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Research Article

Revisiting the type species of the genus *Homidia* (Collembola, Entomobryidae)

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Abstract

Homidia cingula Börner, 1906, the type species of the genus *Homidia* Börner, 1906, is widespread from India to Southeast Asia, but its detailed morphological characteristics have not yet been described. We examined the morphology of specimens of *H. cingula* from Indonesia and southwestern China and confirmed their conspecific status by comparing their DNA barcoding sequences. We also compared the morphology of *H. cingula* with other two closely related species, confirming the valid species status of *H. subcingula* Denis, 1948. Our study provides new taxonomic and molecular data for the genus *Homidia*.

Key words: DNA barcode, *Homidia cingula*, Southeast Asia, southwestern China, taxonomy

Introduction

The genus *Homidia* Börner, 1906 (Collembola, Entomobryidae, Entomobryinae) was first described as a subgenus of *Entomobrya* Rondani, 1861 (Börner 1906). It was later recognized as a distinct genus by Denis (1929). *Homidia*, with 76 reported species, is widely distributed in the Northern Hemisphere, particularly in East and Southeast Asia (Bellinger et al. 1996–2023). *Homidia* can be easily distinguished from other genera of Entomobryinae by several key characteristics, including 8+8 eyes, dental spines in adults, a subapical mucronal tooth larger than the apical one, and eyebrow-like macrochaetae on the anterior part of the fourth abdominal segment (Börner 1906; Szeptycki 1973).

The type species of the genus, *Homidia cingula* Börner, 1906, was originally described from Java (Buiterizor) and has since been recorded in India, Bangladesh, Thailand, Malaya, Singapore, Sumatra, and Vietnam (Yoshii 1989). *Homidia cingula* is characterized by its distinctive colour pattern, which includes pigmented abdominal segments II and III. This colour pattern is also seen in three other related species: *Entomobrya kali* Imms, 1912 from India, *Homidia subcingula* Denis, 1948 from Vietnam, and *Homidia glassa* Nguyen, 2001 also from Vietnam. Handschin (1925) initially questioned whether *H. kali* was a synonym of *H. cingula*, and this was later confirmed by Mitra (1976) who re-examined the syntypes of *E. kali*. However, these species have notable differences in colour pattern based on their original descriptions (Table 1): thoracic

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patches and broad band on Abd. IV posteriorly in *H. cingula*; narrow band on Abd. IV posteriorly and transverse band on Abd. V in *H. subcingula*; and a pair of small metathoracic patches in *H. glassa*. Although Denis (1929) noted differences between *H. cingula* and *H. subcingula* (such as a broad vs narrow stripe on the posterior part of the fourth abdominal segment), Mitra (1976) suggested that *H. cingula* may represent juveniles and *H. subcingula* the darker form of adults. Nguyen (2001) only identified one difference between *H. subcingula* and *H. glassa*: two small patches on the metathorax in the latter. Unfortunately, the type material of *H. cingula* was destroyed (Weidner 1962). Further examination of the type species is essential to resolve the taxonomic uncertainties surrounding these species and to improve our understanding of the genus *Homidia*.

This study focuses on specimens of *H. cingula* collected from Indonesia (Java, Sulawesi) and China, as well as the types of *H. subcingula*. We also employ molecular barcoding techniques to obtain genetic sequences for *H. cingula* specimens from Java and China and compare their genetic distances. A detailed description of *H. cingula* is provided.

Materials and methods

Morphological examination

Juvenile and adult specimens were cleared in lactic acid, mounted in Marc André II solution, and studied using Leica DMLB and Nikon 80i microscopes. Illustrations were enhanced in Adobe Photoshop CS5. Dorsal body chaetae nomenclature follows Szeptycki (1979), Zhang and Deharveng (2015), and Zhang et al. (2019), labial palp nomenclature follows Fjellberg (1999), and labial chaetae nomenclature follows Gisin (1967). The dorsal chaetotaxy is given per half-tergite in the descriptions; the solid and hollow circles represent the primary and secondary chaetae, respectively.

Abbreviations used in this study

Th. I–III	thoracic segment I–III;
Abd. I–VI	abdominal segment I–VI;
Ant. I–IV	antennal segment I–IV;
mac	macrochaeta(-ae);
mes	mesochaetae(-ae);
mic	microchaeta(-ae);
ms	S-microchaeta(-ae) (microsensillum);
sens	ordinary S-chaeta(-ae) on terga;
NJAU	Nanjing Agricultural University;
MNHN	Museum National d'Histoire Naturelle;
NCBI	National Center for Biotechnology Information.

DNA barcoding

DNA was extracted using an Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech, Shanghai, China) following the manufacturer's standard protocols. Primers used were LCO1490/HCO2198, which are commonly used for metazoans (Folmer et al. 1994). PCR amplification of mitochondrial COI was performed in 25 μL volumes containing 12.5 μL of Premix Taq (TaKaRa Taq v. 2.0 plus dye), 1.25 μL of each primer, 8 μL of ddH_2O, 2 μL of template DNA, with PCR programs following Zhang et al. (2014). All PCR products were checked on a 1% agarose gel. Successful products were purified and sequenced in both directions by Majorbio (Shanghai, China) on an ABI 3730XL DNA Analyser (Applied Biosystems). COI sequences for the remaining species were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/). Sequences were preliminarily aligned using MAFFT v. 7.450 by the L-INS-I strategy (Katoh and Standley 2013) and corrected manually, with a final 658-bp alignment. Neighbour-joining (NJ) tree and Kimura 2-parameter (K2P; Kimura 1980) distances were calculated in MEGA v. 7.0 (Kumar et al. 2016). Node supports were evaluated through 1,000 bootstrap replications.

Taxonomy

Order Entomobryomorpha Börner, 1913
Family Entomobryidae Schäffer, 1896
Genus *Homidia* Börner, 1906
***Homidia cingula* Börner, 1906**
***Entomobrya* (*Homidia*) *cingula* Börner, 1906**

***Entomobrya kali* Imms, 1912**
Figs 1–22, Table 1

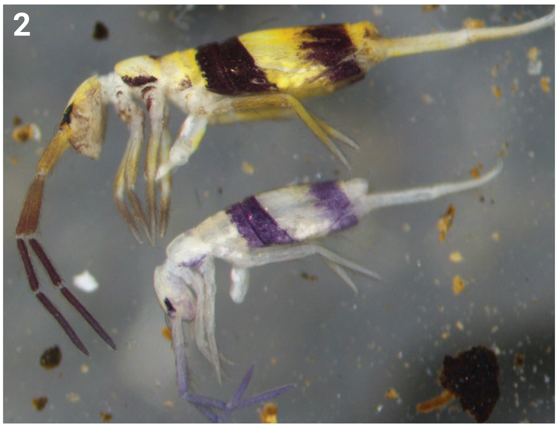
Type locality. Buitenzorg, Bogor, Java Province, Indonesia.

Materials examined. Indonesia • 2 adult females, subadult and 3 juveniles on slide, and four in alcohol; South Sulawesi Province, Kabupaten Bone, Watampone, near Lampo spring; 17 Jul. 1986; Anne Bedos leg.; in litter; sample # Indo-166; four specimens on slide deposited in NJAU and others in MNHN. Indonesia • 1 in alcohol; Jawa Timur; 2 Jul. 2001; Villemant & Daugeron leg.; sample # 03255D01_JAVA05CV03; deposited in MNHN. China • 2 females on slide and 3 in alcohol; Yunnan Province; 26.643°N, 98.905°E; 1,149 m a.s.l.; 11 Oct. 2014; C-Y Qin leg.; in litter; sample # 14YN2. China • 5 in alcohol; Yunnan Province; 27.007°N, 98.869°E; 1,199 m a.s.l.; 12 Oct. 2014; C-Y Qin leg.; sample # 14YN3. All Chinese material deposited in NJAU.

Redescription. Body length up to 2.38 mm. Ground colour pale yellow or pale. Antenna gradually darker towards tip. Eye patches dark blue. Th. II with lateral strips and a small patch on the postero-middle part. Th. III with very pale lateral strips. Coxae and femora weakly pigmented. Two transverse dark bands on Abd. II and III. Posterior half of Abd. IV pigmented (Figs 1, 2).

Antenna 2.5–3.0 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV = 1: 1.2–1.3: 1.2–1.3: 1.6–1.9. Smooth straight mic at antennal base three dorsal and three ventral on Ant. I, one external, one internal and one ventral on Ant. II and absent on Ant. III and IV. Ant. III organ with two rod-like sensilla (Fig. 3). Ant IV with apical bulb bilobed (Fig. 4).

Eyes 8+8. Labral papillae absent. Labral intrusion deeply V-shaped. Prelabral and labral chaetae 4/ 5, 5, 4, all smooth; prelabral chaetae and chaetae of the first row longer than others (Fig. 5). Mandibles with 4+5 teeth (Fig. 6). Five labial papillae A–E with 0, 5, 0, 4, 4 guard chaetae, respectively; lateral process of



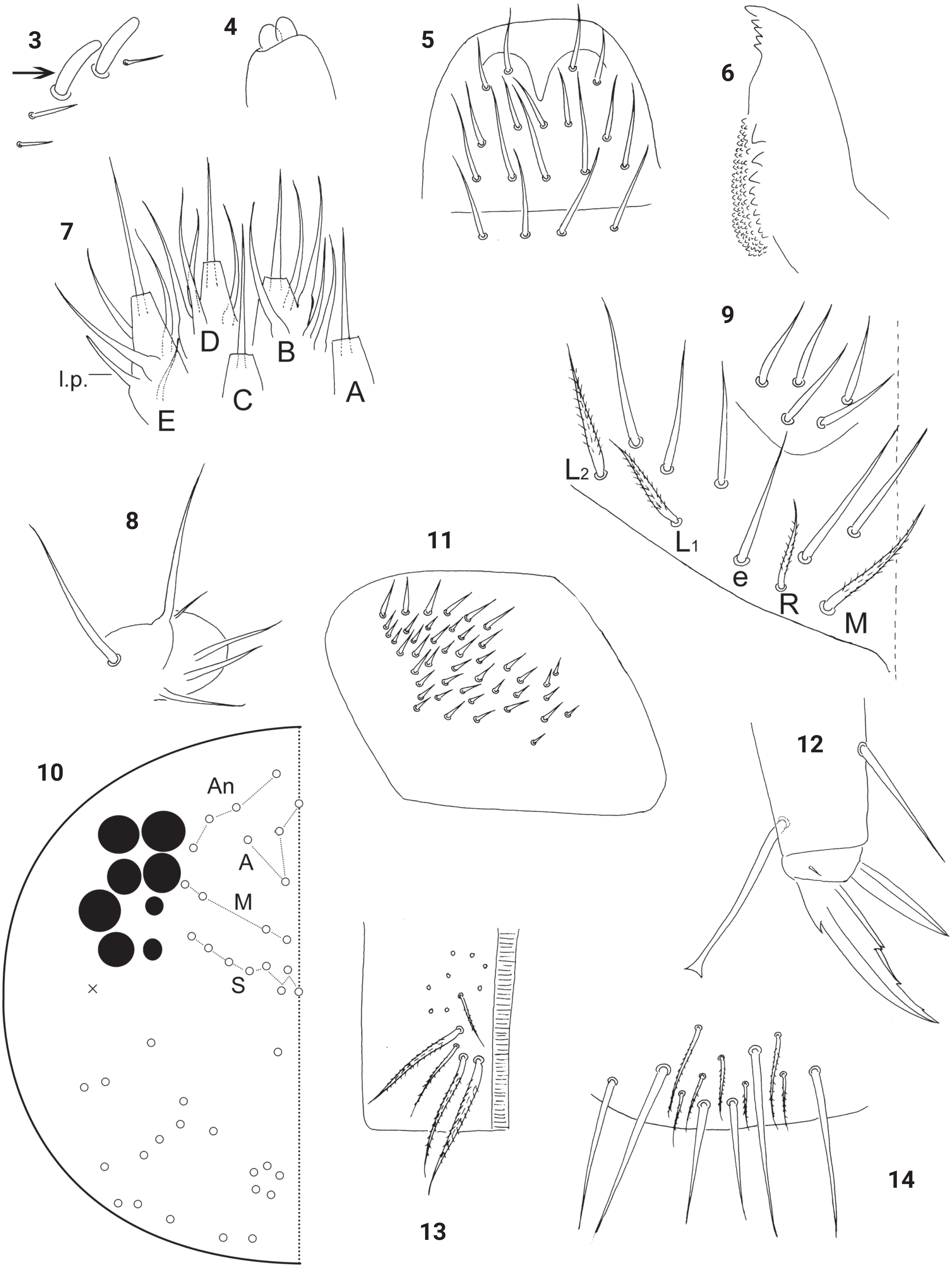
Figures 1, 2. Habitus of *Homidia cingula* Börner, 1906 preserved in ethanol **1** from South Sulawesi (Indonesia) **2** from Yunnan (China).

labial palp thinner than normal chaetae, with tip not reaching apex of papilla E (Fig. 7). Subapical seta of maxillary outer lobe subequal to the apical one; four smooth sublobal hairs on maxillary outer lobe and the lateral one much smaller than others (Fig. 8). Labial base as MReL$_1$L$_2$; chaeta e smooth; proximal area with five smooth chaetae (Fig. 9); modified (leaf-like) chaetae absent on the ventral side. Cephalic dorsal chaetotaxy with four antennal (An), four anterior (A), four medio-ocular (M), and eight sutural (S) chaetae (Fig. 10).

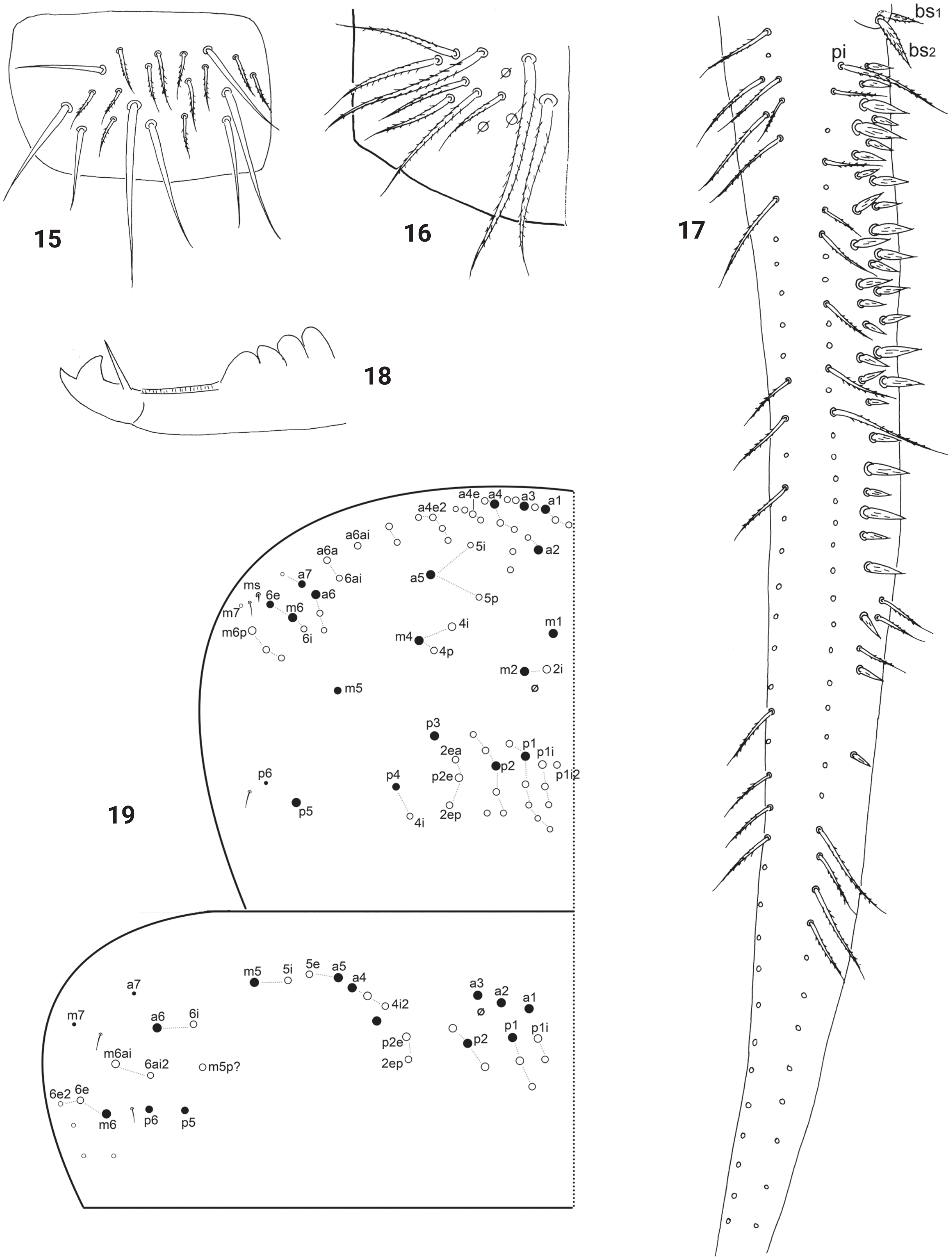
Coxal macrochaetal formula as 3/4+1, 3/4+2. Trochanteral organ with 31–35 smooth, spine-like chaetae (Fig. 11). Unguis with four inner and two lateral teeth; distal inner tooth extremely tiny. Unguiculus acuminate with outer edge smooth. Tenent hair clavate, subequal to unguis (Fig. 12). Abd. IV 4.4–5.1 times as long as Abd. III along dorsal midline. Tenaculum with 4+4 teeth and one large striate chaeta. Ventral tube anteriorly with 9–13 ciliate chaetae on each side, three of them mac (Fig. 13); posteriorly with numerous ciliate chaetae and six distal smooth ones (Fig. 14); each lateral flap with 8–10 smooth and 7–12 ciliate chaetae (Fig. 15). Manubrial plaque with three pseudopores and 7–10 ciliate chaetae (Fig. 16). Posterior face of dens with two longitudinal rows of chaetae; 27–35 spines present internal to the inner row of chaetae; two basal chaetae (following Szeptycki 1973) spiny and multilaterally ciliate, bs$_1$ slightly shorter than bs$_2$; proximal-inner seta (pi) ciliate, apparently thinner and much longer than bs (Fig. 17). Mucro bidentate with subapical tooth much larger than apical one (Fig. 18).

Th. II with three (m1, m2, m2i) medio-medial, three medio-lateral (m4, m4i, m4p), about 23 posterior mac and three lateral s-chaetae; ms internal to al; m7 and p6 as mic. Th. III with about 30 mac and two lateral s-chaetae; a7 and m7 as mic (Fig. 19). Abd. I with nine (m2–4, m2i, a2, a3, a5, m4i, m4p) central mac and two lateral S-chaetae. Abd. II with five (a2, a3, m3, m3e, m3ep) central, one (m5) lateral mac and two S-chaetae; chaeta m3ea as mic. Abd. III with one (m3) central, four (am6, pm6, p6, m7a) lateral macrochaetae and three S-chaetae (Fig. 20). Abd. IV with 8–10 anterior mac arranged in a transverse row, 4–5 (A5, A6, B4, B5, Ae7) centrally posterior mac, about 15 lateral mac and 52–62 S-chaetae; mac Ae7 often absent; B6 as meso or mic (Fig. 21). Abd. V with three S-chaetae (Fig. 22).

Distribution. India, Bangladesh, China (Yunnan), Thailand, Malaya, Singapore, Indonesia (Java, Sumatra, Sulawesi).

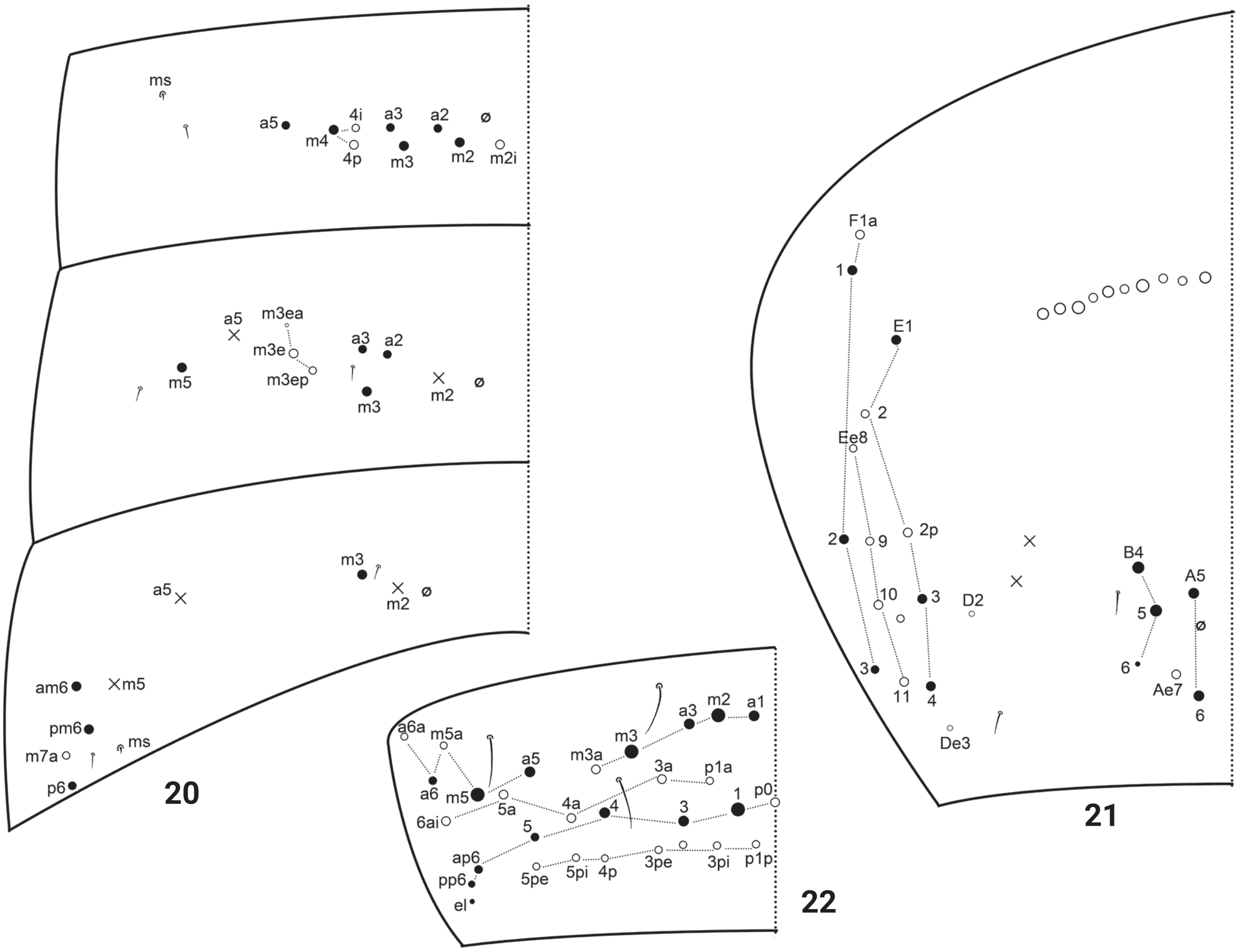


Figures 3–14. *Homidia cingula* **3** Ant. III organ **4** Antennal apical bulb **5** labrum **6** right mandible **7** labial palp **8** maxillary outer lobe **9** labial chaetae **10** dorsal cephalic chaetotaxy **11** trochanteral organ **12** hind claw **13** ventral tube, anterior face **14** ventral tube, distal part of posterior face.



