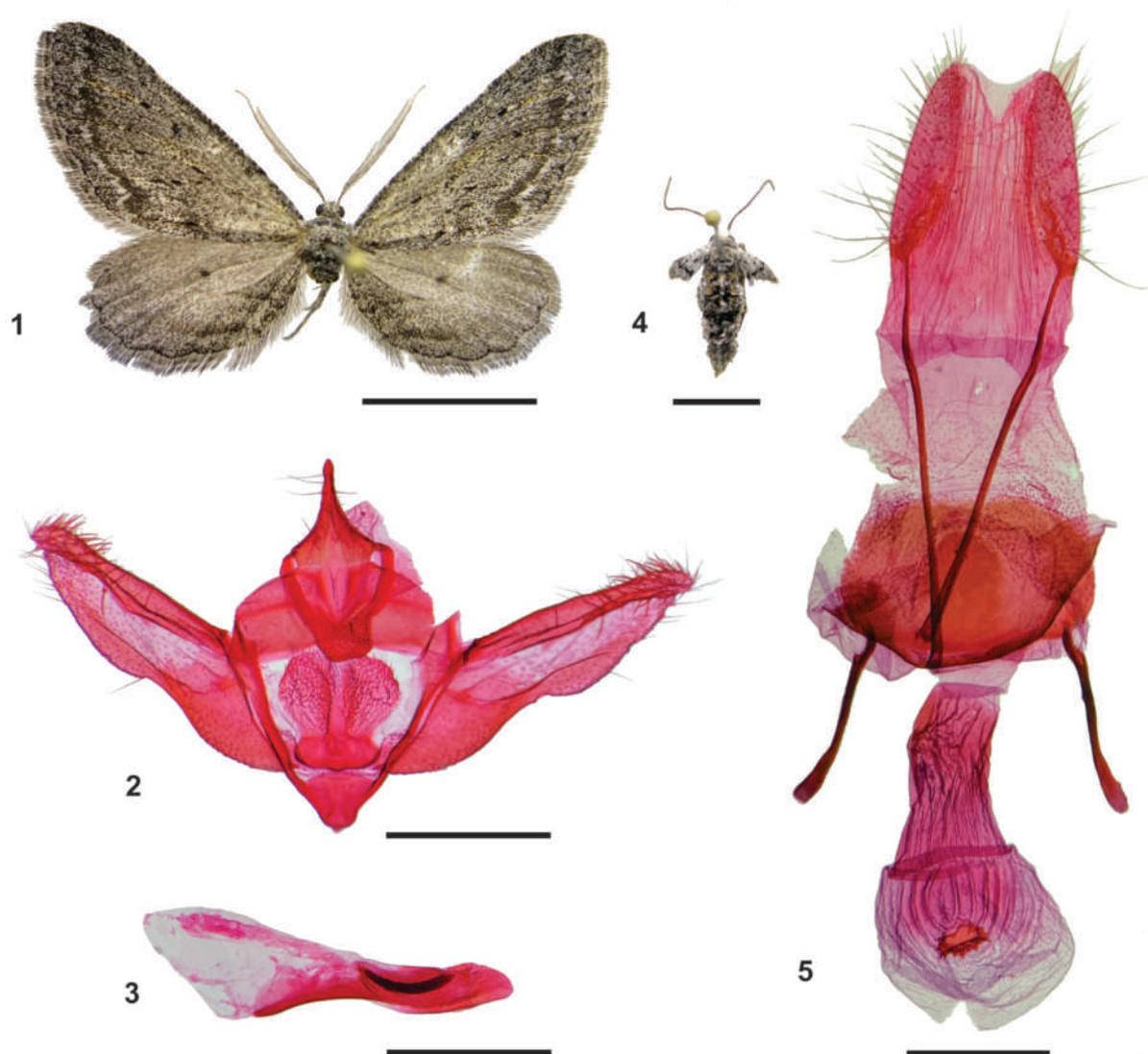
## Materials and methods

Larvae were collected using a beating sheet on the shrubs *Baccharis tola* Phil. (Asteraceae) and *Fabiana ramulosa* (Wedd.) Hunz. & Barboza (Solanaceae) in April, 2023 near Socoroma Village (18°16'42"S, 69°34'15"W), Parinacota Province, at 3400 m elevation on the western slope of the Andes of northern Chile. This is the same sampling site of a paratype of *C. marceloi*, about 10 km northwest of the type locality. The larvae collected were brought to the laboratory in plastic vials with parts of the respective plant and reared until they finished feeding and pupated. The abdomen of each emerged adult was removed and placed in hot KOH 10% for a few minutes for dissection of the genitalia, which were stained with Eosin Y and Chlorazol Black and mounted on slides with Euparal. The specimens studied and their genitalia slides are deposited in the "Colección Entomológica de la Universidad de Tarapacá" (IDEA), Arica, Chile.

Genomic DNA was extracted from legs of two specimens using the QIAamp Fast DNA Tissue Kit (Qiagen). PCR amplification of the barcode region (Hebert et al. 2003) was performed with the primers LCO1490 and HCO2198 (Folmer et al. 1994) using a program of 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 47 °C, 1 min at 72 °C and a final elongation step of 10 min at 72 °C. DNA purification and sequencing were performed at Macrogen Inc. (Santiago, Chile). Additional sequences of *C. marceloi* were downloaded from BOLD (Ratnasingham and Hebert 2007). Sequence alignment with the ClustalW method and assessment of the genetic distances with the Kimura 2-Parameter (K2P) method were performed in the software MEGA11 (Tamura et al. 2021).

## Results

One male and two females were reared from larvae collected on *B. tola*, and one male and seven females from *F. ramulosa*. The emerged adults were identified as *C. marceloi* based on morphology (Figs 1–5).