Figures 15–19. *Homidia cingula* **15** lateral flap of ventral tube **16** manubrial plaque **17** posterior face of dens **18** mucro **19** thoracic chaetotaxy.

Remarks. *Homidia cingula* is characterized by dark transverse bands on Abd. II–III, 5+5 central mac on Abd. II, and 1+1 central mac on Abd. III. It has similar colour pattern to *H. subcingula* from Dalat, Vietnam (dark Abd. II and III). Mitra



Figures 20–22. Abdominal chaetotaxy of *Homidia cingula* **20** Abd. I–III **21** Abd. IV **22** Abd. V.

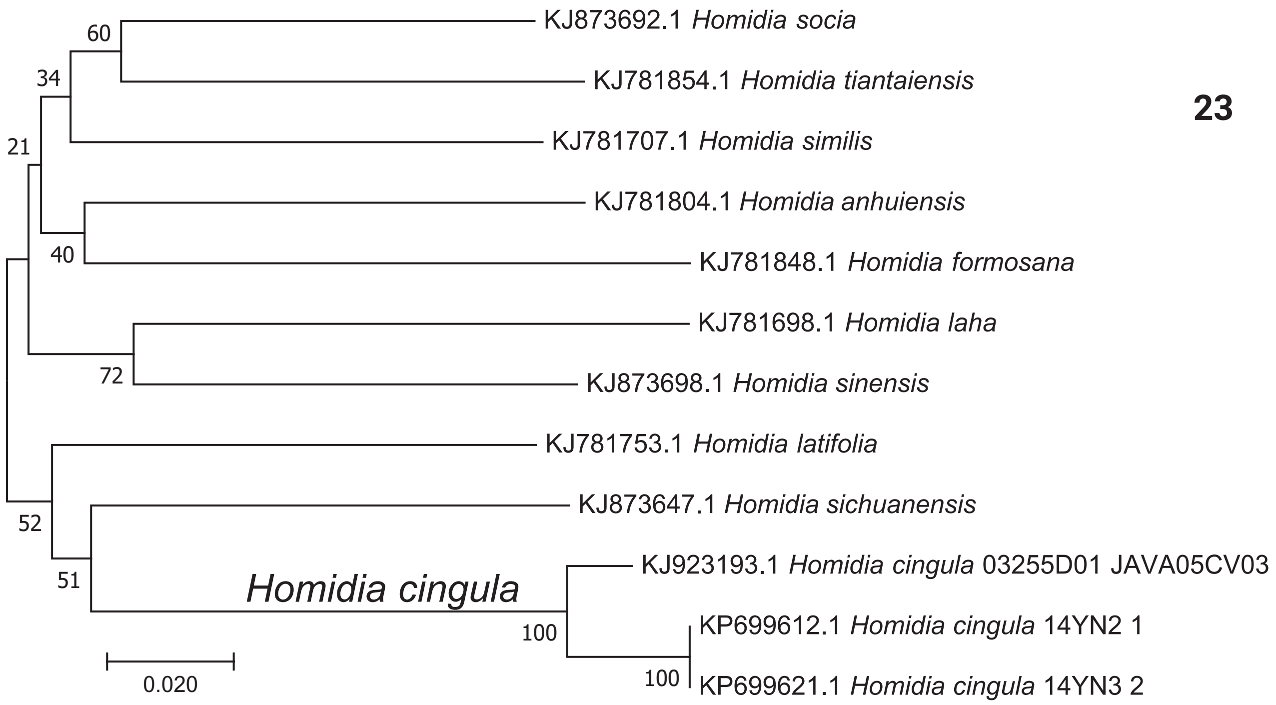


Figure 23. Neighbour-joining tree based on mtDNA COI sequences. Node numbers are bootstrap values.

(1976) considered them as synonyms and doubtfully thought that Börner's *H. cingula* represented juveniles of *H. subcingula*. However, the re-examination of the type specimen (2.4 mm) of *H. subcingula* in MNHN by the senior author (F. Zhang) shows that both taxa are valid species, although the type of *H. cingula* is in poor condition and many details are impossible to visualize. *Homidia subcingula* differs from the *H. cingula* in the narrow strip on posterior Abd. IV, Abd. V pigmented, 11+11 mac on Abd. I, and 6+6 (m3ea present) central mac on Abd. II. In addition, the validation of *H. glassa*, which also possesses pigmented Abd. II–III, is questionable. Chaetotaxy of Abd. I–III of *H. glassa* could be closer to *H. subcingula*, but this differs from that of *H. cingula* (Table 1).

Table 1. Morphological comparison of three *Homidia* species.

Characters	*H. cingula*	*H. subcingula*	*H. glassa*
Middle patch on Th. II posteriorly	Present	Absent	Absent
Lateral stripe on Th. II	Present	Absent	Absent
Two small patches on Th. III	Absent	Absent	Present
Posterior band on Abd. IV	Broad	Narrow	Absent
Transverse band on Abd. V	Absent	Present	Absent
Mac on Abd. I	9	11	10?
Central mac on Abd. II	5	6	6
Central mac on Abd. III	1	2	2
Maximum body length (mm)	2.38	2.40	2.64

Molecular results

Our results show that pairwise genetic distances range from 0.03 to 0.212 among 10 *Homidia* species (Table 2). The genetic distance between specimens of *H. cingula* from Yunnan (China) and Java is 0.03 (Table 2). The small genetic divergence (Hebert et al. 2003) indicates that these specimens belong to the same species (Fig. 23).

Table 2. K2P genetic distances among twelve *Homidia* sequences. YN, Yunnan; JAVA, Java.

GeneBank accession	Species	1	2	14YN2	14YN3	5	6	7	8	9	10	11
KJ781804.1	*Homidia anhuiensis*											
KJ923193.1	*Homidia cingula*_03255D01_ JAVA05CV03	0.205										
KP699612.1	*Homidia cingula*_14YN2_1	0.201	**0.03**									
KP699621.1	*Homidia cingula*_14YN3_2	0.201	**0.03**	0								
KJ781848.1	*Homidia formosana*	0.174	0.191	0.204	0.204							
KJ781698.1	*Homidia laha*	0.212	0.193	0.198	0.198	0.206						
KJ781753.1	*Homidia latifolia*	0.17	0.161	0.173	0.173	0.197	0.187					
KJ873647.1	*Homidia sichuanensis*	0.173	0.157	0.173	0.173	0.204	0.186	0.163				
KJ781707.1	*Homidia similis*	0.158	0.188	0.209	0.209	0.19	0.192	0.161	0.179			
KJ873698.1	*Homidia sinensis*	0.173	0.169	0.185	0.185	0.175	0.158	0.196	0.183	0.157		
KJ873692.1	*Homidia socia*	0.163	0.2	0.198	0.198	0.183	0.171	0.164	0.174	0.146	0.173	
KJ781854.1	*Homidia tiantaiensis*	0.17	0.192	0.199	0.199	0.184	0.203	0.172	0.167	0.157	0.176	0.138

Discussion

Both morphological and molecular analyses confirm that the *Homidia* specimens from Indonesia and southwestern China, collected ca 3,000 km apart, are the same species. Species possessing distinct colour pattern (Abd. II–III and posterior half of Abd. IV pigmented) are widely distributed in Southeast and South Asia. Genetic divergence of the individuals from the most southern and the most northern regions is very low (ca 3%). Their colouration and wide distribution perfectly match the original descriptions and subsequent records of *H. cingula*. Therefore, we consider the species examined in this study to be *H. cingula*, although the type material described by Börner has been destroyed (Weidner 1962). Re-examination of types of *H. subcingula* verifies its validity based on colour pattern and chaetotaxy of Abd. IV (Table 1). The maximum body lengths of *H. cingula* and *H. subcingula* were approximately equal, thus disproving the hypothesis that *H. cingula* represents juveniles of *H. subcingula*. We doubt the validity of *H. glassa*, whose characteristics is very similar to *H. subcingula* except for its rough description of colouration. Applying colouration to distinguish *Homidia* species remains a powerful tool in modern taxonomy. Following this rule, the photographs of "*H. cingula*" from Taiwan (photographer: H.-J. Cheng) seem different from our understanding of *H. cingula*: both anterior and lateral margins are pigmented (anterior part pale in *H. cingula*), and the posterior half patch of Abd. IV is divided into two parts (connected in *H. cingula*). A molecular comparison could easily resolve this problem. Combining with the first instar chaetotaxy (Zhang et al. 2019), our revisiting of *H. cingula* provides valuable information for the diagnoses of the genus *Homidia*. Further collections of *H. cingula* from type locality (Buitenzorg, Indonesia) could be conducted to assign the neotype material.

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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: FZ. Funding acquisition: FZ. Supervision: FZ. Writing - original draft: FZ, GQZ, YXZ.

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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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Research Article

Miradessus gen. nov. from South America described for two species previously in *Amarodytes* Régimbart, 1900 and two new species (Arthropoda, Insecta, Coleoptera, Adephaga, Dytiscidae, Hydroporinae, Bidessini)

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Abstract

Miradessus **gen. nov.** is described for two previously described species, *Amarodytes pulchellus* Guignot, 1955 from Colombia, with new records from Venezuela, and *A. plaumanni* Gschwendtner, 1935, from Brazil, and two previously unknown species, *Miradessus beni* **sp. nov.**, from Bolivia and Peru, and *Miradessus rikae* **sp. nov.** from Ecuador. The genus is characterized by 1) occipital line absent; 2) basal pronotal striae present; 3) basal elytral stria absent; 4) sutural elytral stria absent; 5) transverse carina on elytral epipleuron at humeral angle absent; 6) distinct marginal bead on anterior clypeal margin absent; and 7) male median lobe deeply multilobed with a dorsal portion separate from a unilobed or bilobed ventral portion.

Resumen

Se describe a *Miradessus* **gen. nov.** para dos especies previamente descriptas, *Amarodytes pulchellus* Guignot, 1955 de Colombia, con nuevos registros de Venezuela, y *A. plaumanni* Gschwendtner, 1935, de Brasil, y dos especies previamente desconocidas, *Miradessus beni*, **sp. nov.**, de Bolivia y Perú, y *Miradessus rikae*, **sp. nov.**, de Ecuador. El género se caracteriza por 1) línea occipital ausente; 2) estría pronotal basal presente; 3) estría elitral basal ausente; 4) estría elitral sutural ausente; 5) carena transversa en el ángulo humeral del epipleuron elitral ausente; 6) reborde marcado en el margen anterior del clípeo ausente; y 7) lóbulo medio del macho profundamente multilobado con una porción dorsal separada de una porción ventral unilobada o bilobada.

Key words: Diving beetle, South America, taxonomy, water beetle

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Introduction

The tribe Bidessini Sharp, 1880 includes an unusually large proportion of genera and species of Dytiscidae (Miller and Bergsten 2016). They occur in a great many habitats throughout the world (Miller and Bergsten 2016). New genera have been regularly discovered in recent years through field expeditions in new regions or habitats (e.g. hygropetric or subterranean) and reexamination of historically recognized genera (Miller and Spangler 2008; Hendrich and Balke 2009; Hendrich et al. 2009; Miller and García 2011; Miller 2012, 2016a; Miller and Short 2015; Miller and Wheeler 2015; Balke et al. 2017).

During a revisionary investigation of the genus *Amarodytes* Régimbart, 1900 by the authors it became clear that there are several groups of Bidessini species historically involved in the genus that are seemingly more closely related to other groups in Bidessini than to each other. The type species, *A. percosioides* Régimbart, 1900, is part of a group that includes species with single-segmented lateral lobes that are related to *Hydrodessus* J. Balfour-Browne, 1953 and *Peschetius* Guignot, 1942, which also have single-segmented lateral lobes (Miller et al. 2006; Miller and Bergsten 2014, 2016, 2023; Miller 2016b). However, other species assigned to *Amarodytes* were found to have two-segmented lateral lobes, and, therefore, are misplaced in the genus (Benetti and Régil Cueto 2004). Two of these species were described as *Amarodytes pulchellus* Guignot, 1955 and *Amarodytes plaumanni* Gschwendtner, 1935. Other specimens recently examined from Bolivia and Peru are similar to *A. pulchellus*, but represent a different, undescribed species described here. Finally, two specimens from Ecuador were also found to represent an unknown species. These species together possess a unique set of character states and cannot be placed into any existing Bidessini genus, nor do they belong in *Amarodytes*. For this reason, a new genus is here erected to include them. The systematics of *Amarodytes* will require additional attention to address the *A. percosioides*-, *A. duponti*-, and *A. segrix*-groups which seem unlikely to be appropriately placed in the same genus (Benetti and Régil Cueto 2004).

Materials and methods

Methods for dissections and other treatment of specimens largely follow recommendations by Miller and Bergsten (2016).

Materials

Specimens from nearly every genus of Bidessini were examined, including multiple species from many of them. Specimens of relevant species treated herein were examined primarily from the following collections:

MIZA	Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez, Universidad Central de Venezuela, Maracay, Venezuela (L. Joly);
MSBA	Museum of Southwestern Biology, Division of Arthropods, University of New Mexico, Albuquerque, NM, USA (K.B. Miller);
OLML	Oberösterreichisches Landesmuseum, Linz, Austria (M. Schwarz);

SEMC	Snow Entomological Collection, University of Kansas, Lawrence, KS, USA (A.E.Z. Short);
USNM	United States National Museum, Department of Entomology, Washington, DC, USA (S. Brady);
ZSMG	Zoologische Staatssammlung, Munich, Germany (M. Balke).

Measurements

Measurements were taken with an ocular scale on a Zeiss Discovery V8 dissecting microscope at 50× magnification. Attempts were made to measure the most variable specimens in size and shape to determine the extent of that variation. Measurements include: 1) total length (TL), 2) greatest width across elytra (GW), 3) greatest pronotal width (PW), 4) greatest width of the head (HW), 5) distance between the eyes (EW), 6) greatest length of metatrochanter (RL), and 7) greatest length of metafemur (FL). The ratios TL/GW, HW/EW, and FL/RL were calculated to provide an indication of relative size and shape of certain structures.

Photos and illustrations

Methods for images largely follow Miller and Bergsten (2016).

Results

***Miradessus* Miller, Benetti & Michat, gen. nov.**
https://zoobank.org/1BF90E3E-44AC-4D82-A84C-8953ADCF7798
Figs 1–30

Type species. *Amarodytes pulchellus* Guignot, 1955 by current designation.

Diagnosis. *Miradessus* belongs to the tribe Bidessini based on the presence of bisegmented lateral lobes (Figs 10,12,14,16) and a spermathecal spine (Fig. 17). Within Bidessini, the genus differs from most other genera in the following character combination: 1) occipital line absent (Figs 1–4), 2) basal pronotal striae present (Figs 1–4), 3) basal elytral stria absent (Figs 1–4), 4) sutural elytral stria absent (Figs 1–4), 5) transverse carina on elytral epipleuron at humeral angle absent, and 6) distinct marginal bead on anterior clypeal margin absent (Figs 1–4). Other genera share these features including *Novadessus* Miller, 2016, *Bidessodes* Régimbart, 1900, *Neobidessodes* Hendrich & Balke, 2009, the *Amarodytes duponti* group, and *Amarodytes segrix* Guignot, 1950. But *Miradessus* differs from all of these in the distinctive shape of the male genitalia (Figs 9–16). The male median lobe is very characteristically apically multilobed with a single, dorsal elongate and slender portion and a ventral section that is broad and bilobed (Figs 9, 11, 15) or flattened and elongate (Fig. 13). The lateral lobes are robust, and the bases are large and, in at least some species, covered with conspicuous tubercles (Figs 9, 13). Members of the genus are also superficially quite distinctive from other Bidessini (see more below under Discussion).

Etymology. This genus is named *Miradessus* from the Latin *miror*, meaning to admire, for the impressive color pattern of these beetles, and *dessus*, a common root for genera in the tribe Bidessini (gender masculine).

Discussion. The species in this new genus are characterized by lateral lobes that are distinctly two-segmented like the majority of Bidessini taxa (Biström 1988; Miller et al. 2006; Miller and Bergsten 2014; 2016; Miller 2016b;). Members of typical *Amarodytes* (including the type species, *Amarodytes percosioides* Régimbart, 1900) have single-segmented lateral lobes and belong within a clade sister to a clade characterized by two- or three-segmented lateral lobes (Benetti & Miller unpublished). Within the bisegmented lateral-lobe clade, these specimens do not fit well into any other genera (see Diagnosis above). They share some character combinations with *Novadessus*, *Bidessodes*, *Neobidessodes*, the *Amarodytes duponti* group, and *Amarodytes segrix*. In some ways they are most superficially similar to members of *Bidessodes* Régimbart, but specimens in that genus have series of very fine denticles along the posterior margins of the abdominal ventrites (Miller 2017), which are lacking in *Miradessus*, and also have distinctly different male genitalia (Miller 2017). *Miradessus* are similar to Neobidessodes, but that genus has simple male median lobes unlike the multilobed condition in *Miradessus* (Figs 9, 11, 13, 15). Otherwise, they are not similar to other genera in the tribe and are unique because of the prominently apically multilobed male median lobe (Figs 9, 11, 13, 15) which is not found in other genera of Bidessini.

Miradessus beni **Miller, Benetti & Michat, sp. nov.**

https://zoobank.org/6A3EEA04-9DEA-41DA-A2F4-40E795270841
Figs 1, 5, 9, 10, 18–20, 30

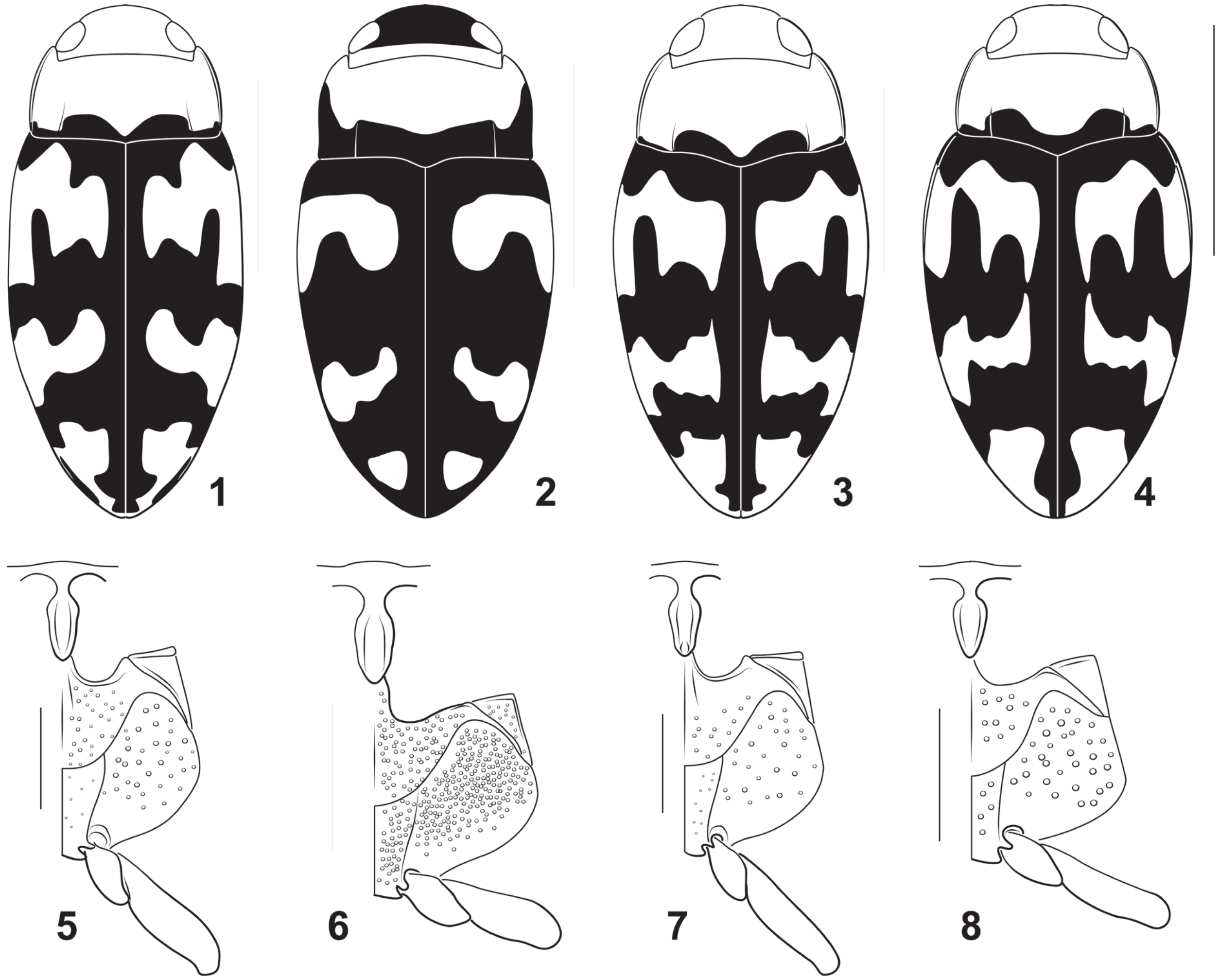
Type locality. Bolivia, Departmento de La Paz, Provincia de Abel Iturralde, San Miguel del Bala, 14°30.602'S, 67°29.555'W.

Diagnosis. This species and *M. pulchellus* are extremely similar externally, with similar overall shape, color pattern, and other features (Figs 1, 3). The external differences between them are subtle, including the shape of the prosternal process, which is more prominently laterally carinate and deeply sulcate in *M. pulchellus* than in *M. beni*. Also, the ventral surface is darker in most specimens of *M. pulchellus* than in *M. beni*. The main differences between these species are in the male genitalia, which are distinctive and characteristic. Both have the median lobe trilobed (with two side portions and a middle portion), but in *M. pulchellus* the middle portion is nearly as long as the side portions and the apex has a distinct, fine pencil of setae on each side that are divergent (Fig. 13). In *M. beni* the middle portion is considerably shorter than the side portions and has a series of setae along its apex (Fig. 9). In lateral aspect, the lateral lobe of *M. pulchellus* has the apical segment nearly as long as the basal segment and it is curved ventrad (Fig. 14). In *M. beni* the lateral lobe has the apical segment only about 1/3 the length of the basal segment and it is curved dorsad (Fig. 10).

Description. ***Measurements*.** TL = 2.7–2.8 mm, GW = 1.3–1.4 mm, PW = 1.0–1.1 mm, HW = 0.7–0.8 mm, EW = 0.3–0.4 mm, TL/GW = 2.0–2.1, HW/EW = 1.8–1.9, FL/RL = 2.2–2.3.

***Habitus*.** Body shape elongate-oval, lateral outline somewhat discontinuous between pronotum and elytron, posterior apex narrowly rounded (Fig. 1).

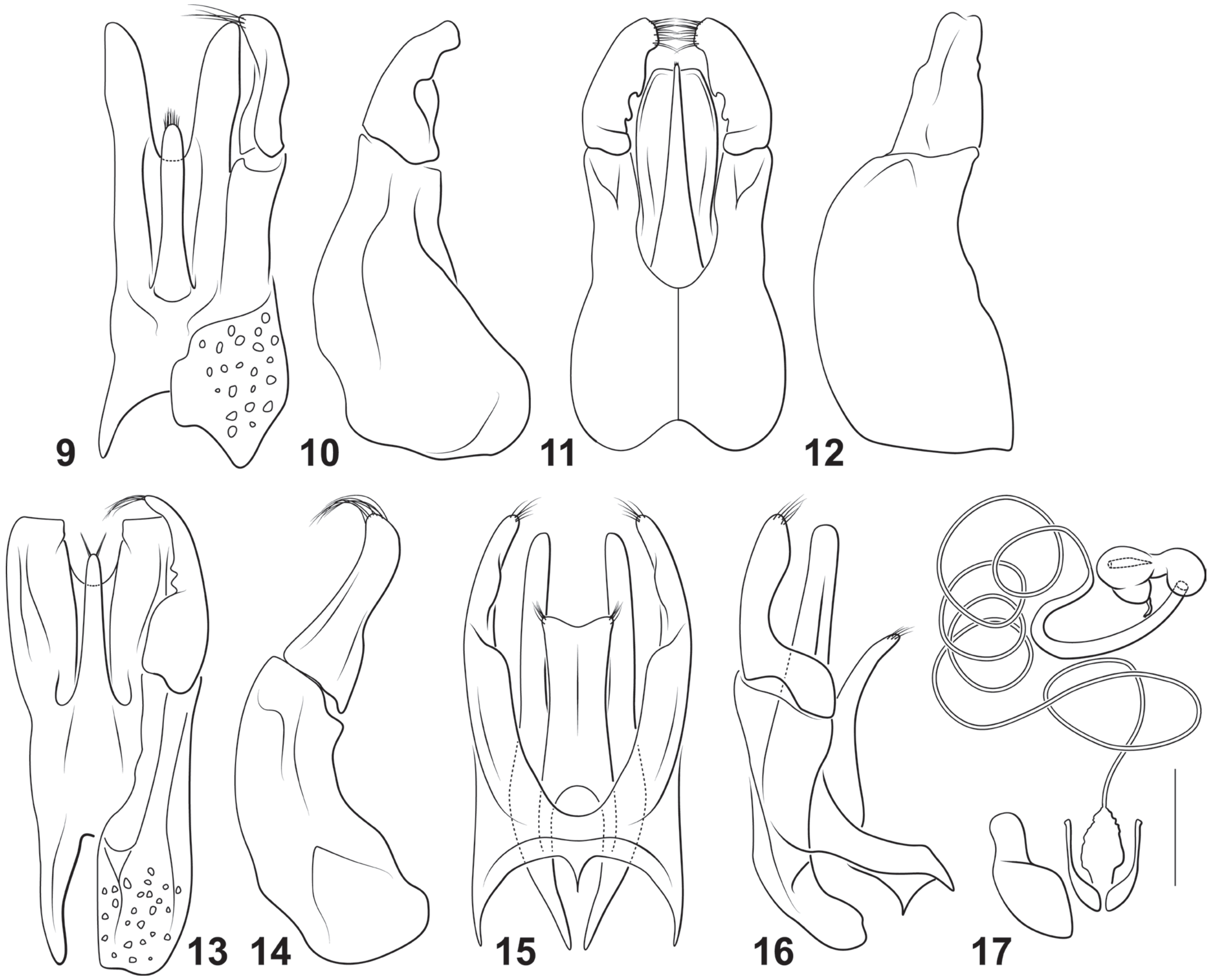
Coloration (Fig. 1). Head yellow-orange. Pronotum yellow-orange with a narrow, rounded lobe of black broadly along each side of posterior margin. Elytron maculate, yellow-orange and black, margins of maculae strongly demarcated;



Figures 1–8. *Miradessus* species **1–4** dorsal habitus **1** *M. beni* **2** *M. plaumanni* **3** *M. pulchellus* **4** *M. rikae* **5–8** prosternal process left surfaces of metaventrite metacoxa metatrochanter and metafemur **5** *Miradessus beni* **6** *M. plaumanni* **7** *M. pulchellus* **8** *M. rikae*. Scale bars: 1.0 mm (**1–4**); 0.5 mm (**5–8**).

with large, transverse yellow-orange regions anteriorly, medially, and apically, not extending medially to suture, with narrow band of black along entire length of suture, margins of maculae irregular and variously lobed (Fig. 1). Ventral surfaces mostly orange; legs, epipleuron, and lateral areas of prothorax and head lighter orange-yellow, some sutural margins darker, infuscate, mesothoracic ventrites and prosternal process strongly infuscate to nearly black.

***Sculpture and structure*.** Head shiny and smooth, nearly impunctate, with small micropunctures sparsely distributed; eyes moderately large (HW/EW = 1.8–1.9); antennae slender, unmodified. Pronotum with lateral margins moderately curved anteriorly, shallowly curved posteriorly; with narrow bead along entire margin; surface shiny, moderately and evenly punctate; lateral pronotal plica weakly impressed, sublinear, extending about 1/3 distance across pronotum. Elytron with lateral margin evenly and broadly rounded; surface shiny, moderately and evenly punctate. Prosternum medially moderately broad, medially not protruberant, mediolaterally somewhat granular; prosternal process moderately broad medially with slight tubercle, apical blade large, covered with fine setae, laterally with prominent rounded ridges along entire length, medially longitudinally sulcate, lateral margins slightly convergent to narrowly rounded apex (Fig. 5). Metaventer and metaven-



Figures 9–17. *Miradessus* species **9, 10** *M. beni*, male aedeagus **9** median lobe and left lateral lobe, dorsal aspect **10** right lateral lobe, right lateral aspect **11, 12** *M. plaumanni*, male aedeagus **11** median lobe and lateral lobes, dorsal aspect **12** right lateral lobe, right lateral aspect **13, 14** *M. pulchellus*, male aedeagus **13** median lobe and left lateral lobe, dorsal aspect **14** right lateral lobe, right lateral aspect **15, 16** *M. rikae*, male aedeagus **15** median lobe and lateral lobes, dorsal aspect **16** median lobe and right lateral lobe, right lateral aspect **17** *M. pulchellus*, female genitalia, ventral aspect. Scale bar: 0.25 mm.

tral wings smooth and shiny, covered with shallow punctures (Fig. 5). Metacoxa with medial portion moderately broad, metacoxal lines distinct, evenly divergent anteriorly to posterior margin of metaventrite, lateral portion large, medially and anteriorly covered with shallow punctures; metatrochanter about 1/3 length of metafemur (Fig. 5). Abdominal ventrites impunctate except II and III laterally with shallow, indistinct punctures; VI strongly concave in lateral aspect, apex pointed.

***Male genitalia*.** Median lobe in ventral aspect broad, trilobed, with dorso-medial, elongate, slender, apically pointed ramus and another ventral, elongate, apically bilobedramus, with each ramus elongate and slender, medial dorsal ramus much shorter than ventral ramus (Fig. 9); lateral lobe in lateral aspect robust, basal segment very broad, robust, apical segment short, robust, curved dorsad, apex obliquely truncate (Fig. 10).

***Sexual dimorphism*.** Males have the pro- and mesotarsomeres I–III slightly but distinctly broader than in females. Abdominal ventrite VI distinctly concave in lateral aspect in females, but medially more expanded and apically somewhat depressed in males.

***Variation*.** There is some minor variation in shape and extent of coloration of the dorsal and ventral surfaces but otherwise specimens are similar.

Etymology. This species is named *beni* after the name Río Beni, the river at the type locality of the species. The name is a noun in apposition.

Distribution. *Miradessus beni* is known from the type locality in lowland Departmento de La Paz, Bolivia and from two sites in lowland Peru (Fig. 30).

Habitat. The type locality is a heavily forested area of the Andean foothills next to a large river. However, nothing is known of the specific collection habitat of this species.

Material examined. *Type material*. *Holotype* (Figs 18–20) in MIZA, male labeled, "BOLIVIA: La Paz Dept. Ituralde [sic] Prov., San Miguel 14°30.602'S, 67°29.555'W, 24–30 Sept. 2007 KB Miller KBMC24090701/ HOLOTYPE *Miradessus beni* Miller, Benetti & Michat, 2023 [red label with black line border]."

Paratypes, 41 total; 6 paratypes (MIZA, MSBA) labeled same as holotype;" 8 paratypes (USNM) labeled, "PERU:Dpt. Cuzco Prov. Quispicanchis Quincemil 6-II-X-1976 Robert Gordon"; 12 paratypes (ZSMG) labeled, "Bidessus cruciatus ? [handwritten] / Amarodytes pulchellus [handwritten] / Peru, Prov. Huanuco, Rio Yuyapichis, Biol. Stat. Panguana. Östl. Ort. 9°37'S, 74°56'W 6.–17.April 2003, Malaise [handwritten], leg. H., J. u. E. -G. Burmeister"; 2 paratypes (ZSMG) labeled, "PERU, Dept. Huanuco, Panguana ACP, Rio Yuyapichis 9°37'S – 74°56'W, at blacklight, XII.2015 J. Monzon leg.";4 paratypes (ZSMG) same as previous except ".../230 m, blacklight trap/ Hydrodessus sp. 1 [handwritten], Hendrich dt. 2020"; 3 paratypes (ZSMG) same as previous except ".../I.2016"; 4 paratypes (ZSMG) same as previous except ".../V–VI.2017/ Dytiscidae ?Hydrodessus sp. [handwritten], H.J. Bremer det. 2018"; 2 paratypes (ZSMG) same as previous except ".../ Lux – 20.9.–9.10.2007 leg. Burmeister." Each paratype with ".../PARATYPE *Miradessus beni* Miller, Benetti & Michat, 2023 [blue label with black line border]."

Miradessus plaumanni (Gschwendtner, 1935), comb. nov.

Figs 2, 6, 11, 12, 21–23, 30

Amarodytes plaumanni Gschwendtner, 1935: 152; Young 1969: 2; Trémouilles 1995: 47; Nilsson and Hájek 2022: 101.
Bidessus plaumanni: Blackwelder 1944: 76.

Type locality. Brazil, Santa Catarina State, Nova Teutônia.

Diagnosis. This species differs considerably from the other known species in the genus. The anterior area of the dorsal surface of the head is testaceous in this species (Fig. 2), but pale yellow in the other species (Figs 1, 3, 4). The ventral portion of the male median lobe in *M. plaumanni* is broad and unilobate (Fig. 11) instead of strongly bilobate as in the other species (Figs 9, 13, 15). Other differences from other species in the group include: 1) lateral pronotal margins nearly straight posteriorly (Fig. 2) instead of broadly curved (Figs 1, 3, 4), 2) the pronotum and elytron different in color pattern (Fig. 2) from the other, more uniformly-patterned species (Figs 1, 3, 4), and 3) the lateral portions of the metaventrite and metacoxa more coarsely and densely punctate (Fig. 6) than in other species (Figs 5, 7, 8). The general shape and dorsal coloration of specimens are rather different as well (Figs 1–4).

Description. ***Measurements*.** TL = 2.3 mm, GW = 1.2 mm, PW = 0.9 mm, HW = 0.7 mm, EW = 0.4 mm; TL/GW = 2.00, HW/EW = 1.7, FL/RL = 1.9.

***Habitus*.** Body shape elongate-oval, lateral outline discontinuous between pronotum and elytron, posterior apex somewhat acuminate (Fig. 2).

Coloration (Fig. 2). Head brown with a narrow orange band along posterior margin. Pronotum yellow-orange with broad dark band along posterior margin and narrow band along lateral margins. Elytron maculate, yellow-orange and black, margins of maculae strongly demarcated; with transverse yellow-orange regions anteriorly, medially and apically, not extending to suture, with broad band of black along entire length of suture, margins of basal maculae posteriorly bilobed. Ventral surfaces mostly dark orange-brown; legs, epipleuron, and ventral areas of prothorax and head lighter orange-yellow, some sutural margins darker, infuscate, mesothoracic ventrites and prosternal process strongly infuscate.

***Sculpture and structure*.** Head smooth, nearly impunctate, with small micropunctures sparsely distributed; eyes large (HW/EW = 1.7); antennae slender, unmodified. Pronotum with lateral margins moderately curved anteriorly, almost straight posteriorly; with narrow bead along entire margin; surface moderately punctate, punctures more concentrate along posterior margin; lateral pronotal plica strongly impressed, almost straight, extending more than 1/3 distance across pronotum. Elytron with lateral margin evenly and broadly rounded; surface finely and evenly punctate, with a slightly marked line of punctures with short setae extending medially from base to apex. Prosternum medially moderately broad, medially not protruberant, mediolateral surface somewhat granular; prosternal process moderately broad medially with slight tubercle, apical blade large, laterally with low rounded ridges along entire length, medially longitudinally shallowly sulcate, lateral margins almost straight to narrowly rounded apex (Fig. 6). Metaventer and metaventral wings smooth and shiny, covered with coarse, dense, and evenly impressed punctures. Metacoxa with medial portion moderately broad, metacoxal lines distinct, evenly divergent anteriorly to posterior margin of metaventer; lateral portion large, evenly covered with dense, coarse punctures; metatrochanter about 1/3 length of metafemur (Fig. 6). Abdominal ventrites finely punctured with fine setae.

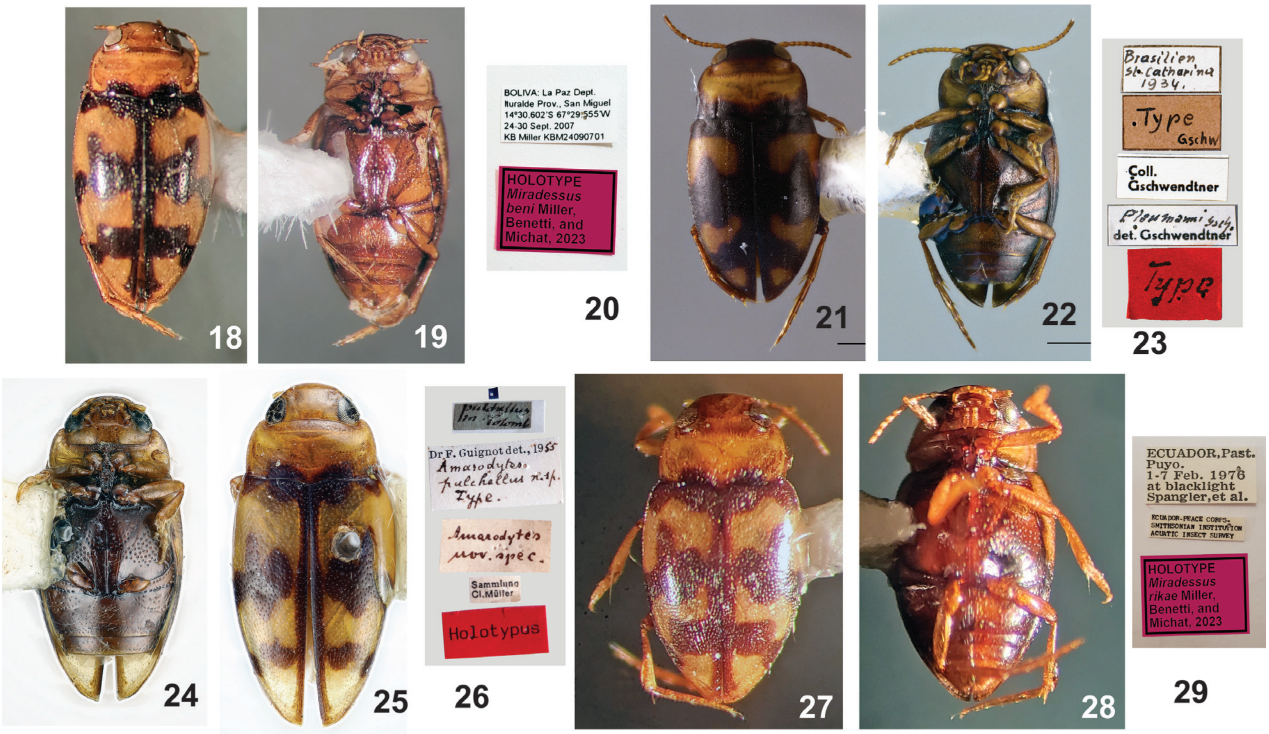
***Male genitalia*.** Median lobe in ventral aspect broad, with medial, dorsal elongate very slender, apically sharply pointed portion and ventral, broad, flattened, apically broadly rounded portion, dorsal portion slightly longer than ventral portion (Fig. 11); lateral lobe in lateral aspect robust, basal segment very broad, robust, apical segment short, robust, straight, apex broad, slightly bilobed (Fig. 12).

***Sexual dimorphism and variation*.** Males have the pro- and mesotarsomeres I–III slightly but distinctly broader than in females.

Distribution. This species is only known from the type locality, Brazil, Santa Catarina state, Nova Teutônia (Fig. 30).

Habitat. Nothing is known of the habitat of this species.

Material examined. ***Type specimens*.** Five syntype specimens are in OLML. One male specimen is labeled, “Brasilien S. Catharina 1934 [handwritten]/ Type Gschw [handwritten]/ Coll. Gschwendtner/ Plaumanni Gsch. [handwritten] det. Gschwendtner/ Type [red label].” This single specimen with the red type label is hereby designated as lectotype for the series (Figs 21–23). The other four specimens become paralectotypes and are mounted on two pins, two are labeled, “Brasilien, St Catharina 1934 [handwritten with black line border]/ Coll Gschwendtner/ Amarodytes plaumanni Gschw Det. Gschwendt. [first three



Figures 18–29. *Miradessus* species, primary type specimens and labels **18–20** *M. beni*, holotype specimen **18** dorsal habitus **19** ventral habitus **20** specimen labels **21–23** *M. plaumanni*, lectotype specimen **21** dorsal habitus **22** ventral habitus **23** specimen labels **24–26** *M. pulchellus*, holotype specimen **24** dorsal habitus **25** ventral habitus **26** specimen labels **27–29** *M. rikae*, holotype specimen **27** dorsal habitus **28** ventral habitus **29** specimen labels. **21–23** courtesy of M. Schwarz (OLML) **24–26** courtesy of M.A. Balke (ZSMG).

words handwritten]/ Paratype Gschw [brown label with black line border]/ Paratype [handwritten, red label, lower right corner torn away]; other two labeled, "Brasilien, St Catharina 1934 [handwritten label with black line border]/ Amarodytes plaumanni Gschw Det. Gschwendt. [first three words handwritten]/ plaumanni Gschw. det. Gschwendtner [two of these labels]/ Coll. Gschwendnter [three of these labels]/ Paratype Gschw [brown label with black line border]/ Paratype [handwritten, red label]." No additional specimens were examined.

Miradessus pulchellus **(Guignot, 1955), comb. nov.**

Figs 3, 7, 13, 14, 17, 24–26, 30

Amarodytes pulchellus Guignot, 1955: 273; Young 1969: 2; Trémouilles 1995: 47; Nilsson and Hájek 2022: 101.

Type locality. Colombia.

Diagnosis. See above under the very similar *M. beni* for diagnosis.

Description. ***Measurements*****.** TL = 2.6–2.8 mm, GW = 1.3–1.4 mm, PW = 1.0–1.2 mm, HW = 0.6–0.8 mm, EW = 0.3–0.4 mm, TL/GW = 1.9–2.1, HW/EW = 1.7–1.8, FL/RL = 2.2–2.3.

Habitus**.** Body shape elongate-oval, lateral outline somewhat discontinuous between pronotum and elytron, posterior apex narrowly rounded (Fig. 3).

Coloration (Fig. 3). Head yellow-orange. Pronotum yellow-orange with a narrow, rounded lobe of black broadly along each side of posterior margin. Elytron maculate, yellow-orange and black, margins of maculae strongly demarcated; with large, transverse yellow-orange regions anteriorly, medially, and apically, not extending medially to suture, with narrow band of black along entire length of suture, margins of maculae irregular and variously lobed. Ventral surfaces mostly dark orange-brown, legs, epipleuron, and ventral areas of prothorax and head lighter orange-yellow, some sutural margins darker, infuscate, mesothoracic ventrites and prosternal process strongly infuscate to nearly black.