**Figures 1–5.** Adults of *Cataspilates marceloi* reared from larvae collected on two new host plants **1** male, abdomen removed, dorsal view, reared from *Baccharis tola* Phil. (Asteraceae) **2** male genitalia, phallus removed, ventral view **3** phallus, lateral view **4** female, dorsal view, reared from *Fabiana ramulosa* (Wedd.) Hunz. & Barboza (Solanaceae) **5** female genitalia, ventral view. Scale bars: 10 mm (**1**); 0.5 mm (**2, 3, 5**); 5 mm (**4**).

Two identical DNA barcodes were obtained from the male reared from *B. tola* (BOLD Process ID CATMA003-23) and one female from *F. ramulosa* (BOLD Process ID CATMA004-23). The genetic divergence of these two specimens with the holotype (BOLD Process ID CATMA001-22) and one paratype (BOLD Process ID CATMA002-22) of *C. marceloi* was 1.4–1.5% (K2P). As indicated in the original description, the holotype and paratype of *C. marceloi* were reared from larvae collected on the shrub *A. spinosissima* near Murmuntani. The sequences from Socoroma and Murmuntani were assigned to different BINs (BOLD:AFL2832 and BOLD:AEW3146, respectively) with a genetic divergence of 1.4% (K2P) between the two clusters.

Although DNA barcode analysis is extremely useful for taxonomic identifications, different BINs not always correspond to different species (Prieto et al. 2021; Meier et al. 2022). A few cases of interspecific DNA barcode divergence similar to that found between the two BINs of *C. marceloi* have been

described for geometrid moths (e.g. Wanke et al. 2019). However, genetic divergence between congeneric species of this moth family is generally deeper (e.g. Hausmann et al. 2009; Hausmann and Huemer 2011; Wanke et al. 2020). Accordingly, the two BINs recognized by BOLD for *C. marceloi* are here considered as conspecific, as no additional differences were found between the specimens from Murmuntani (type locality) and Socoroma. The genetic divergence between specimens from the two localities could be due to the low dispersal capacity of the flightless females.