***Sculpture and structure*.** Head shiny and smooth, nearly impunctate, with small micropunctures sparsely distributed; eyes large (HW/EW = 1.7–1.8); antennae slender, unmodified. Pronotum with lateral margins moderately curved anteriorly, shallowly curved posteriorly; with narrow bead along entire margin; surface shiny, moderately and evenly punctate; lateral pronotal plica weakly impressed, sublinear, extending about 1/3 distance across pronotum. Elytron with lateral margin evenly and broadly rounded; surface shiny, moderately, and evenly punctate. Prosternum medially moderately broad, medially not protruberant, mediolaterally somewhat granular; prosternal process moderately broad medially with slight tubercle, apical blade large, with fine setae, laterally with low rounded ridges along entire length, medially longitudinally shallowly sulcate, lateral margins slightly convergent to narrowly rounded apex (Fig. 7). Metaventrite and metaventral wings smooth and shiny, covered with shallow punctures. Metacoxa with medial portion moderately broad, metacoxal lines distinct, evenly divergent anteriorly to posterior margin of metaventrite; lateral portion large, medially and anteriorly covered with shallow punctures; metatrochanter about 1/3 length of metafemur (Fig. 7). Abdominal ventrites impunctate except II and III laterally with shallow, indistinct punctures; VI strongly concave in lateral aspect, apex pointed.

***Male genitalia*.** Median lobe in ventral aspect broad, trilobed, with medial, dorsal elongate slender, apically pointed portion and ventral, elongate apically bilobed portion, with each ramus elongate and apically truncate, medial dorsal portion nearly as long as ventral portions (Fig. 13); lateral lobe in lateral aspect robust, basal segment moderately broad, curved, apical segment elongate, slightly curved ventrad, apex rounded (Fig. 14).

Female genitalia (Fig. 17). Bursa copulatrix short; spermathecal duct extremely long, slender, somewhat coiled, broadly expanded in elongate section before receptacle; receptacle similar in size to spermatheca, intermediate duct between receptacle and spermatheca broad, short; spermatheca spherical with broad, somewhat more sclerotized expansion at opening to spermathecal duct, spermathecal spine prominent, elongate, and broad; fertilization duct slender, irregularly curved, heavily sclerotized.

***Sexual dimorphism*.** Males have the pro- and mesotarsomeres I–III slightly but distinctly broader than in females. Abdominal ventrite VI strongly concave in lateral aspect in females, but medially somewhat swollen and apically with a broadly rounded depression in males.

***Variation*.** There is some minor variation in extent of coloration of the dorsal surface, but otherwise specimens are similar.

Distribution. *Miradessus pulchellus* was described from Colombia, without greater specificity. Specimens were examined from numerous localities throughout northwestern Venezuela (states of Barinas, Guarico, Lara, Portuguesa, Tachira, Trujillo, and Zulia) (Fig. 30).

Habitat. Specimens have been collected mainly from exposed and sunny areas in lotic margins (small rivers and streams) and nearby pools. They are often numerous in these habitats.

Material examined. ***Type specimens*. *Holotype*** male (Figs 24–26) in ZSMG labeled, "pulchellus in Colomb [HW]/ Sammlung C.L. Müller/ Amarodytes nov. speci. [handwritten]/ Type [red label]/ Holotypus [red label]/ Dr. F. Guignot det., 1955 Amarodytes pulchellus n. sp. Type. [handwritten]." The holotype has the male genitalia and apical abdominal segments dissected. No other type specimens accompany the holotype in ZSMG (D.A. Balke pers. comm.).

***Other material examined*.** 134 total examined, all from Venezuela (SEMC), with the following data (SEMC accession numbers in Table 1); 7, Barinas, Rio Caramuca, E of El Corozo, 8°35.449'N, 70°19.427'W, 213 m, 14 Jul 2009, Short et al., river margins, VZ09-0714-04A; 1, Barinas, Rio Paguey at Los Rozos, 8°30.764'N, 70°27.233'W, 190 m, 24 Jan 2012, Short, Arias and Gustafson, river margins, VZ12-0214-03A; 7, Barinas, Rio Sta Barbara, E Sta Barbara, 7°50.028'N, 71°11.188'W, 177 m, 26 Jan 2012, Short, Arias and Gustafson, sandy sidepool in floodplain, VZ12-0126-01B; 6, Barinas, river nr Bum Bum, 8°18.033'N, 70°45.201'W, 216 m, 15 Jul 2009, Short et al., river margins, VZ09-0715-02A; 40, Guarico, Rio San Antonio, N Dos Caminos, 9°46.320'N, 67°21.177'W, 280 m, 8 Jan 2009, Miller and Short, side stream, VZ09-0108-02B; 6, Guarico, Rio San Antonio, N Dos Caminos, 9°46.320'N, 67°21.177'W, 280 m, 8 Jan 2009, Short, Miller, García, Camacho and Joly, river margin, VZ09-0108-02A; 4, Guarico, Rio San Antonio, N Dos Caminos, 9°46.320'N, 67°21.177'W, 280 m, 8 Jan 2009, Short, Miller and García, river margin, VZ09-0108-02A; 39, Lara, Rio Salado, W of Arenales, 10.15433333°N, 69.95763333°W, 490 m, 22 Jan 2009, Short, Camacho, Garcia, gravel stream, VZ09-0122-01X; 1, Portuguesa, Rio Are at Aparición, 9°22.900'N 69°23.153'W, 220 m, 22 Jan 2012, Short, Arias, river margins, VZ12-0122-02A; 3, Portuguesa,

Table 1. SEMC (University of Kansas) accession numbers for *Miradessus pulchellus* specimens.

Species	Accession numbers
Miradessus pulchellus	SEMC1029297, SEMC1029305, SEMC1029315, SEMC1029333, SEMC1029334, SEMC1029343, SEMC1044663, SEMC1044678, SEMC1044683, SEMC1044698, SEMC1044732, SEMC1044735, SEMC1044741, SEMC1044744, SEMC1044745, SEMC1044977, SEMC1044981, SEMC1045044, SEMC1045069, SEMC1045633, SEMC852659, SEMC852661, SEMC852662, SEMC852666, SEMC852669, SEMC852670, SEMC852674, SEMC852675, SEMC852677, SEMC852679, SEMC852680, SEMC852686, SEMC852690, SEMC852695, SEMC852702, SEMC852703, SEMC852708, SEMC852711, SEMC852712, SEMC852723, SEMC852726, SEMC852730, SEMC852745, SEMC852746, SEMC852752, SEMC852753, SEMC852762, SEMC852763, SEMC852765, SEMC852768, SEMC852783, SEMC852784, SEMC852785, SEMC852786, SEMC852789, SEMC852806, SEMC852816, SEMC852821, SEMC856675, SEMC856676, SEMC856678, SEMC856680, SEMC856681, SEMC856682, SEMC856683, SEMC856685, SEMC856686, SEMC856687, SEMC856688, SEMC856689, SEMC856692, SEMC856693, SEMC856694, SEMC856695, SEMC856696, SEMC856697, SEMC856699, SEMC856700, SEMC856701, SEMC856702, SEMC856703, SEMC856704, SEMC856707, SEMC856718, SEMC856720, SEMC856721, SEMC856724, SEMC856726, SEMC856727, SEMC856728, SEMC856734, SEMC856737, SEMC856738, SEMC856739, SEMC856740, SEMC856758, SEMC856759, SEMC856760, SEMC856761, SEMC857535, SEMC857537, SEMC857548, SEMC857550, SEMC857551, SEMC857552, SEMC864033, SEMC864036, SEMC864077, SEMC864082, SEMC875224, SEMC875225, SEMC875247, SEMC875248, SEMC875250, SEMC875254, SEMC876023, SEMC876024, SEMC876056, SEMC876058, SEMC876060, SEMC876064, SEMC876078, SEMC876080, SEMC876095, SEMC876313, SEMC879014, SEMC880761, SEMC880779, SEMC880782, SEMC880785, SEMC880799, SEMC880802, SEMC880810

Aparición by highway, 9°22.268'N, 69°23.062'W, 213 m, 22 Jan 2012, Short, Arias & Gustafson, roadside pond, VZ12-0122-01A; 10, Trachira, El Tamá National Park, 7°35.038'N, 72°10.340'W, 472 m, 16 Jul 2009, Short, Sites, García, Inciarte, Gustafson and Camacho, HG Vapor light, VZ09-0716-07A; 3, Trujillo, Rio Jiripara nr. Sabana Grande, 9°42.307'N, 70°32.570'W, 199 m, 29 Jan 2012, Short, river margins, VZ12-0129-02B; 6, Trujillo, Rio Jiripara nr Saban Grande, 9°42.307'N, 70°32.570'W, 199 m, 29 Jan 2012, Short, Arias and Gustafson, muddy pool in floodplain, VZ12-0129-02A; 1, Zulia, Perija NP, Tukuko, Rio Tukuko, 9°50.513'N, 72°48.334'W, 252 m, 5 Jul 2009, Short and Gustafson, riffle/rocks in river, VZ09-0705-01B.

Miradessus rikae **Miller, Benetti & Michat, sp. nov.**

https://zoobank.org/DE04E790-A93F-4ED8-8D63-4B6BA7653AE3
Figs 4, 8, 15, 16, 27–30

Type locality. Ecuador, Pastaza, Puyo.

Diagnosis. This species is shorter and more robust (Fig. 4) than either *M. pulchellus* or *M. beni* (Figs 1, 3) although the dorsal color pattern is similar to them (Fig. 4). The male genitalia are diagnostic. The median lobe in *M. rikae* is trilobed like other *Miradessus*, but the median portion is uniquely short, broad, and apically broadly subtruncate with the ventral portions elongate, slender and apically narrowly rounded (Fig. 15).

Description. *Measurements*. TL = 2.2 mm, GW = 1.2 mm, PW = 0.9 mm, HW = 0.6 mm, EW = 0.4 mm, TL/GW = 1.8, HW/EW = 1.5, FL/RL = 2.0.

***Habitus*.** Body shape elongate-oval, lateral outline discontinuous between pronotum and elytron, posterior apex narrowly rounded (Fig. 4).

Coloration (Fig. 4). Head yellow-orange. Pronotum yellow-orange with a narrow, rounded lobe of black broadly along each side of posterior margin. Elytron maculate, yellow-orange and black, margins of maculae strongly demarcated; with large, transverse yellow-orange regions anteriorly, medially and apically, not extending medially to suture, with narrow band of black along entire length of suture, margins of maculae irregular and variously lobed. Ventral surfaces mostly orange; legs, epipleuron, and lateral areas of prothorax and head lighter orange-yellow.

***Sculpture and structure*.** Head shiny and smooth, nearly impunctate, with small micropunctures sparsely distributed; eyes large (HW/EW = 1.5); antennae slender, unmodified. Pronotum with lateral margins moderately curved; with narrow bead along entire margin; surface shiny, moderately and evenly punctate; lateral pronotal plica distinctly impressed, sublinear, extending about 1/3 distance across pronotum. Elytron with lateral margin strongly curved anteriorly in dorsal aspect, evenly curved to apex; surface shiny, moderately and evenly punctate. Prosternum medially moderately broad, medially not protruberant, mediolaterally somewhat granular; prosternal process moderately broad medially with slight tubercle, apical blade large, laterally with prominent rounded ridges along entire length, medially longitudinally sulcate, lateral margins slightly convergent to narrowly rounded apex. Metaventrite and metaventral wings smooth and shiny, covered with shallow punctures (Fig. 8). Metacoxa with medial portion moderately broad, metacoxal lines distinct, evenly divergent anteriorly to posterior margin of metaventrite; lateral portion large, medially and anteriorly covered with small, shallow punctures; metatrochanter about 1/3 length of metafemur (Fig. 8). Abdominal ventrites nearly impunctate except II and III laterally with shallow, indistinct punctures; VI with apex pointed.

***Male genitalia*.** Median lobe in ventral aspect conspicuously trilobed, with medial portion short and broad, apically expanded and subtruncate, apicolateral angles with short setae, ventral portions elongate, slender, apically narrowly rounded (Fig. 15); lateral lobe in lateral aspect robust, basal segment somewhat broad and robust, apical segment broad basally, with deep emargination along dorsal margin, apex rounded with series of setae (Fig. 16).

***Sexual dimorphism*.** Only males are known.

***Variation*.** The two specimens exhibit slight variation in the shape and extent of maculation on the dorsal surface, but they are otherwise similar.

Etymology. This species is named *rikae* after Ms Rikelle Timpe, close friend of the first author.

Distribution. *Miradessus rikae* is known from two sites in Ecuador (Fig. 30).

Habitat. The two known specimens were collected at blacklights, so nothing is known of the specific habitat. The two collection localities are in forested regions of lowland Ecuador.

Material examined. ***Type material*. *Holotype*** male (Figs 27–29) in USNM labeled, "ECUADOR,Past. Puyo. 1-7 Feb. 1976 at blacklight Spangler,et al./ ECUADOR-PEACE CORPS- SMITHSONIAN INSTITUTION AQUATIC INSECT SURVEY/ HOLOTYPE *Miradessus rikae* Miller, Benetti & Michat, 2023 [red label with double black line border]." One paratype male in USNM labeled "ECUADOR,NAPO, Lago Agrio(5 Km N) 26 Sept.1975 at blacklight Andrea Langley/ PARATYPE *Miradessus rikae* Miller, Benetti & Michat, 2023 [blue label with black line border]." Both the holotype and paratype have the male genitalia dissected and placed in microvials mounted on the pins.

Key to species of *Miradessus*

1 Anterior surface of head brown (Fig. 2); surface of metacoxa densely covered with punctation (Fig. 6); ventral portion of male median lobe approximately unilobate (Fig. 11) .... ***Miradessus plaumanni***

– Anterior surface of head yellow-orange (Figs 1, 3, 4); surface of metacoxa with punctation sparsely distributed (Fig. 5, 7, 8); ventral portion of male median lobe apically strongly bilobate, each ramus elongate and relatively slender (Figs 9, 13, 15) **2**

2 Body short and robust (Fig. 4; TL/GW = 1.8); male median lobe with medial portion broad and apically subtruncate (Fig. 15) .... ***Miradessus rikae***

– Body more elongate and slender (Figs 1, 3; TL/GW = 1.9–2.1); male median lobe with medial portion slender and apically narrowly rounded (Figs 9, 13) **3**

3 Male median lobe with medial portion elongate, nearly as long as ventral portions, with distinct laterally divergent pencils of setae apically (Fig. 13); lateral lobes with apical segment nearly as long as basal segment, curved ventrad (Fig. 14) .... ***Miradessus pulchellus***

– Male median lobe with medial portion much shorter than ventral portions, apex with series of setae (Fig. 9); lateral lobes with apical segment only about 1/3 length of basal segment, curved dorsad (Fig. 10) .... ***Miradessus beni***

Species in the genus *Miradessus*

Miradessus beni Miller, Benetti & Michat, sp. nov. (Bolivia, Peru, Fig. 30)
Miradessus plaumanni (Gschwendtner, 1935), comb. nov. (Brazil, Fig. 30)
Miradessus pulchellus (Guignot, 1955), comb. nov. (Colombia, Venezuela, Fig. 30)
Miradessus rikae Miller, Benetti & Michat, sp. nov. (Ecuador, Fig. 30)

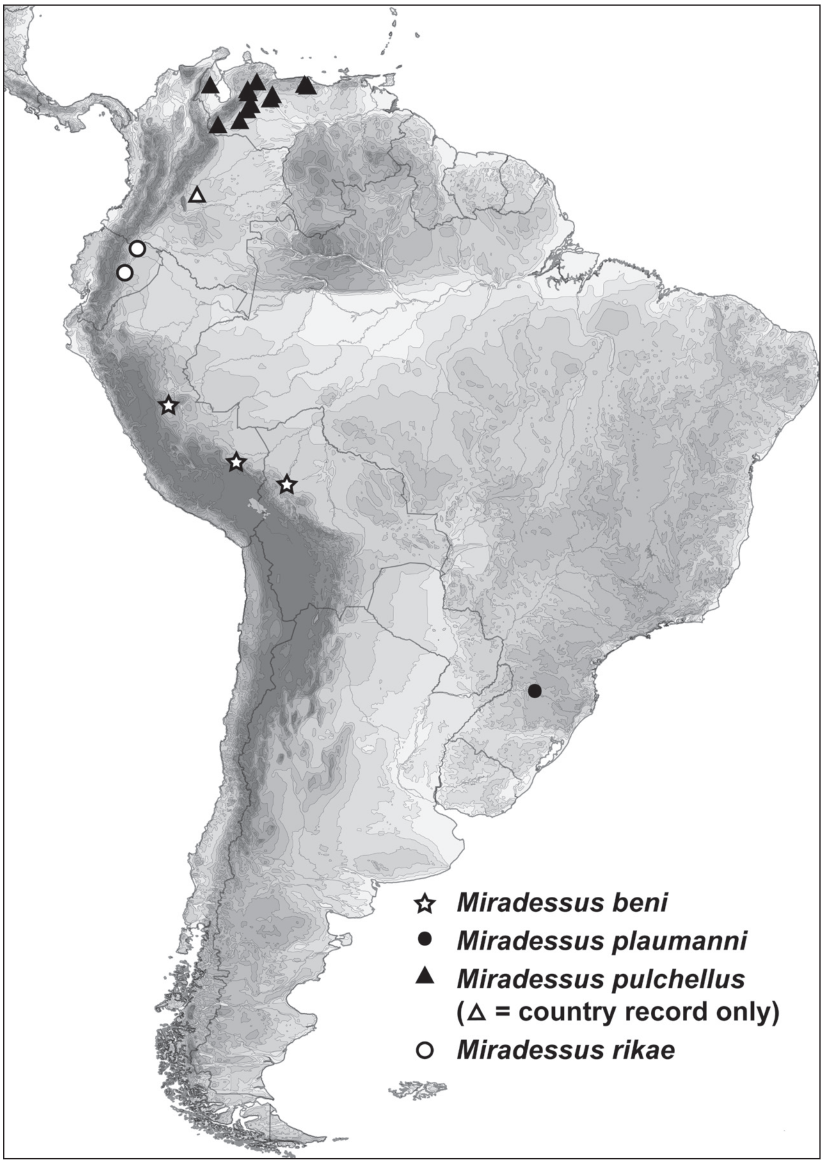


Figure 30. *Miradessus* species, distributions.

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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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Research Article

Sexual dimorphism of feeding stylets in some Thysanoptera – Phlaeothripinae, with description of two new species of *Haplothrips* from China

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Abstract

Sexual dimorphism in feeding stylets is recorded among some Phlaeothripinae that have maxillary stylets long and close together in females but wider apart in males. These atypical long feeding stylets have been found in two new species of *Haplothrips* from China, both taken on *Artemisia* from Plateau zone. Two species are described and illustrated: *H. helanshanensis* **sp. nov.** from Helanshan Mountain and *H. longistylus* **sp. nov.** from Tibet. There is no evidence of any association between feeding behaviors and feeding stylet orientation.

Key words: Haplothripini, *Haplothrips helanshanensis*, *H. longistylus*, maxillary stylets

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Introduction

Sexual dimorphism is widespread throughout the insect order Thysanoptera, such that it is likely to have been a plesiotypic condition inherited from the ancestors of the group. It is recorded in species from Cretaceous amber from Burma (Ulitzka 2022) and occurs in both suborders of living thrips, including members of families that are generally considered to be the least derived, such as Merothripidae and Aeolothripidae. Sexual dimorphism can be so marked that Trybom (1912) erected *Mitothrips* for two males of a species that was subsequently recognized as a species of *Franklinothrips* (Back 1912). In this genus, females are ant-like in appearance and behavior, whereas males are slender, as in most Aeolothripidae. Sexual dimorphism is generally associated with some difference in the behavior between the sexes, particularly in mating behavior (Mound 2005). In contrast, the purpose of this article is to discuss a much less obvious example of sexual dimorphism involving differences between the sexes in the position of the feeding stylets.

Sexual dimorphism in feeding stylet positions

This curious example of sexual dimorphism came to the attention of the authors in describing below two new species of *Haplothrips* from China. In these species the feeding stylets of females are close together medially on the head, whereas those of males are positioned considerably further apart (Figs 1–8). At present there are no observations to help suggest why these males and females should have their feeding stylets positioned differently on the head, although an obvious correlation might be the precise feeding site, such as in flowers versus on leaves.

Bhatti (1979) was the first person to record a thrips species as having the maxillary stylets of females closer together than those of conspecific males. He recorded that the separation between the maxillary stylets of females ranged from 13–19 μm in contrast to 30–40 μm in males in *Ananthakrishnana euphorbiae* (Priesner). Subsequently, Bhatti (1997) indicated a similar situation in another species of Haplothripini that he described from India. In this species, *Apterygothrips banyan* Bhatti, the distance between the maxillary stylets of females he gave as 30–38 μm, in contrast to 37–39 μm in males. The only other published reference to this type of sexual dimorphism among thrips is by Okajima and Urushihara (1995). These authors illustrated from Thailand the previously unknown male of *Stephanothrips occidentalis* Hood & Williams, a species that is distantly related within Phlaeothripidae to the two Haplothripini discussed above. Females of *S. occidentalis* have been found worldwide and have the stylet separation about 25 μm, but the males from Thailand have the stylets 34–39 μm apart.

Whilst studying the two new *Haplothrips* species described below, further instances of maxillary stylet position varying between sexes were observed in two species of this genus available in the Australian National Insect Collection. The first, *Haplothrips stofbergi* Faure, was described from South Africa on various grasses (Faure 1958), but the lengthy description did not include any comparison of stylet position between the sexes. However, 10 females and 10 males of this species have been studied in ANIC that were taken in quarantine in Australia from grasses collected in South Africa. The species can be distinguished from other *Haplothrips* species by having only three sense cones on the fourth antennal segment. Females of these specimens have the maxillary stylets about 25 μm apart, but males have this separation about 40 μm (Figs 1, 2). The second species, *Haplothrips* (*Trybomiella*) *timori* Mound & Minaei was described from Darwin in northern Australia based on a single male (Mound and Minaei 2007). Subsequently, one female and three males were collected on Badu Island in the Torres Strait, two females were taken on Timor Leste at Dili, and two females with one male have been seen from near Kuala Lumpur, Malaysia. The species is possibly associated with the widespread weed, *Euphorbia hirta*. In females the maxillary stylet separation is about 25 μm, but in males it is nearer 50 μm (Figs 3, 4).

Material and method

For study, thrips specimens are mounted onto microscope slides usually in Canada balsam (ThripsWiki 2023). The body contents of each specimen are generally cleared using a weak solution of sodium hydroxide in order that surface sculpture can be seen clearly. This process requires considerable patience because the stylets become disrupted out of their natural position if the hydroxide solution is too strong or if the specimens are heated. The descriptions and photomicrograph images are produced from slide-mounted specimens with a Leica DM2500 using DIC illumination and processed with Automontage and Photoshop software. The abbreviations used for the pronotal setae are as follows: am – anteromarginal, aa – anteroangular, ml – midlateral, epim – epimeral, pa – posteroangular. The unit of measurements in this study is micrometre. All specimens studied here are deposited in the School of Bioscience and Engineering, Shaanxi University of Technology (**SNUT**), Hanzhong, China, and in Australian National Insect Collection (**ANIC, CSIRO**), Canberra, Australia.

Taxonomy

Haplothrips helanshanensis **Dang & Mound, sp. nov.**
https://zoobank.org/ED751D73-E3C4-477B-8324-9086CD996E81
Figs 5, 6, 9, 11, 13, 15, 17, 18

Materials examined. ***Holotype*****,** ♀ (SNUT), China, Inner Mongolia, Helan Mountain National Nature Reserve, on *Artemisia* sp., 04.viii.2010, L.H. Dang. ***Paratypes***, 2♀2♂ (SNUT), with the same data as holotype; 1♀1♂ (ANIC), with the same data as holotype.

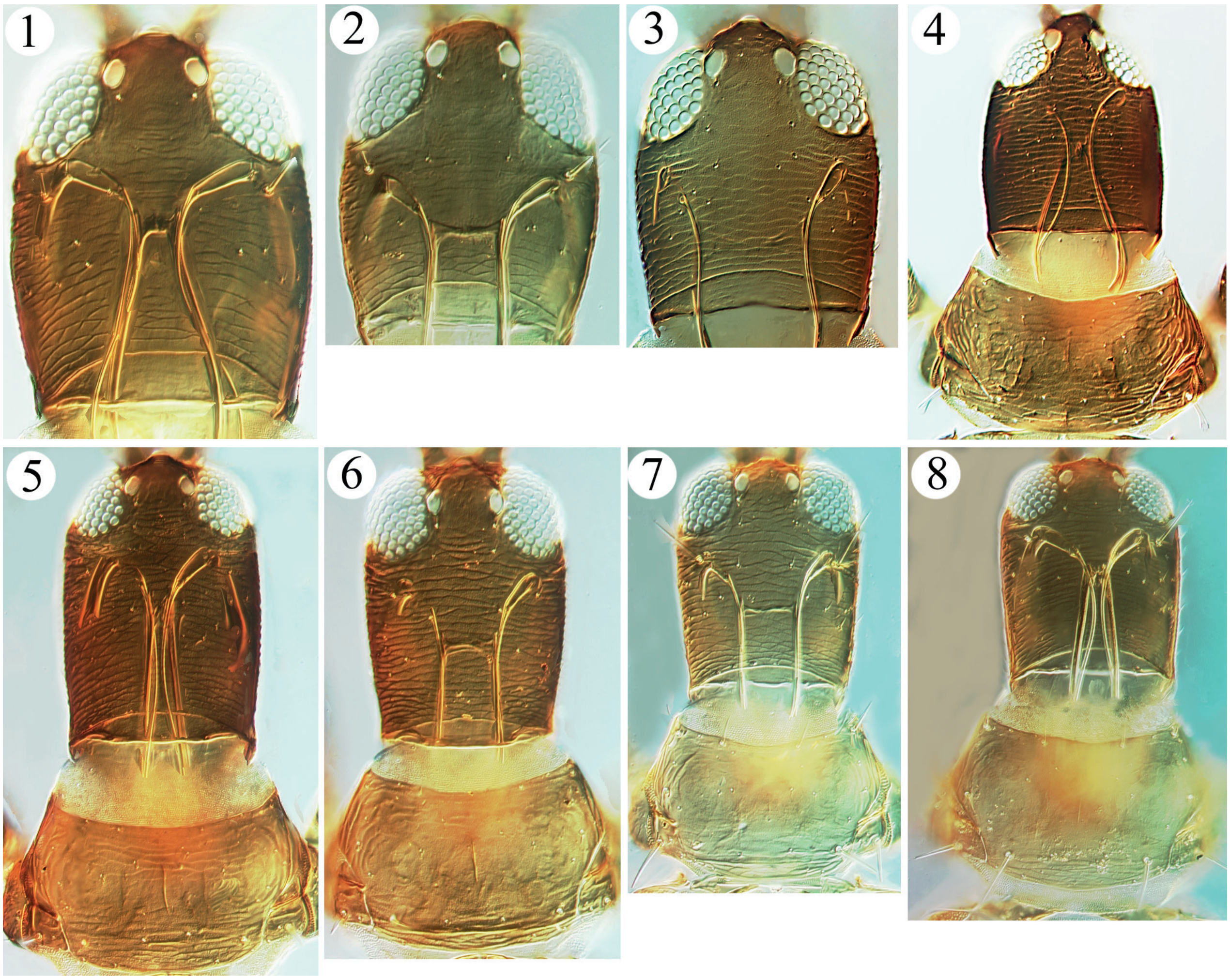
Description. Female macroptera. Body brown. All legs brown, with brownish yellow on fore tarsi and extreme apices of fore tibiae. Antennal segments uniformly brown, with III yellowish brown (Fig. 9). Wings and body setae hyaline.

Head**.** Head elongate, about 1.5 times as long as wide (Fig. 5); dorsal surface sculptured with transverse striae; eyes small, about one-quarter of head length, postocular setae minute, similar to other cheek setae (Fig. 5); cheeks parallel. Mouth-cone rounded, maxillary stylets long, retracted into postocular setae, close together, the narrowest separation about 5 μm, bridge stout and short, 15 μm wide (Fig. 5). Antennae 8-segmented, sense cones on segments III–IV small, about half breadth of this segment, III with 1+1, IV with 2+2 (Fig. 9).

Thorax**.** Pronotum weakly sculptured, notopleural sutures complete (Fig. 5); am, aa and ml minute, epim and pa setae developed, pointed at apex, epim the longest (Fig. 5); mesopresternum eroded medially, reduced to two small, lateral, triangular plates (Fig. 11); metanotum weakly reticulate at middle and smooth laterally (Fig. 13), metathoracic sternopleural sutures absent. Fore tarsi with a tiny tooth (Fig. 17).

Abdomen**.** Pelta acutely triangular and weakly reticulate, with a pair of campaniform sensilla (Fig. 13); abdominal tergites II–VII with two pairs of wing-retaining setae; S1–S3 on tergite IX short, much shorter than tube, pointed at apex (Fig. 15); tube about 0.4 times as long as head, 1.8 times as long as basal width, anal setae shorter than tube.

Measurements (holotype female in μm). Body length 2050. Head length 255, width just behind eyes 175; eye length 60, postocular setae length 10; the narrowest separation between maxillary stylets 5, width of bridge 15. Antenna length 370, segments I–VIII length (widest) 35(30), 50(32), 60(32), 60(30), 60(30), 45(25), 40(20) and 30(12), sensoria on segment III length 15. Fore wing



Figures 1–8. *Haplothrips* spp. Heads of *H. stofbergi* (**1, 2**) **1** female **2** male; *H. timori* (**3, 4**) **3** head, male **4** head and pronotum, female; head and pronotum of *H. helanshanensis* sp. nov. (**5, 6**) **5** female **6** male; head and pronotum of *H. longistylus* sp. nov. (**7, 8**) **7** male **8** female.

length 88. Pronotum length125, width 235, length of pronotal setae, am 5, aa 5, ml 5, epim 23, pa 15. Pelta length 95, width 85; tergite IX posteromarginal setae S1–S3, 75, 65, 45; tube length 105, basal width 60, apical width 35; anal setae length 85.

Male macroptera. Similar to female; but maxillary stylets wider apart, about one-quarter of head width (Fig. 6); postocular setae slightly longer than other cheek setae; fore tarsal tooth bigger than in females (Fig. 18); abdominal tergite IX setae S2 short and stout, sternites without a pore plate.

Measurements (paratype male in μm). Body length 1820. Head length 225, width just behind eyes 160; eye length 70, postocular setae length 15; the narrowest separation between maxillary stylets 40, width of bridge 40. Antenna length 375, segments I–VIII length (widest) 30(30), 35(25), 55(22), 55(30), 55(25), 45(20), 45(20) and 30(12), sensoria on segment III length 15. Pronotum length 115, width 220, length of pronotal setae, am 10, aa 10, ml 10, epim 25, pa 20. Fore wing 80. Pelta length 80, width 75; tergite IX posteromarginal setae S1–S3, 85, 35, 80; tube length 130, basal width 55, apical width 35; anal setae length 85.

Etymology. This species name is based on its collecting location.

Comments. This new species is similar to the Australian species, *H. salicorniae* Bournier, in having the postocular setae minute, mesopresternum eroded medially, antennal segments III–IV with two and four sense cones respectively, S1 on tergite IX much shorter than tube, fore tarsal tooth tiny in female, metanotum sculptured with reticulation, and the colour pattern of legs and antennae. In contrast, because the fore wings of *H. salicorniae* have no duplicated cilia, that species is placed in the subgenus *Trybomiella*. The new species from China has several duplicated cilia on fore wings and is placed in the subgenus *Haplothrips*. In addition, in *H. salicorniae* the pronotum has three pairs of well-developed setae (aa, epim and pa) that are expanded at the apex, whereas in *H. helanshanensis* sp. nov. these setae are pointed at the apex and the aa and pa are minute (Figs 5, 6). In both species the females have similarly shaped heads and maxillary stylets, but unfortunately, males of *H. salicorniae* remain unknown (Mound and Minaei 2007).

Haplothrips longistylus Dang & Mound, sp. nov.

https://zoobank.org/983CCC68-5539-4486-BC33-DAB3AE3AEE88
Figs 7, 8, 10, 12, 14, 16, 19, 20

Materials examined. ***Holotype***, ♀ (SNUT), China, Tibet, Lasa city, Nanshan Park, on *Artemisia gmelinii*, 03.viii.2019, L.H. Dang. ***Paratypes***, 1♀1♂ (SNUT), with the same data as holotype; 1♀1♂ (ANIC), with the same data as holotype.

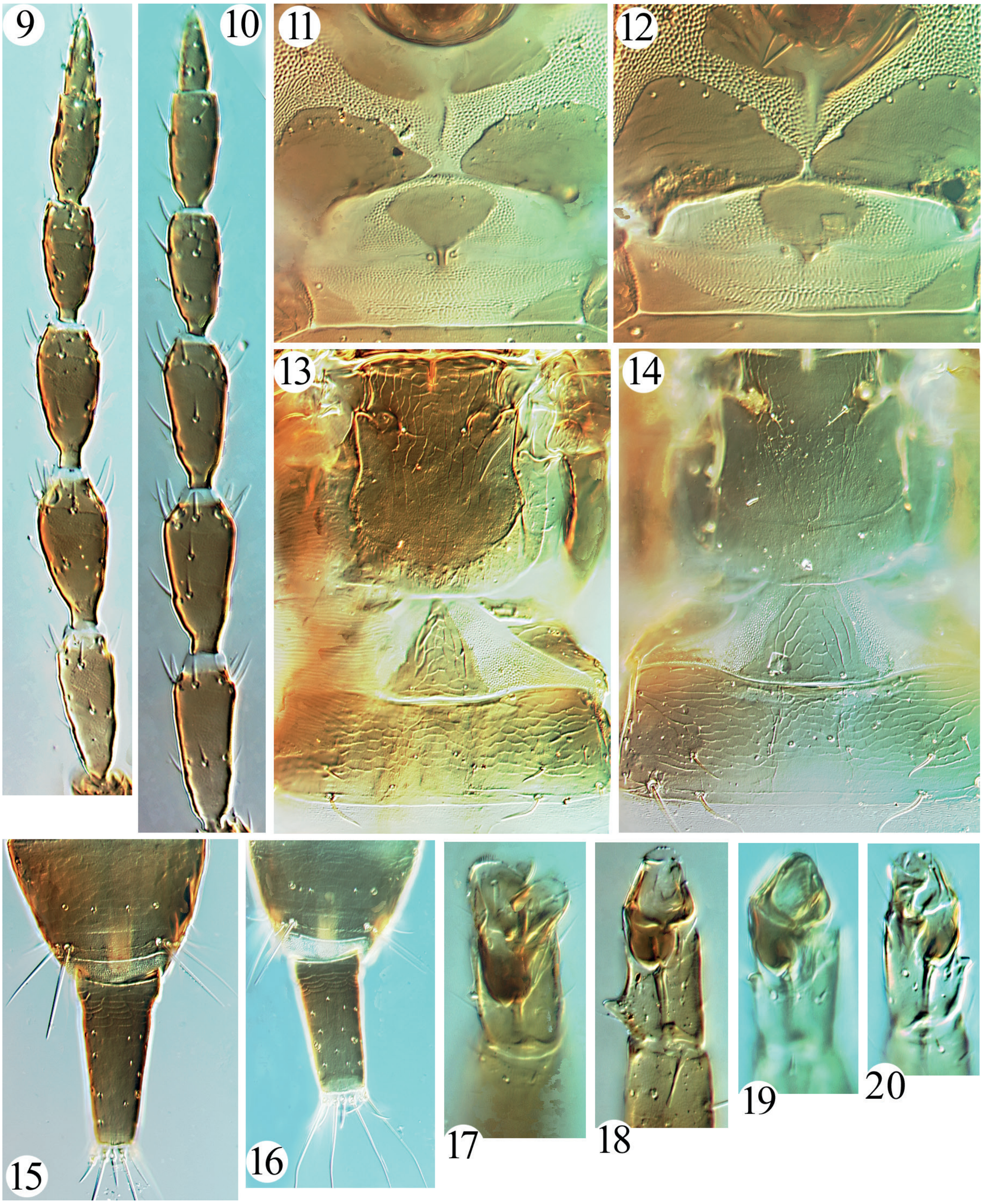
Description. Female macroptera. Body brown. All legs brown with fore tarsi and extreme apices of fore tibiae brownish yellow. Antennal segments uniformly brown, III brown with pale at base (Fig. 10). Wings hyaline with brown around subbasal setae, body setae hyaline.

***Head*.** Head elongate, about 1.3 times as long as wide (Fig. 8); dorsal surface weakly sculptured with transverse striae; eyes small, about one-quarter of head length, postocular setae well developed, about as long as eyes (Fig. 8); cheeks almost parallel. Mouth-cone rounded, maxillary stylets long, retracted to postocular setae, close together, the narrowest separation 5 μm, bridge stout and short, 15 μm wide apart (Fig. 8). Antennal 8-segmented, sense cones on segments III–IV small, about half as broad as its segment, III with 1+1, VI with 2+2 (Fig. 10).

***Thorax*.** Pronotum almost smooth, notopleural sutures complete (Fig. 8); five pairs of major setae well developed, pointed at apex, pa the longest (Fig. 8); mesopresternum strongly eroded medially, reduced to two small, lateral, triangular plates (Fig. 12); metanotum very weakly reticulate at middle and smooth laterally, metathoracic sternopleural sutures absent. Fore tarsi with a tiny tooth (Fig. 19).

***Abdomen*.** Pelta triangular and weakly reticulate, with a pair of campaniform sensilla (Fig. 14); abdominal tergites II–VII with two pairs of wing-retaining setae; S1–S3 on tergite IX short, much shorter than tube, pointed at apex (Fig. 16); tube about 0.6 times as long as head, 2.0 times as long as basal width, anal setae shorter than tube.

Measurements (holotype female in μm). Body length 2450. Head length 215, width just behind eyes 160; eye length 65, postocular setae length 65; the



Figures 9–20. *Haplothrips* spp. antennae (**9, 10**) **9** *H. helanshanensis* sp. nov. **10** *H. longistylus* sp. nov.; prosternum and mesopresternum (**11, 12**) **11** *H. helanshanensis* sp. nov. **12** *H. longistylus* sp. nov.; metanotum and tergites I–II (**13, 14**) **13** *H. helanshanensis* sp. nov. **14** *H. longistylus* sp. nov.; tergites IX–X (**15, 16**) **15** *H. helanshanensis* sp. nov. **16** *H. longistylus* sp. nov.; fore tarsal tooth of *H. helanshanensis* sp. nov. (**17, 18**) **17** female **18** male; fore tarsal tooth of *H. longistylus* sp. nov. (**19, 20**) **19** female **20** male.

narrowest separation between maxillary stylets 10, width of bridge 15. Antenna length 410, segments I–VIII length (widest) 35(35), 50(30), 62(30), 60(30), 60(30), 50(25), 45(20) and 30(12), sensoria on segment III length 15. Fore wing length 1070. Pronotum length160, width 280, length of pronotal setae, am 35, aa 35, ml 30, epim 55, pa 60. Pelta length 100, basal width 130; tergite IX posteromarginal setae S1–S3, 75, 75, 80; tube length 135, basal width 65, apical width 40; anal setae length 100.

Male macroptera. Similar to female; but smaller, maxillary stylets wide apart, about one-third of head width (Fig. 7); fore tarsal tooth small (Fig. 20); abdominal tergite IX setae S2 short and stout, sternites without a pore plate. One larger male with body length 2050, its separation between maxillary stylets 25 μm, about one-seventh of head width.

Measurements (paratype male in μm). Body length 1720. Head length 200, width just behind eyes 155; eye length 65, postocular setae length 55; the narrowest separation between maxillary stylets 50, width of bridge 50. Antenna length 360, segments I–VIII length (widest) 35(30), 40(25), 55(22), 55(25), 55(25), 45(20), 45(20) and 30(12), sensoria on segment III length 15. Pronotum length 120, width 200, length of pronotal setae, am 25, aa 30, ml 30, epim 50, pa 50. Fore wing length 760. Pelta length 75, width 80; tergite IX posteromarginal setae S1–S3, 75, 35, 95; tube length 125, basal width 55, apical width 45; anal setae length 95.

Etymology. This species name refers to the elongate maxillary stylets.

Comments. The new species is similar to *H. pharao* Priesner from Egypt in having major setae pointed at apices, postocular setae slightly shorter than eyes, the mesopresternum divided into two lateral triangles, and the fore tarsal tooth tiny in females. However, it can be differentiated in colour pattern of antennae and legs and length of S1 on tergite IX. In *H. longistylus* sp. nov., all legs are brown except fore tibiae with extreme apices and fore tarsi brownish yellow, antennal segments are uniformly brown, but III pale at base (Fig. 10) and S1 on tergite IX is much shorter than tube (Fig. 16) (in *H. pharao*, at least all tarsi yellow, and antennal segments III–IV uniformly yellow, IV–VI yellow with brown at apex, and S1 on tergite IX about as long as tube). The new species is also similar to *H. stofbergi* from Africa in the shape of the major setae, mesopresternum, fore tarsal tooth, S1 on tergite IX, and pelta, but it differs in having metathoracic sternopleural sutures absent and antennal segments III–IV with two and four sense cones, respectively (Fig. 10), whereas in *H. stofbergi* the metathoracic sternopleural sutures are very long and antennal segments III–IV have one and three sense cones. Only one female of *H. pharao* is available in ANIC, and maxillary stylets of the male were not mentioned in the original description (Priesner 1930), but both sexes of *H. stofbergi* are checked here, and sexual dimorphism in the maxillary stylets confirmed (Figs 1, 2).

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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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Research Article

Descriptions of two new stick insect species of *Cnipsomorpha* Hennemann, Conle, Zhang & Liu (Phasmatodea) from China based on integrative taxonomy

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Abstract

Accurate taxonomical identification is an extremely important basis for stick insect research, including evolutionary biology but also applied biology such as pest control. In addition, genetic methods are a valuable identification auxiliary technology at present. Therefore, this paper used morphological and molecular data to investigate five stick insect specimens from the genus *Cnipsomorpha* in Yunnan, successfully identifying two new species: *Cnipsomorpha yunnanensis* Xu, Jiang & Yang, **sp. nov.** and *C. yuxiensis* Xu, Jiang & Yang, **sp. nov.** A phylogenetic tree was constructed through their *28S* and *COI* genes in order to infer the phylogenetic position of the two new species. Photographs of the new species and a key to all known *Cnipsomorpha* species are provided.

Key words: *28S*, Biodiversity, *COI*, morphology, taxonomy, stick insects

Introduction

Cnipsomorpha Hennemann, Conle, Zhang & Liu, 2008 (Phasmatodea: Clitumninae: Medaurini) represents a genus of stick insects that was published based on specimens from China, also including one undescribed species from Vietnam (Ho 2021). Fifteen species have been published to date: *C. apteris* (Liu & Cai, 1992), *C. bii* Ho, 2017, *C. colorantis* (Chen & He, 1996), *C. daliensis* Ho, 2017, *C. erinacea* Hennemann, Conle, Zhang & Liu, 2008, *C. inflexa* Ho, 2021, *C. jinpingensis* Ho, 2021, *C. kunmingensis* Chen & Pan, 2009, *C. nigromaculata* Ho, 2021, *C. nigrospina* Ho, 2021, *C. polyspina* Ho, 2021, *C. serratitibia* Ho, 2021, *C. trituberculata* Ho, 2021, and *C. viridis* Ho, 2021, all distributed in Yunnan (Liu and Cai 1992; Chen and He 1996; Hennemann et al. 2008; Chen and Pan 2009; Ho 2017, 2021) and *C. maoershanensis* Ho, 2017 found only in Guangxi (Ho 2017).

Reconstructing the phylogenetic relationships of phasmatodeans has been based mostly on molecular data in many previous studies (Sandoval et al. 1998; Wheeler et al. 2001; Whiting et al. 2003; Terry and Whiting 2005; Kômoto et al. 2010; Bradler et al. 2014; Simon et al. 2019) which facilitated the revisions that were mostly based on traditional morphological classification (Glaw et al. 2019; Madeira-Ott et al. 2020; Bank et al. 2021a; Cumming et al. 2021). *Cnipsomorpha* is considered to pertain to the clade Clitumninae sensu Hennemann et al. (2008), that was first corroborated by molecular results in Simon et al (2019) and subsequently recovered by Tihelka et al. (2020). Therefore, more research is needed.