### Material examined

CHILE • 1 ♂; Parinacota, Socoroma; May 2023; H.A. Vargas leg.; ex-larva; *Baccharis tola*; April 2023; HAV-1666 [genitalia slide]; CATMA003-23 [BOLD Process ID] • 1 ♀; same collection data as for preceding; HAV-1667 [genitalia slide] • 1 ♀; same collection data as for preceding • 1 ♂; same collection data as for preceding; *Fabiana ramulosa*; April 2023; HAV-1679 [genitalia slide] • 1 ♀; same collection data as for preceding; HAV-1674 [genitalia slide]; CATMA004-23 [BOLD Process ID] • 6 ♀♀; same collection data as for preceding; all in IDEA.

### Discussion

The results reveal that the previous record of a single host underestimated the host range of *C. marceloi*. The discovery of its larvae feeding on shrubs belonging to two additional families (Asteraceae and Solanaceae) is a clear demonstration of polyphagy for this little-known Neotropical geometrid moth.

Agreeing with the hypothesis of Snäll et al. (2007) for evolution of flightlessness in Operophterini, Wahlberg et al. (2010) proposed that the winged ancestor of lineages with flightless females among the Boarmiini of the Holarctic forests would have been a slow flying moth with polyphagous larvae that used deciduous trees as hosts. The discovery of larval polyphagy in *C. marceloi* suggests that this attribute might also have been important in the transition to flightlessness among Neotropical Boarmiini. Together with larval polyphagy, Wahlberg et al. (2010) mentioned spring larval feeding, overwintering as egg or pupa, and adults flying late/early season as permissive traits for evolution of wing reduction among Holarctic Boarmiini. All these traits would have reduced the importance of female flight, facilitating the transition from winged to wingless females (Snäll et al. 2007). The last three attributes would have allowed the phenology to adjust to the food availability in the Holarctic forests. In contrast, larvae of *C. marceloi* were collected in autumn, adults reared from these larvae emerged in the same season, and it remains unknown whether it has any overwintering stage, suggesting that the set of permissive traits for evolution of flightlessness among Neotropical Boarmiini could be at least partially different from that recognized for the Boarmiini of Holarctic forests.

The ecological context of the area inhabited by *C. marceloi* (Fig. 6) differs drastically from that of the Holarctic forests. The high elevation shrubland of the arid western slope of the central Andes has a tropical xeric climate with most rain concentrated in summer (Luebert and Plischoff 2006), which provides water input for fast development of a highly seasonal vegetation cover (Muñoz



**Figure 6.** Sampling site of larvae of *Cataspilates marceloi*. A shrubland at 3400 m elevation on the arid western slope of the central Andes, in the surroundings of Socoroma Village, Parinacota Province, northern Chile.

and Bonacic 2006). The phenology of geometrid moths has been little explored in this area, but the available information suggests that different patterns co-exist. Two cases of pupal dormancy have been recorded, in one of which larval feeding appears to be synchronized with highest vegetation cover (Vargas 2021), while larval feeding and adult emergence throughout the year has been recorded for two species (Vargas et al. 2020, 2022). Although the highest level of vegetation cover typically occurs shortly after summer rains, some shrubs of this area, including the hosts of *C. marceloi*, maintain some growth during the dry season (Muñoz and Bonacic 2006), providing food substrate for associated phytophagous Lepidoptera. Thus, a strict adjust in the phenology of a given life stage to a narrow period of the year might have been unnecessary for evolution of wing reduction in *C. marceloi*.

Scientific interest in the South American fauna of Geometridae has increased during the last 20 years, improving the understanding of biodiversity patterns and evolutionary relationships (e.g. Brehm 2002; Zamora-Manzur et al. 2011; Brehm et al. 2016, 2019; Ramos-González et al. 2019; Moraes et al. 2021; Murillo-Ramos et al. 2021; Machado et al. 2022). Further studies on the natural history and phylogeny of *C. marceloi* and close relatives are encouraged to disentangle the evolutionary history of wing reduction among Neotropical geometrid moths of the tribe Boarmiini.

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## Additional information

### Conflict of interest

The author has declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

The author solely contributed to this work.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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