In this study, we found two new species *Cnipsomorpha yunnanensis* Xu, Jiang & Yang, sp. nov. and *C. yuxiensis* Xu, Jiang & Yang, sp. nov., and carried out the molecular study of *Cnipsomorpha* for the first time. We demonstrate that *Cnipsomorpha* together with *Parapachymorpha* and *Spinoparapachymorpha* form the sister group to Pharnaciini.

Materials and methods

Sample collection and treatment

A total of four specimens was collected in China in 2015 by net-sweeping of ferns, fixed in 75% ethanol, and brought back to the laboratory for storage in a -80 °C refrigerator. Images were taken using a Canon EOS 60D suite (Canon Inc., Tokyo, Japan). Morphological terms follow Bragg (1997) and Ho (2021), and relevant literature information was obtained from the Phasmida Species File database (Brock et al. 2021). The type specimens were deposited at the College of Forestry, Guizhou University, China.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from femoral tissue using the Ezup Column Animal Genomic DNA Purification Kit (Sangon Biological Engineering Co., LTD, Shanghai, China; hereafter, SG). First, reagents were prepared according to the instructions. 0.2 mg of muscle tissue was taken, placed in a 1.5 mL centrifuge tube, and 80 μl ACL buffer (all buffers from Ezup Column Animal Genomic DNA Purification Kit) were added, and the tube placed in a bath at 56 °C for 0.5 h. 100 μl ACL buffer and 20 μl Proteinase K were added and again placed in the water bath at 56 °C for 1 h; the subsequent operations were carried out according to the instructions of the kit.

The *28S* and *COI* target fragments were amplified and sequenced using the PCR primers listed in Table 1. Reagents were added for the PCR reaction test with reference to 2X SanTaq PCR Mix (SG): 15 μL 2X SanTaq PCR Mix, 1 μL forward primer, 1 μL reverse primer, 2 μL template, and 11 μL sterilized dd H_2O. PCR employed the following temperature cycles through a KQ60 thermal cycler (Hangzhou Lattice Scientific Instrument Co., LTD, Zhejiang, China): 5 min

Table 1. Primers used for PCR and sequencing.

Gene	Primers*	Sequences(5'–3')	References
COI	C1-J-2195	TTGATTTTTTGGTCATCCAGAAGT	Simon et al. 1994
COI	TL2-N-3014	TCCAATGCACTAATCTGCCATATTA	Simon et al. 1994
28S	*28S* Road 1a	CCCSCGTAAYTTAGGCATAT	Terry and Whiting 2005
28S	*28S* Road 4b	CCTTGGTCCGTGTTTCAAGAC	Terry and Whiting 2005

* Their annealing temperature is 45 °C.

Table 2. Sequence information and GenBank accession numbers. New species are in bold.

Subfamily	Tribe	Species	*28S*	*COI*
Clitumninae	Medaurini	***Cnipsomorpha yunnanensis***	**MZ486038**	**MZ435970**
Clitumninae	Medaurini	***Cnipsomorpha yuxiensis***	**MZ486045**	**MZ435977**
Clitumninae	Clitumnini	*Ramulus thaii*	FJ474166.1	FJ474322.1
Clitumninae	Clitumnini	*Ramulus artemis*	KJ024395.1	
Clitumninae	Clitumnini	*Ramulus nematodes*	MN925497.1	MN925741.1
Clitumninae	Clitumnini	*Cuniculina cuniculus*	MK291890.1	
Clitumninae	Clitumnini	*Lobofemora scheirei*	MN925432.1	
Clitumninae	Clitumnini	*Rhamphophasma spinicorne*	MK291839.1	
Clitumninae	Medaurini	*Medauroidea extradentata*	KT426670.1	KT426637.1
Clitumninae	Medaurini	*Medauromorpha foedata*	MN925435.1	MN925689.1
Clitumninae	Medaurini	*Parapachymorpha spinigera*	MK291850.1	
Clitumninae	Medaurini	*Spinoparapachymorpha spinosa*	MK291851.1	
Clitumninae	Pharnaciini	*Pharnacia ponderosa*	MN925409.1	MN925665.1
Clitumninae	Pharnaciini	*Phobaeticus serratipes*	MK291836.1	
Clitumninae	Pharnaciini	*Phobaeticus foliatus*	MN925378.1	MN925636.1
Clitumninae	Pharnaciini	*Phobaeticus heusii*	AY125324.1	
Clitumninae	Pharnaciini	*Phobaeticus kirbyi*		KT426649.1
Clitumninae	Pharnaciini	*Tirachoidea westwoodii*	MK291837.1	
Timematinae	Timematini	*Timema californicum*	KM853347.1	AF410061.1

of initial denaturation at 94 °C, followed by 35 cycles of 30 s of denaturation at 90 °C, 30 s of alignment at 45 °C, and 30 s of extension at 72 °C, finishing with 10 min of 72 °C and stored at 4 °C. High-quality PCR products (no clean-up) were bidirectional sequenced by Sanger sequencing technology of SG.

Phylogenetic reconstruction

The obtained sequences from SG were viewed, checked, and edited by BIOEDIT v. 7.0.9.0 (Alzohairy 2011), then compared and edited through DNAMAN v. 6.0.3.99 to obtain high-quality sequences (Wang 2016). A BLAST search was used to compare high-quality sequences with the NCBI database (Li et al. 2014). The sequences were uploaded to GenBank and the accession numbers are provided in Table 2. Then, all available sequences of Clitumninae were downloaded from the NCBI database (Table 2; Law and Crespi 2002; Whiting et al. 2003; Buckley et al. 2009; Djernæs et al. 2011; Bradler et al. 2014, 2015; Song et al. 2015; Robertson et al. 2018; Bank et al. 2021b).

All sequences were aligned by MAFFT v. 7.149 (Katoh and Standley 2014), then trimmed with the GBLOCKS v. 0.91b (Talavera and Castresana 2007). The best-fit substitution model of reconstructed *28S* and *COI* trees was selected using the AIC criterion with JMODELTEST v. 2.1.7 (Darriba et al. 2012). According to the divergence between *Timema* and the Euphasmatodea that occurred more than 120 Myr ago (Simon et al. 2019), *Timema* was selected as outgroup. Based on the best-fit substitution model with 1000 bootstrap replicates using MEGA v. 7.0.26, maximum likelihood (ML) trees for each gene were reconstructed separately (Kumar et al. 2016) and visualized in FIGTREE v. 1.4.3 (https://github.com/rambaut/figtree).

Results

Taxonomic account

Clitumninae Brunner von Wattenwyl, 1893
Medaurini Hennemann & Conle, 2008

***Cnipsomorpha* Hennemann, Conle, Zhang & Liu, 2008**

Type species. *Cnipsomorpha erinacea* Hennemann, Conle, Zhang & Liu, 2008.

Distribution. Guangxi, Yunnan, China.

Note. The two new species small to medium in size. Spinose. Apterous. The head is round or rectangular, with spines. Occiput raised, with spines. The antennae are short, shorter than the femoral segment of the forelegs, with distinct segments. Thoracic spines or tubercles. Pronotum is nearly trapezoidal. Meso- and metapleurae with small spines. Abdomen cylindrical, with triangular extension posterolaterally of abdominal terga II–VII in females, inconspicuous in males. In females, the sternum VII has an distinct praeopercular organ, the posterior edge of the anal segment is slightly concave, and the male's 10th abdominal segment is dorsally divided into two semi-tergites. This combination of characteristics distinguishes it from species of other genera and determines that the new taxa belong to *Cnipsomorpha*.

***Cnipsomorpha yunnanensis* sp. nov.**
https://zoobank.org/DD51B013-D5F5-43CB-8760-14D47F5C4FCB
Fig. 1

Type material. ***Holotype*.** One Female. Fenshuiling Nature Reserve, Jinping County, China, alt. 2100 m, 18–19 May 2015, leg. Bin Yan. Specimen code: YNJP150517001. Specimen used for DNA extraction.

Differential diagnosis. *Cnipsomorpha yunnanensis* sp. nov. is similar to *Cnipsomorpha erinacea* Hennemann, Conle, Zhang & Liu, 2008 (Hennemann et al. 2008). In *C. yunnanensis* sp. nov., the vertex of the head has only two pairs of spines, where *C. erinacea* has more than two. In *C. yunnanensis* sp. nov., the mesonotum is not convex or swollen, and is armed with two pairs of spines and three pairs of tubercles, whereas in *C. erinacea* the mesonotum is convex, swollen, and armed with twelve prominent spines. Finally, in *C. yunnanensis* sp. nov., the middle parts of the terga II–IV are not raised and are armed with a single pair of spines in the posterior region while terga I–IX are expanded posterolaterally and are almost of the same length as the abdomen width; those of *C. erinacea* are raised and armed with several prominent spines, and shorter than the abdomen width.

Cnipsomorpha yunnanensis sp. nov. also similar to *C. jinpingensis* Ho, 2021. In *C. yunnanensis* sp. nov., the lateral margins of the pronotum possess a spine medially, and the posterior margin of the anal segment exhibit a large trapezoidal concave edge. But in *C. jinpingensis*, the lateral margins of pronotum are without a spine medially, and the posterior margin of anal segment bear two to three small emarginations (Ho 2021).

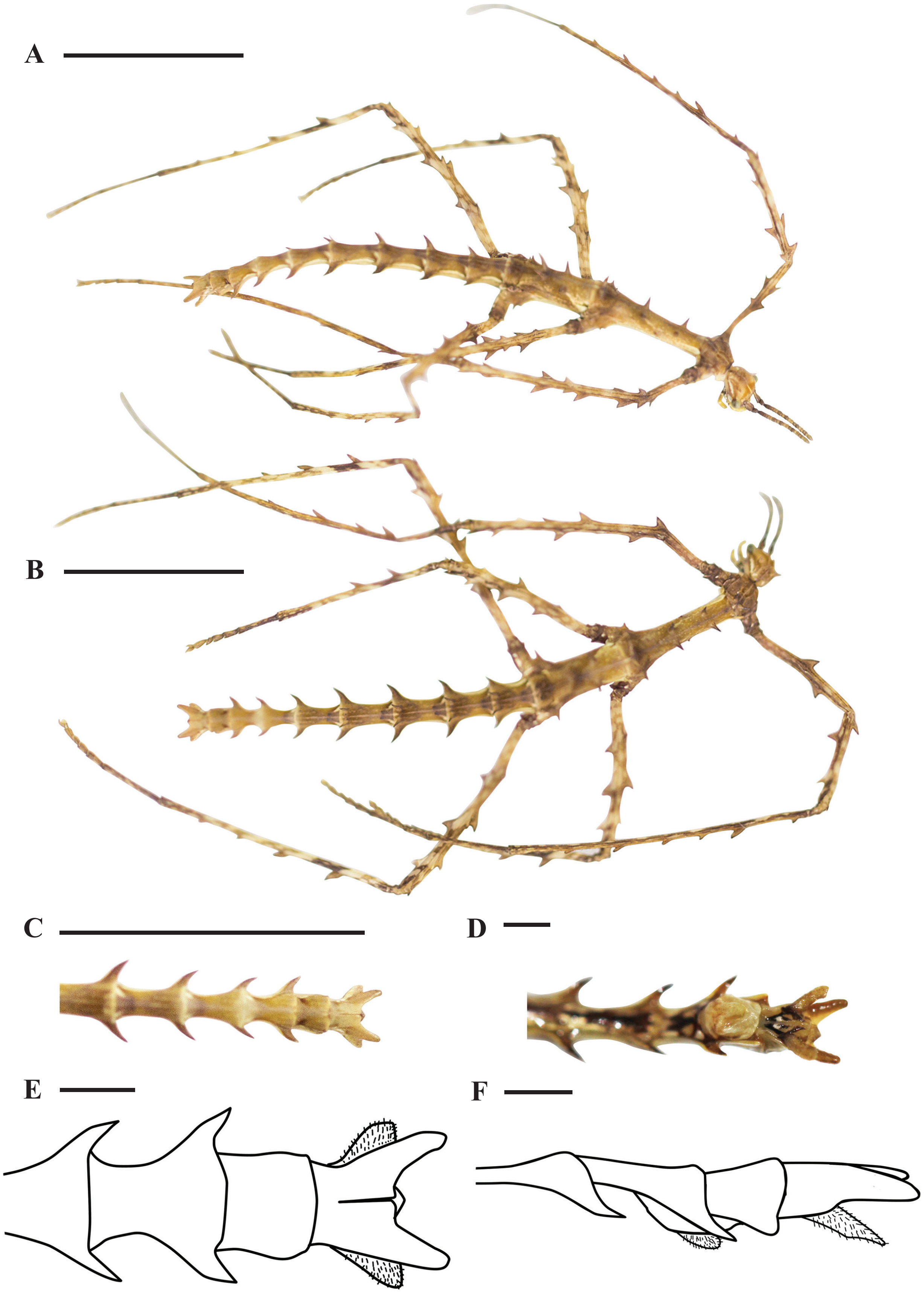

Figure 1. Features of *Cnipsomorpha yunnanensis* sp. nov. Female **A** body, lateral view **B** body, dorsal view **C** hind part of abdomen, dorsal view **D** hind part of abdomen, ventral view **E** end of abdomen, dorsal view **F** end of abdomen, lateral view. Scale bars: 10 mm (**A–C**); 1 mm (**D–F**).

Description. Female (Fig. 1). Apterous. Body slender, with some sparse, small granules. General color of specimen in alcohol is yellow. Expanded terga I–IX are curved and spine-like. Legs with serrations.

Head oval, with irregular granules, without distinct tubercles. Compound eyes are spherical and protruding. There is one pair of spines on the vertex of the head, the apex leans forward. Occiput significantly reduced, with one pair of spines. Antennae filamentous, distinctly segmented, 18 segments, bristly, shorter than profemora, scapus oval, strongly compressed basally, longer than the third segment, third segment longer than the pedicellus, and apical segment roughly the same as the third segment.

Thorax slender, midline distinct, with sparse granules and spines. Pronotum shorter than head, midline distinct, and slight bulge on both sides of the midline, one pair of spines in the posterior region, interspersed with one or two spine-like tubercles. Mesonotum longer than the head and pronotum combined, with one pair of small tubercles in the anterior region, two pairs of spines in the posterior region, one pair of tubercles near both sides in the middle region, and one pair of spine-like tubercles in the posterior region. Metanotum shorter than the mesonotum, anterior margins lacking spines, middle region with two pairs of thorns, posterior region with one pair of thorns tilted backward, and rear edge near both sides with one pair of small thorns. Meso- and metapleurae with granules, and each with one spine before the base segment of the mesocoxa. Meso- and metasternum with granules.

Abdomen slender, with sparse granules, terga with spines, apex of these spines towards the apex of abdomen, terga II–IX with expanded posterolateral angles (Fig. 1C, D). Median segment shorter than metanotum, and width greater than length. Terga I–V with one pair of spines posteromedially. The 1st–5th pairs of spines gradually increase. Fifth pair of spines smaller than the 2nd pair of spines but larger than the 1st pair of spines. Terga II–VIII with expanded posterolateral angles, gradually increasing in size from the 2nd–4th pair, the 5th–7th gradually decreasing in size, 8th pair larger than the 7th pair but smaller than the 6th pair, 9th pair distinctly expanded, the shape of spine-like tubercles. Sternum VII with an indistinct preopercular organ (Fig. 1D, F). Anal segment longer than tergum IX and shorter than tergum VIII, posterior margins with a large trapezoidal concave edge, apex rounded. Cerci flat, leaf-shape, apex rounded (Fig. 1E). Subgenital plate with one spine-like tubercle, extending approximately to the posterior margin of tergum IX (Fig. 1E).

Legs slender and with serrations. Profemora incurved basally, with two serrations of the anterdorsal carina, without serrations of the posterodorsal carina, with three serrations of the antero- and posteroventral carina. Antero- and posterodorsal carina with three serrations of the mesofemora, middle serration the largest, antero- and posteroventral carina with three serrations roughly the same size. Antero- and posterodorsal carina with three serrations of the metafemora, middle serration the largest, antero- and posteroventral carina with three serrations, middle serration the largest. All tibial anterodorsal and ventral carina unarmed. Posterodorsal carina of protibiae with six serrations, gradually smaller towards the apex, interspersed with large spines occasionally. Posterodorsal carina of meso- and metatibiae with two serrations. Mesobasitarsus shorter than the rest combined, pro- and metabasitarsus longer than the rest combined.

Male and **eggs** unknown.

Measurements are provided in Table 3.

Distribution. Jinping, Yunnan, China.

Etymology. This species is named after the province type locality, Yunnan, China.

Comments. Only known from one female; the specimen is now missing the mesofemur due to DNA extraction. This new species is flatter than other species in this genus, which may have been caused by insufficient nutrition prior to being collected.

***Cnipsomorpha yuxiensis* sp. nov.**

https://zoobank.org/C6CA7BAF-4697-42AA-A3B7-D6B98E97F281
Fig. 2

Type material. ***Holotype*.** One female. Ailaoshan Primeval Forest, Gasha, Xinping, Yuxi, Yunnan, China, alt. 2400 m. 8 May 2015. Collectors: Bin Yan, Yunfei Wu. Specimen code: YNYX150508001-1. ***Allotype*.** One male. Same data as holotype. Specimen code: YNYX150508001-2. Specimen used for DNA extraction. ***Paratype*.** One female. Jinshanyakou, Ailaoshan, Xinping, Yuxi, Yunnan, China. alt. 2377–2413 m, 17 May 2015, leg. Bin Yan. Specimen code: YNYX150507002.

Differential diagnosis. The female of *C. yuxiensis* sp. nov. is similar to *C. trituberculata* Ho, 2021. In *C. yuxiensis* sp. nov., the anterodorsal and posterodorsal carinae of femora bear inconspicuous serrations, where *C. trituberculata* exhibits distinct serrations. In *C. yuxiensis* sp. nov., the seventh abdominal sternum bears an indistinct preopercular organ, where *C. trituberculata* has a distinct preopercular organ. In *C. yuxiensis* sp. nov., the female middle area of the mesonotum shows nine spine-like tubercles, where *C. trituberculata* has twelve. The male of *C. yuxiensis* sp. nov. is similar to the *C. viridis* Ho, 2021. In *C. yuxiensis* sp. nov., the sixth abdominal tergum is unarmed, where *C. viridis* has paired posterior medial spines. In *C. yuxiensis* sp. nov., the spines on the metanotum are not paired and are sparse, where *C. viridis* has0 paired posterior medial and pre-median spines.

Description. Female (Fig. 2A, B, D, E). Slender and granulated. Apterous. The color of the specimen in alcohol is generally yellowish to brown, with black markings.

Head nearly spherical, with sparse irregular granules. Compound eyes large, spherical, and protruding. Vertex of head with one pair of spines, apex pointed. Occiput without swelling, with six spine-like tubercles, one pair close to the edge, middle pair larger. Antennae filamentous, distinctly segmented, bristly, shorter than profemora. Scapus oval, longer than the third segment, third segment longer than the pedicellus.

Thorax slender, with distinct granules. Pronotum shorter than head, slightly longer than wide, transverse sulcus in middle area, longitudinal sulcus indistinct, posterior margins raised, with a pair of spine-like tubercles, anterior and posterior regions slightly convex. Mesonotum longer than length of head and pronotum combined, mesonotum raised, anteromedially with eleven tubercles. Midline of mesonotum with seven spine-like tubercles, six of which form three pairs, one tubercle in the area between two pairs of large spine-like tubercles, the first smaller and the remaining four on both sides. Posterior region of mesonotum with one pair of spine-like tubercles. Middle area of metanotum raised, with three pairs of spine-like tubercles, two pairs near the midline, one pair in the middle area near the margins, rear pair largest, and rear margins with one pair of spine-like tubercles. Meso- and metapleurae and meso- and metasternum with granules.

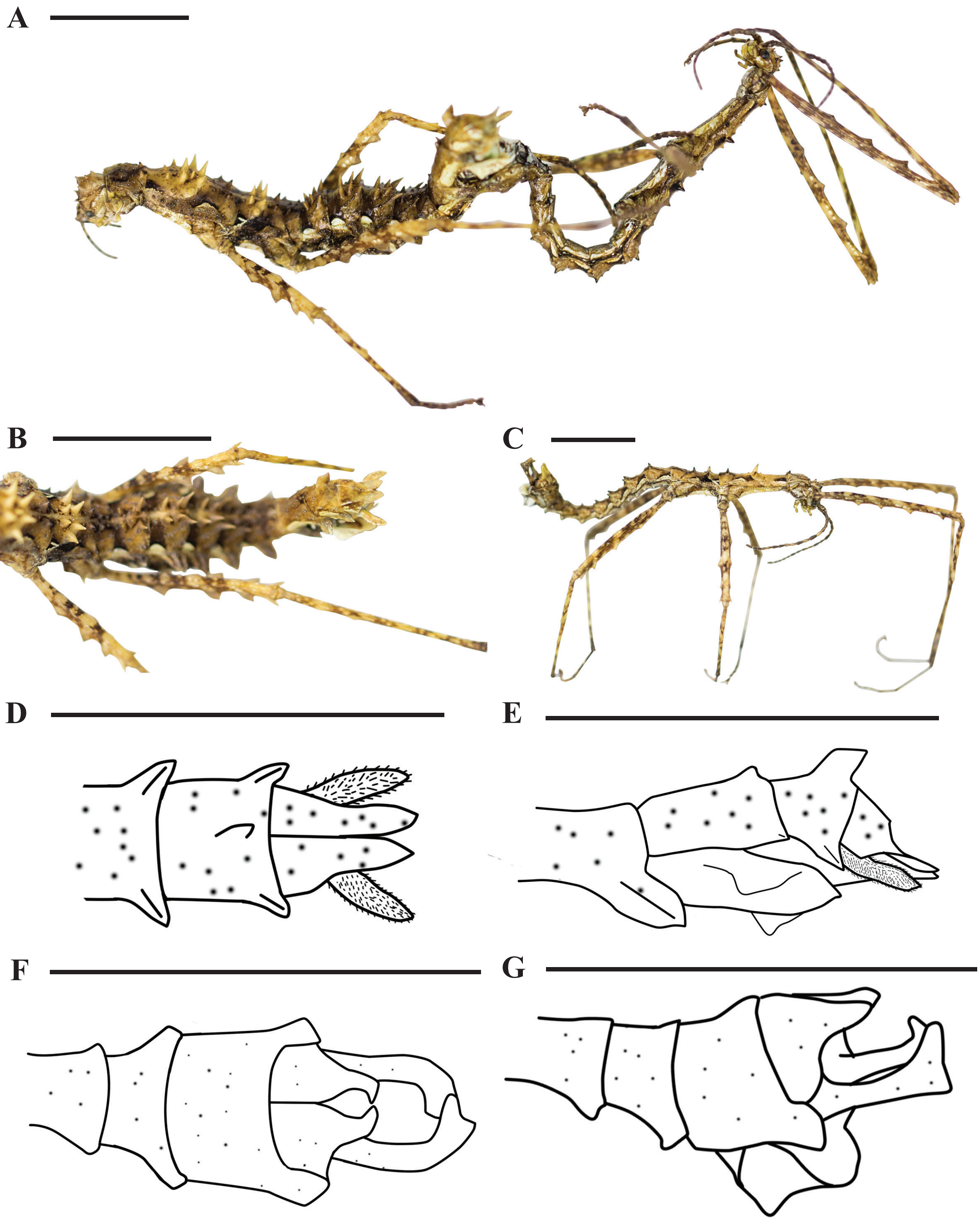

Figure 2. Features of *Cnipsomorpha yuxiensis* sp. nov. Female (**A, B, F, G**) and male (**A, C–E**) **A** male and female, in copulation, lateral view **B** body, dorsal view **C** body, dorsal view **D** end of abdomen, drawing of dorsal view **E** end of abdomen, lateral view **F** end of abdomen, dorsal view **G** end of abdomen, lateral view. Scale bars: 10 mm.

Abdomen slender with distinct granules, and with spine-like tubercles whose apexes lean towards the apex of abdomen. Median segment shorter than metanotum, with two pairs of very small spine-like tubercles, with distinct granules.

Terga II–V with three pairs of spine-like tubercles, gradually increasing in size to tergum IV (largest), middle pair closest to midline, anterior pair next closest to midline, posterior pair closest to posterior margins (Fig. 2B). Tergum V tubercles smaller than those of tergum II. Posterior margins of tergum VIII with one spine-like tubercle, and posterior area of tergum IX with parallelogram-like bulge (Fig. 2E). Expanded posterolateral angles of terga I–IX spade-like, 1st indistinct, 2nd–7th gradually increasing in size, 8th–9th gradually decreasing in size. Sternum VII with indistinct preopercular organ. Anal segment longer than tergum IX but shorter than tergum VIII, posterior margins with deep concavity, and posterior area dilated with two long, distinct, spade-like segments. Subgenital plate boat-like, with three carina, each ridge (except middle one) with a peak, apex pointed but does not surpass posterior margins of anal segment. Cerci distinct, slightly surpassing posterior margins of anal segment, apex blunt (Fig. 2D, E).

Legs lender and with serrations, all antero- and posterodorsal carina of femora apex extend and pointed. All coxa with tubercles, one or two small spine-like tubercles (or none). Profemora incurved basally, antero- and posterodorsal carina with five indistinct peaks, antero- and posteroventral carina with five serrations, base spine very small, with rest basically the same size. Antero- and posterodorsal carina of mesoforma with three peaks, antero- and posteroventral carina with three serrations. Antero- and posterodorsal carina of metaforma with five peaks, antero- and posteroventral carina with three serrations. All tibiae unarmed, with two or three peaks, except for prominent mesotibial bulge, with rest relatively shallow. Each basitarsus shorter than the rest of the tarsus combined.

Male (Fig. 2A, C, F, G) more slender than female, granulated. Apterous. Color of specimen in alcohol is generally yellowish to brown, with black markings.

Head nearly spherical, with sparse irregular granules. Vertex of head with one pair of spines. Compound eyes spherical and protruding. Occiput without swelling, with four pairs of spine-like tubercles, two close to the middle, one each on anterior and posterior margins, one pair (different sizes) in posterior area. Antennae filamentous, distinctly segmented, bristly, shorter than profemora; scapus shorter than third segment but longer than pedicellus.

Thorax slender with irregular granules. Pronotum shorter than head, anterior margins with distinct transverse sulcus, middle area raised, posterior area slightly sunken, and posterior area raised with one pair spine-like tubercles. Mesonotum longer than head and pronotum combined. Middle and posterior areas with one pair each of spine-like tubercles, one pair largest in the middle. Metanotum shorter than mesonotum, and tubercles same size as mesonotum. Meso- and metapleurae with granules. Meso- and metasternum with granules and sparse, irregular, spine-like tubercles.

Abdomen slender, cylindrical, with granules, and with irregular black ring. Terga II–V with one pair of spine-like tubercles posteromedially, their apexes directed towards the apex of abdomen (Fig. 2C). Terga I–IX with expanded posterolateral angles with spine-like shape and pointed apex. Posterior margins of tergum IX expand outward and backward. With Y-shaped vomer of abdominal segment IX, and apex exceeding the posterior margins of anal segment (X) (Fig. 2F, G). Anal segment with deep concavity, and posterior area dilated as two distinct segments, the two side plates bent inwards, their apexes in contact. Poculum with pocket-like shape, middle area with peak, and apex blunt and not exceeding the posterior margins of anal segment (Fig. 2G).

Legs. slender, with granules. All coxae of legs with one or two small spine-like tubercles (or none). Profemora incurved basally, dorsal carina wavy but indistinct, antero- and posteroventral carina with four small serrations, posteroventral carina serrations larger than anteroventral carina. Dorsal carina of meso- and metafemora wavy and distinct, antero- and posteroventral carina with three serrations, larger serrations in posteroventral carina. Tibiae without distinct serrations, wavy but indistinct. Each basitarsus shorter than the rest of them combined.

Measurements are given in Table 3.

Eggs unknown.

Distribution. Yuxi, Yunnan, China.

Etymology. This species is named after the type locality, Yuxi, Yunnan, China.

Comments. One of the females lacks the mesofemur due to DNA extraction. It is very rare to be able to collect the specimens in a mating state in the wild but fortunately we were able to do so in this case (Fig. 2A). The Y- shaped vomer of the male in this new species is quite different from that of all other species of *Cnipsomorpha*.

Table 3. Measurements (mm) of *Cnipsomorpha yunnanensis* sp. nov. and *C. yuxiensis* sp. nov.

	***C. yunnanensis*, Holotype, female**	***C. yuxiensis*, Holotype, female**	***C. yuxiensis*, Allotype, male**
Body	33.00	47.00	31.50
Head	1.90	3.83	2.98
Antennae	3.18	5.39	8.09
Pronotum	1.38	3.12	2.20
Mesonotum	5.81	7.09	6.24
Metanotum	4.56	3.90	4.68
Median segment	1.11	2.28	2.49
Profemur	13.13	12.76	17.03
Mesofemur	8.39	7.73	11.80
Metafemur	10.92	9.01	11.97
Protibiae	16.18	13.10	18.09
Mesotibiae	8.85	9.86	12.59
Metatibiae	13.41	13.05	12.20

Key to all known species of *Cnipsomorpha* (revised from Ho 2021)

Females

1 Anterodorsal and posterodorsal carinae of femora with distinct serrations..**2**
– Anterodorsal and posterodorsal carinae of femora unarmed or with indistinct serrations **9**
2 Tibiae without serrations ....***C. apteris***
– Tibiae with serrations..**3**
3 Lateral margins of pronotum without spine medially **4**
– Lateral margins of pronotum with a spine medially **6**

4 Posterior margin of anal segment with a deep emargination ...... ***C. inflexa***
– Posterior margin of anal segment with 2 or 3 small emarginations **5**
5 Anteroventral and posteroventral carinae of tibiae with indistinct serrations .... ***C. jinpingensis***
– Anteroventral and posteroventral carinae of tibiae without serrations .... ***C. colorantis***
6 Body length > 60 mm .... ***C. wenxuani***
– Body length < 60 mm **7**
7 Middle area of mesonotum with 6 pairs of medial spines...... ***C. trituberculata***
– Middle area of mesonotum with 2 or 4 pairs of medial spines **8**
8 Middle area of mesonotum with four pairs of medial spines ..... ***C. serratitibia***
– Middle area of mesonotum with 2 pairs of medial spines .... ***C. yunnanensis* sp. nov.**
9 Seventh abdominal sternum with indistinct preopercular organ .... ***C. yuxiensis* sp. nov.**
– Seventh abdominal sternum with distinct preopercular organ **10**
10 Preopercular organ is flattened **11**
– Preopercular organ is cylindrical **13**
11 Preopercular organ posterior margin rounded .... ***C. kunmingensis***
– Preopercular organ posterior margin emarginated **12**
12 Preopercular organ posterolateral apices is blunt .... ***C. viridis***
– Preopercular organ posterolateral apices is pointed .... ***C. polyspina***
13 Preopercular organ hump-like .... ***C. daliensis***
– Preopercular organ cylindrical **14**
14 Preopercular organ short, apically rounded and tubercle-like....... ***C. erinacea***
– Preopercular organ elongate, apically pointed and knife-like **15**
15 Pronotum without paired anterior medial spines .... ***C. nigromaculata***
– Pronotum with paired anterior medial spines .... ***C. bii***

Males

1 Cerci longer than anal segment .... ***C. colorantis***
– Cerci shorter than anal segment **2**
2 Body length > 60 mm .... ***C. wenxuani***
– Body length < 60 mm **3**
3 Median segment without posterior spines **4**
– Median segment with posterior spines **8**
4 Pronotum without posterior medial spines .... ***C. daliensis***
– Pronotum with posterior medial spines **5**
5 Sixth abdominal tergites with paired posterior medial spines ...... ***C. viridis***
– Sixth abdominal tergites without paired posterior medial spines **6**
6 Semi anal tergites strongly incurved .... ***C. inflexa***
– Semi anal tergites weakly incurved **7**
7 Abdomen without vomer .... ***C. nigromaculata***
– Abdomen with vomer .... ***C. yuxiensis* sp. nov.**
8 Tibiae without serrations .... ***C. kunmingensis***
– Tibiae armed with serrations **9**
9 Metanotum without median spines .... ***C. jinpingensis***
– Metanotum with paired median spines .... ***C. maoershanensis***

Phylogenetic analysis

The best-fit substitution models were used to reconstruct the ML trees of *28S*, *COI*, and *28S* + *COI* are GTR+G (AIC value: 7301), GTR+G+I (AIC value: 6903) and GTR+G (AIC value: 11127). The reliability of the ML trees was tested by running 1,000 ultrafast bootstrap pseudoreplicates (Figs 3–5).

Cnipsomorpha form a clade with *Parapachymorpha* and *Spinoparapachymorpha* which together are the sister group to Pharnaciini (support values = 41) to several species of Clitumnini and Medaurini in the *28S* ML tree (Fig. 3), which confirms the work of Hennemann et al. (2008). Albeit, the *Cnipsomorpha* species have their own derived characteristics (autapomorphies) that define the taxon, namely the posterolateral extensions of the abdominal terga II–VII in females. In *Parapachymorpha* only the posterior expansion of the tergum VII of *P. jinpingensis* and the posterior expansion of the tergum VIII of *P. xishuangbannaensis* are present. The morphological characteristics of the two new species are quite different from those of *Parapachymorpha*.

In Fig. 4, *Cnipsomorpha* together with *Medauroidea* and *Medauromorpha* are the sister group of Pharnaciini. However, in *Medauroidea* and *Medauromorpha* there are no obvious spines or tubercles on the body surface of the latter, and without the posterolateral extensions of abdominal terga II–VII in females (Hennemann et al. 2008).

We think that the phylogenetic position of *Cnipsomorpha* should be closer to that of *Paraachymorpha* and *Spinoparapachymorpha*, because their body shape is more similar and the body shorter than that of *Medauroidea* and *Medauromorpha*. Moreover, in Fig. 5, the phylogenetic position of *Cnipsomorpha* is closer to the Clinumnini, and the *Parapachymorpha* and *Spinoparapachymorpha* belong to Clinumnini.

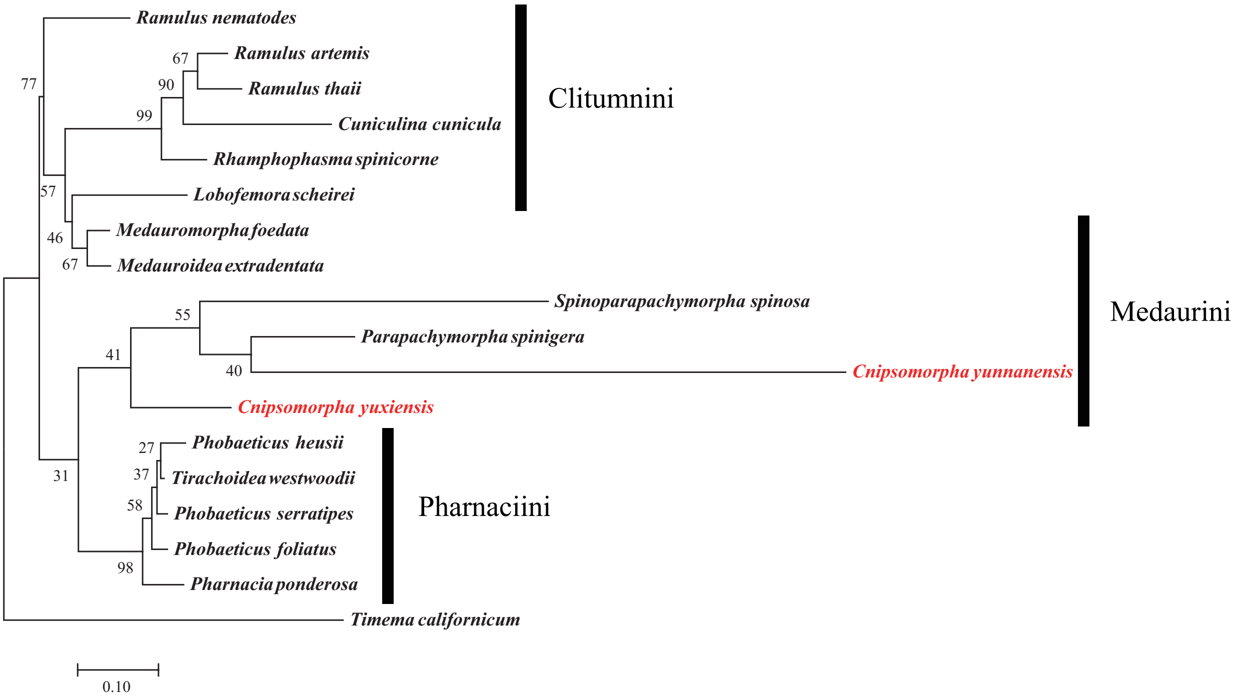

Figure 3. Maximum likelihood tree based on *28S* rDNA of available species of Clitumninae. GTR+G model with 1000 bootstraps. The outgroup is *Timema californicum*.

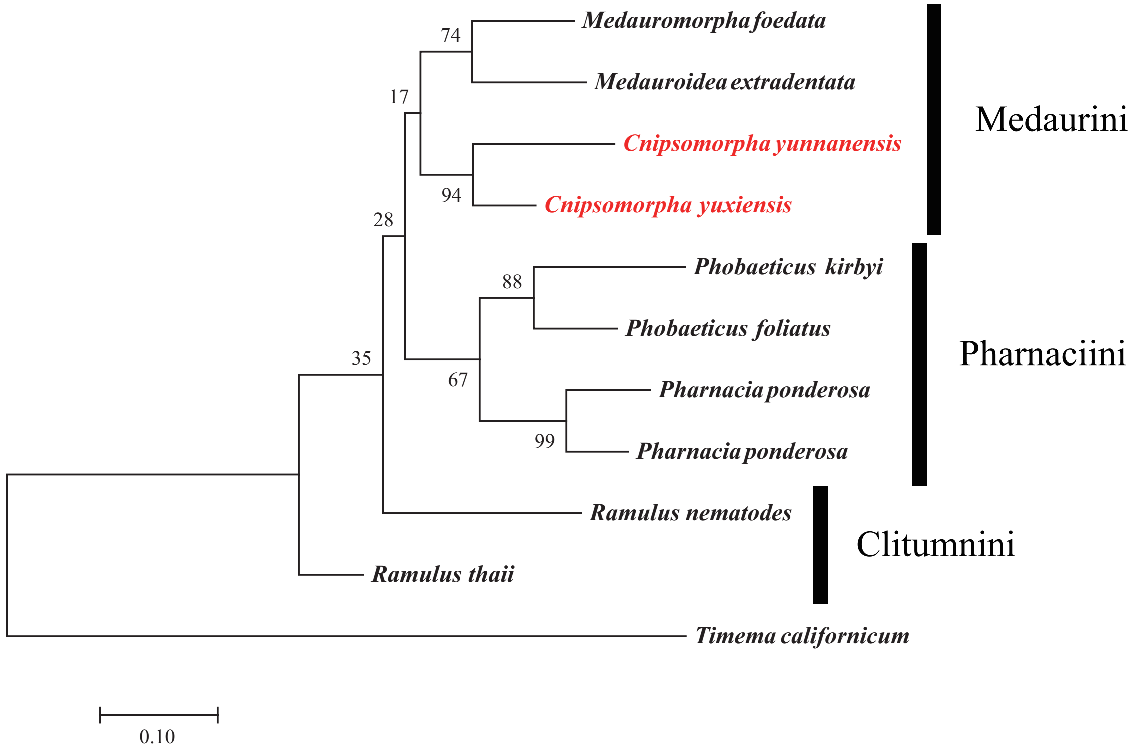

Figure 4. Maximum likelihood tree based on *COI* sequence data of available species of Clitumninae. The outgroup is *Timema californicum*, GTR+I+G model with 1000 bootstraps.

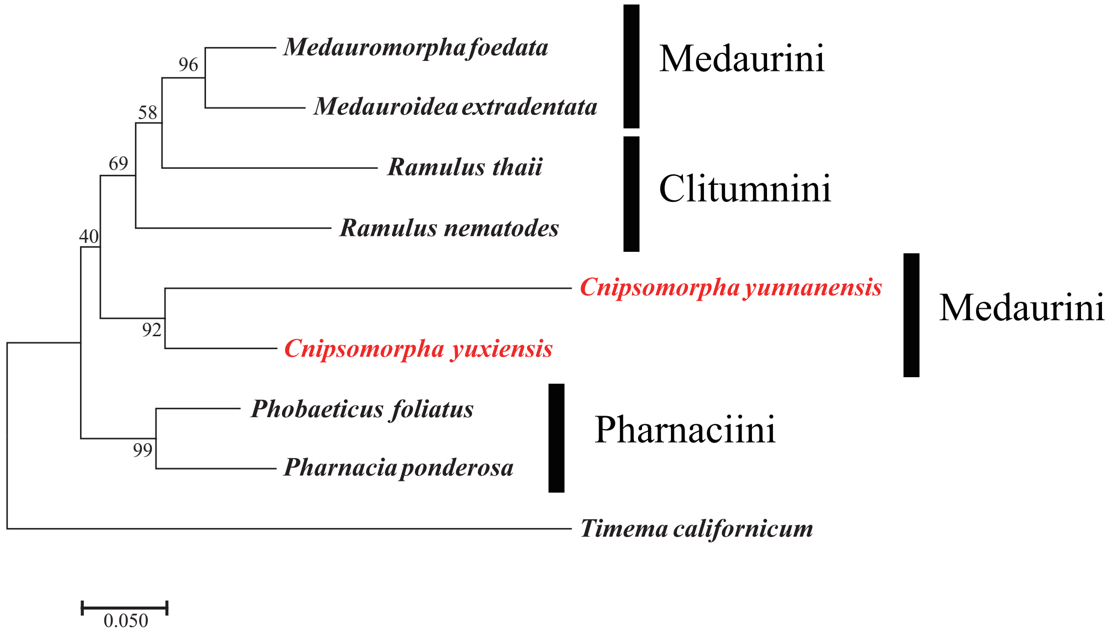

Figure 5. Maximum likelihood tree based on *28S* rDNA + *COI* of available species of Clitumninae. The outgroup is *Timema californicum*, GTR+G model with 1000 bootstraps.

Besides, Medaurini can be divided into two parts as in the study of Bank and Bradler (2022). Medaurini I is close to Gratidiini, Medaurini II is close to Clitumnini, and the two new species of *Cnipsomorpha* belong to Medaurini I in the *28S* ML tree, which shows the same topology as in Bank and Bradler (2022). That is to say, *Cnipsomorpha* together with *Parapachymorpha* and *Spinoparapachymorpha* form the sister group to Pharnaciini, but *Cnipsomorpha* does not cluster together with the bulk of Medaurini species. Comprehensive research based on more taxa and data is necessary to corroborate these phylogenetic assumptions.

Conclusions

We report two new species of *Cnipsomorpha*, *C. yunnanensis* sp. nov., and *C. yuxiensis* sp. nov., based on morphological characteristics, and generated molecular data for these two species. According to the results of our phylogenetic analysis, we can conclude that the phylogenetic position of the two new species is closer to *Parapachymorpha* than to other Medaurini, and that this clade is the sister group of Pharnaciini.

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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

Fangling Xu conceived and designed the experiments and approved the final draft. Yingjie Jiang performed the experiments, analyzed the data, prepared figures and tables, and authored or reviewed drafts of the paper. Maofa Yang conceived and designed the experiments, performed analyses, and contributed reagents.

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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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Research Article

Unravelling the diversity of the genus *Afronurus* Lestage, 1924 (Ephemeroptera, Heptageniidae) in Thailand

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Abstract

The genus *Afronurus* in Thailand is investigated using an integrative approach (morphology based, ootaxonomy and molecular data) for species delimitation. A total of four species of *Afronurus* was identified; *A. cervina* (Braasch & Soldán, 1984), *A. gilliesiana* (Braasch, 1990), *A. rainulfiana* (Braasch, 1990), and *A. rubromaculata* (You et al., 1981). The subimago of *A. gilliesiana* is described for the first time based on reared specimens. The egg structure of all four species is also described for the first time. Morphological and molecular data strongly support their species delimitation. The egg chorionic structure of the genus *Afronurus*, together with other morphological characters, is useful for species identification. A key to mature nymphs of the known species is provided.

Key words: COI, mayfly, systematics

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Introduction

Members of the family Heptageniidae are among the most abundant and common components of benthic communities in running waters, and many heptageniid species have been used as indicators of anthropogenic disturbance. This family is distributed mainly in the Holarctic, Oriental, and Afrotropical regions, with around 509 described species (Sartori and Brittain 2015). Heptageniidae from Thailand have previously been investigated (Braasch 1990; Sites et al. 2001; Sangpradub et al. 2002; Wang and McCafferty 2004; Webb and McCafferty 2008; Braasch and Boonsoong 2010; Boonsoong and Braasch 2013; Boonsoong et al. 2021), where ten genera and approximately 26 species have been recorded (Boonsoong 2022).

The genus *Afronurus* was established by Lestage (1924) from the African representatives assigned to the genus *Ecdyonurus*. Wang and McCafferty (2004) and Kluge (2004) synonymised the Asian genus, *Cinygmina* Kimmins, 1937, with *Afronurus*. The nymphs of the genus *Afronurus* can be distinguished from those of other genera by the presence of two rows of long, fine setae on the mid and hind tibiae, simple scattered setae on the ventral surface of the maxillae, and a slightly thickened anterior margin of the head capsule (Webb and

McCafferty 2008). The genus *Afronurus* is the third largest heptageniid genus, with more than 65 known species from the Oriental (44 species), Afrotropical (15 species), and Palearctic (6 species) realms (Sartori and Brittain 2015; Yanai et al. 2017; Zhang et al. 2021). In Southeast Asia, many species are known only as nymphs, or described as adults (Braasch and Soldán 1984; Braasch 1990; Nguyen and Bae 2003; Braasch 2011; Braasch and Jacobus 2011).

The nymphs of the genus *Afronurus* are among the most common heptageniids encountered in Thai streams (Boonsoong and Braasch 2013). Six species of this genus have been reported in Thailand, namely *A. cervina* (Braasch & Soldán, 1984), *A. dama* (Braasch & Soldán, 1987), *A. gilliesiana* (Braasch, 1990), *A. namnaoensis* Braasch & Boonsoong, 2010, *A. rainulfiana* (Braasch, 1990), and *A. rubromaculata* (You et al., 1981) (Boonsoong and Braasch 2013). However, information is lacking regarding the association between the nymphal and imaginal stages and the genetic identity of known *Afronurus* species in Thailand. The aim of this paper is to study the systematics of the genus *Afronurus* in Thailand using morphology-based and mitochondrial COI analyses.

Materials and methods

Morphological observation

Afronurus nymphs were collected from different microhabitats in streams in Thailand. The nymphs were fixed and kept in absolute ethanol. Some nymphs with dark wing pads were selected for rearing to the adult stage for association. Photographs were taken using a Nikon SMZ800 stereoscopic microscope. The eggs of *Afronurus* were dried in a critical point drier (Polaron Range CPD7501) and coated with gold (Polaron Range SC7620) for examination of the chorionic structures by scanning electron microscopy (Quanta 450). Voucher specimens are deposited in the Aquatic Insects Collection of the Zoological Museum Kasetsart University (**ZMKU**), Bangkok, Thailand.

Molecular analysis

Total genomic DNA was extracted from the legs on one side of a nymph using a Genomic DNA Purification Kit (NucleoSpin, Macherey-Nagel, Germany) following the manufacturer's protocol. The COI gene was amplified using primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'), designed by Folmer et al. (1994). The polymerase chain reaction (PCR) conditions as were follows: a 50 µL final reaction volume containing 25 µL of PCR Master mix Solution (i-Taq), 1 µL (10 µm) of each primer, 2 µL of total DNA and 21 µL of nuclease-free sterile water, using the PCR conditions described by Gattolliat et al. (2015). Amplification cycles were as follows: 5 min at 94 °C, then 30 s at 94 °C, 30 s at 48 °C and 60 s at 72 °C (40 cycles), and a final elongation step at 72 °C for 10 min. The PCR products were purified and sequenced by Macrogen, Inc., Korea.

Sequence alignment and editing were performed using MEGA X (Kumar et al. 2018). The best-fit models were selected based on MEGA X. The General Time Reversible Model and Gamma distributed with invariant sites (GTR+G+I) were used for the MrBayes analysis, v. 3.1.2 (Huelsenbeck and Ronquist 2001). Analyses of four MCMC chains run for one million generations with trees sampled every 100 generations were used for BI. The stationary nucleotide frequencies, the alpha shape parameter of the gamma distribution, the relative rate of substitution and the proportion of invariant sites were unlinked across partitions. The first 25% were discarded as a burn-in after visually verifying that likelihood curves had flattened out and that the independent runs converged using Tracer 1.5 (Drummond and Rambaut 2007). Nucleotide sequences obtained in this study are deposited in the GenBank database (Table 1). Other analysed sequences from *Afronurus* and related genera, obtained from the Barcode of Life Data System (BOLD), were *A. mnong* (OP347112.1) and *A. meo* (OP347111.1) from Vietnam, *A. hyalinus* (LC377346.1) and *A. rubromaculata* (MK642294.1) from China and *Anapos zebratus* (HG935069.1) as the outgroup. We followed all guidelines of the Animal Ethics Committee of Kasetsart University (approval no. ACKU63-SCI-005 and ACKU66-SCI-017) for collecting the mayfly specimens.

Table 1. List of sequences of four Thai *Afronurus* species at each sampling site.

Species	Code	Collection locality	Date	GenBank accession number
A. cervina (Braasch & Soldán, 1984)	SP04LE	Loei	17 Dec 2018	OP729860
	SP04LE2	Loei	17 Dec 2018	OP729859
	SP04LE3	Loei	17 Dec 2018	OP729861
	SP04LE4	Loei	17 Dec 2018	OP729862
	SP01KN	Kanchanaburi	31 Jan 2019	OP729856
	SP01KN2	Kanchanaburi	11 Apr 2015	OP729857
	SP01KN3	Kanchanaburi	31 Jan 2019	OP729852
	SP01KN4	Kanchanaburi	31 Jan 2019	OP729858
	SP01PC	Petchburi	25 Feb 2018	OP729850
	SP01PK	Prachuap Khiri Khan	19 Apr 2019	OP729848
	SP01PK2	Prachuap Khiri Khan	19 Apr 2019	OP729853
	SP01PK3	Prachuap Khiri Khan	19 Apr 2019	OP729854
	SP01RB	Ratchaburi	24 Nov 2018	OP729855
	SP01RB2	Ratchaburi	24 Nov 2018	OP729849
	SP01TK	Tak	26 Dec 2018	OP729851
A. gilliesiana (Braasch, 1990)	SP02CR	Chiang Rai	6 May 2019	OP729846
	SP02CR2	Chiang Rai	6 May 2019	OP729845
	SP02CR3	Chiang Rai	6 May 2019	OP729843
	SP02CR4	Chiang Rai	7 May 2019	OP729847
	SP02CR5	Chiang Rai	7 May 2019	OP729844
A. rainulfiana (Braasch, 1990)	SP03KN	Kanchanaburi	31 Jan 2019	OP729836
	SP03NT	Nakhon Si Thammarat	2 July 2016	OP729834
	SP03NW	Narathiwat	22 Apr 2018	OP729838
	SP03PC	Phetchaburi	24 Feb 2019	OP729837
	SP03RN	Ranong	20 Apr 2018	OP729833
	SP03RB	Ratchaburi	24 Nov 2018	OP729835
A. rubromaculata (You et al., 1981)	SP05CT	Chanthaburi	5 Jun 1028	OP729840
	SP05KN	Kanchanaburi	31 Jan 2019	OP729839
	SP05NA	Nan	5 Dec 2017	OP729842
	SP05RB	Ratchaburi	24 Nov 2018	OP729841

Taxonomic accounts

Family Heptageniidae
Genus *Afronurus* Lestage, 1924

Afronurus cervina (Braasch & Soldán, 1984)

Figs 1A–E, 2A–E, 3A–E, 4A–E, 17A, B

Cinygmina cervina Braasch & Soldán, 1984: 196, figs 14–32, original description (male and female imago, nymph); Venkataraman and Sivaramakrishnan 1989: 118, figs 7, 10 (nymph).
Afronurus cervina – Boonsoong and Braasch 2013: 85.

Material examined. 13 nymphs, Chanthaburi Prov., Rattanaburi resort, 12°31'39.9216"N, 102°10'38.9064"E 41 m, 4.V.2023, B. Boonsoong leg. (ZMKU); 5 nymphs, Kanchanaburi Prov., Huai Pilok, 14°39'52.7"N, 98°33'00.3"E, 132 m, 31.I.2019, W. Anuntaya leg. (ZMKU); 4 nymphs, Kanchanaburi Prov., Ban Tao Tan, 14°38'58.199"N, 98°34'55.8006"E, 166 m, 31.I.2019, W. Anuntaya leg. (ZMKU); 7 nymphs, Tak Prov., Oum Yom, 16°48'15.7"N, 99°00'08.3"E, 249 m, 26.XII.2018, W. Anuntaya leg. (ZMKU); 24 nymphs, Ratchaburi Prov., Bor Klueng, 13°31'27.3612"N, 99°14'39.3606"E, 180 m, 24.XI.2018, W. Anuntaya leg. (ZMKU); 9 nymphs, Loei Prov., Nam Thob, 17°15'36.50"N, 101°34'52.90"E, 376 m, 17.XII.2018, W. Anuntaya leg. (ZMKU); 17 nymphs, Ratchaburi Prov., Kang Som Maew, 13°24'22.32"N, 99°6'43.74"E, 207 m, 24.XI.2018, W. Anuntaya leg. (ZMKU); 6 nymphs, Phetchaburi Prov., Huai Sat Yai, 12°38'13.5"N, 99°30'59.34"E, 162 m, 25.II.2018, W. Anuntaya leg. (ZMKU); 7 nymphs, Prachuap Khiri Khan Prov., Huai Sam Rong, 12°3'49.68"N, 99°37'38.76"E, 103m, 25.II.2018, W. Anuntaya leg. (ZMKU).

Description. Nymph. See Braasch and Soldán (1984: 196–197, 199, figs 17–32, original description).

Adult. See Braasch and Soldán (1984: 196–197, 199, figs 14–16, original description), Braasch (1990: 8).

Diagnosis. Nymph of *Afronurus cervina* (Fig. 1A) has unique characteristics such as a brown abdomen with pale mark and tergites III-IX with two pairs of longitudinal marking on median and lateral. Markings fused on tergites VIII–IX whereas tergite X is dark (Fig. 1B), and no marking on sternites (Fig. 1C). In addition, no marking on anterior margin of head (Fig. 1D). Gill V (Fig. 2A) and gill VI (Fig. 2B) obliquely rounded, triangular, with small projection, and gill VII (Fig. 2C) broad, asymmetrically oval. Marking pattern of hind femur as Figs 1E, 2D. Bristles on the dorsal face of the hind femur are blunt in shape (Fig. 2E).

Adult of *Afronurus cervina* can be distinguished from other *Afronurus* species by its abdominal patterns, dark brown in a band down the middle and yellow along the margin, tergites III–VIII with a pair of thick stripes on the submedian, all tergites with a longitudinal median dark band (Fig. 3A, B). Genital plate emarginated, penis lobes enlarged with terminal edge jagged, between each lobe with cone shaped tubercle (Fig. 3C). The titillators are short, canine tooth-like (Fig. 17A, B). Subanal plate of female adult tongue-shaped and slightly truncated at tip (Fig. 3D). Fore and hind wing as Fig. 3E.

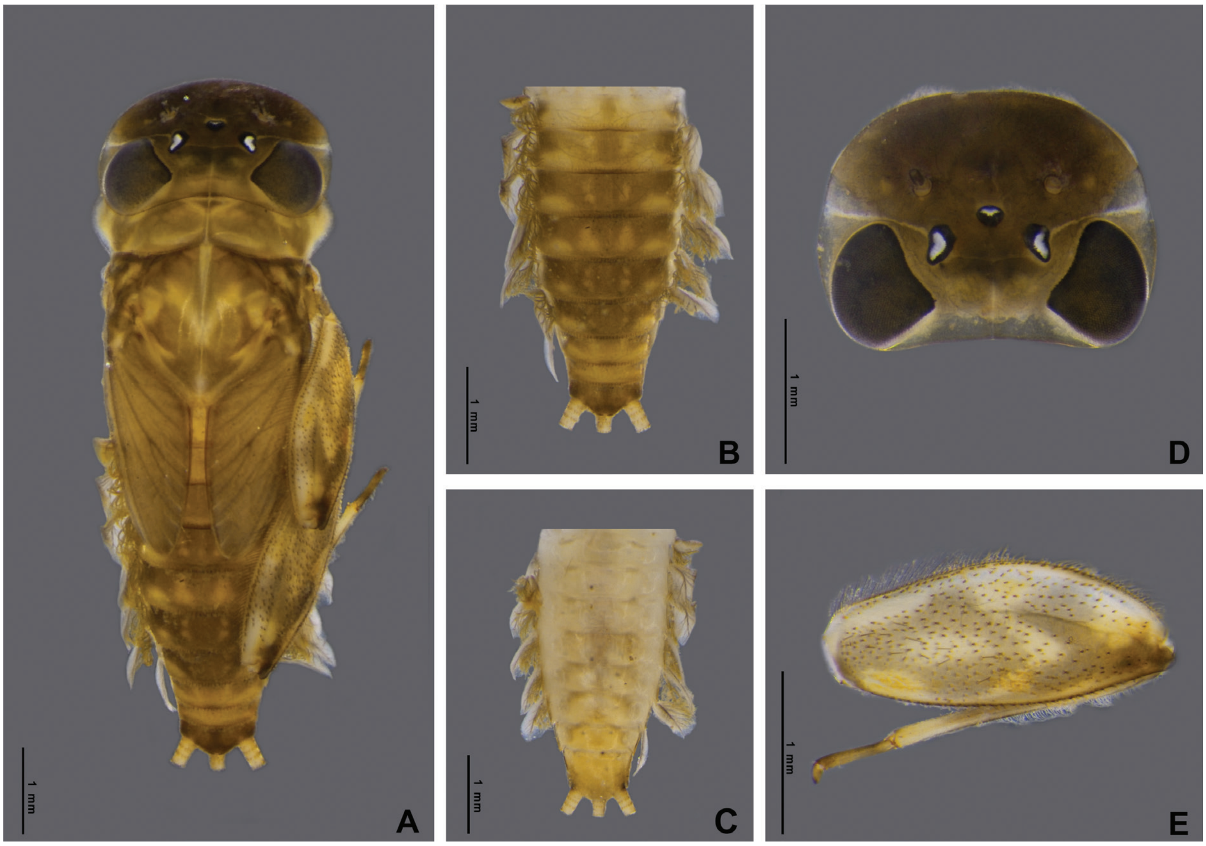

Figure 1. *Afronurus cervina* (Braasch & Soldán, 1984), larval morphology **A** female habitus **B** tergites I–X **C** sternites I–X **D** head **E** hind leg. Scale bars: 1 mm.

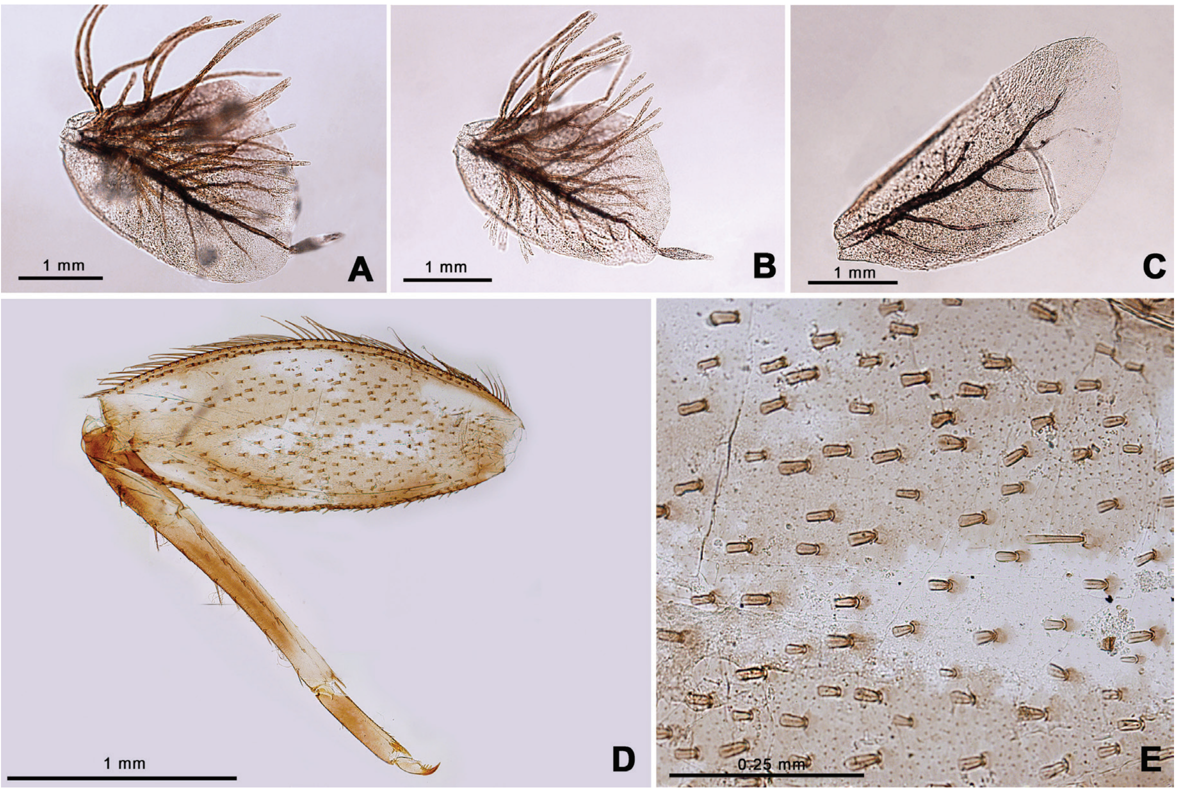

Figure 2. *Afronurus cervina* (Braasch & Soldán, 1984), larval morphology **A** gill V **B** gill VI **C** gill VII **D** hind leg **E** bristles on the dorsal face of the hind femur (middle part). Scale bars: 1 mm (**A–D**); 0.25 mm (**E**).

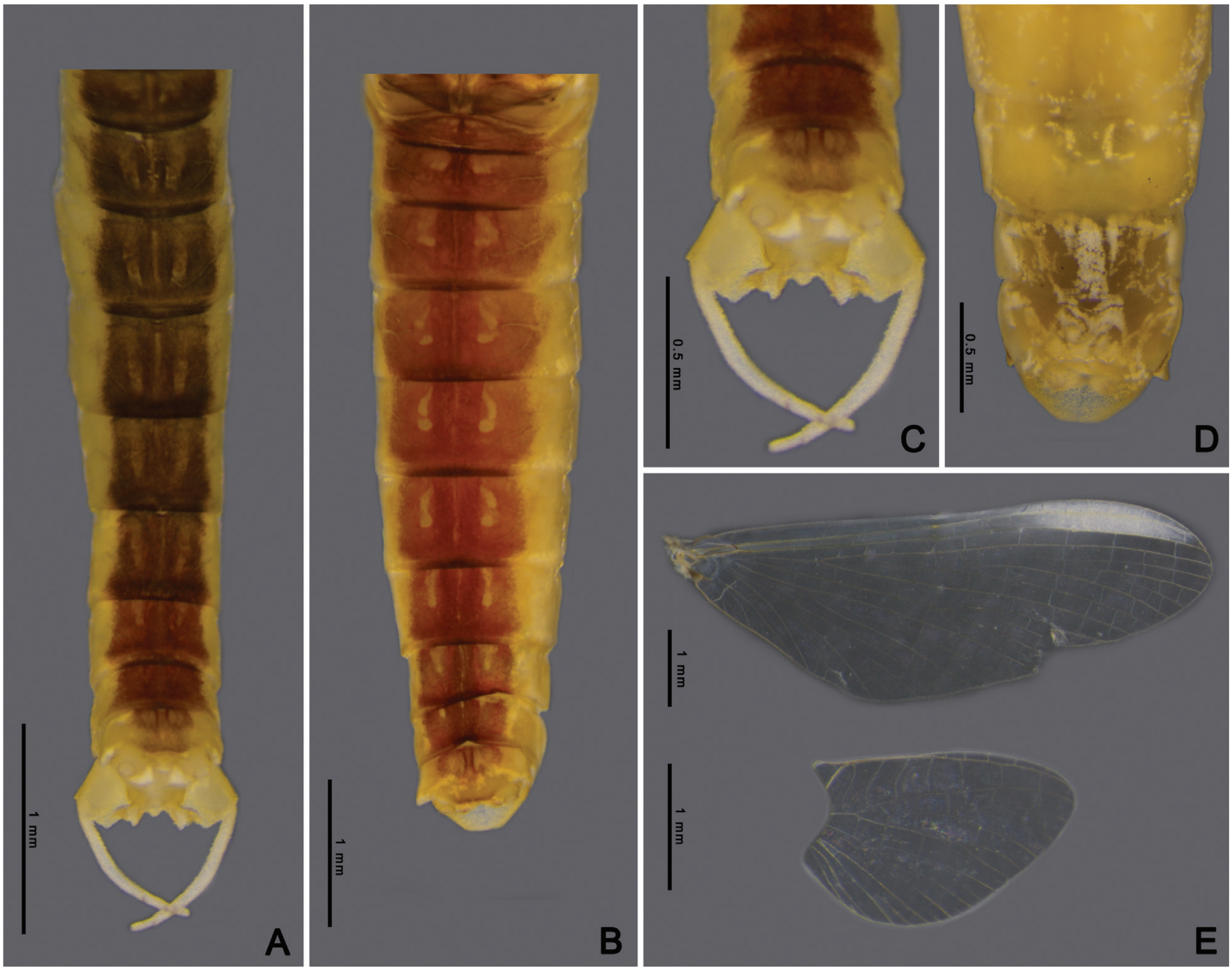

Figure 3. *Afronurus cervina* (Braasch & Soldán, 1984), imaginal morphology **A** male tergites II–X **B** female tergites I–X **C** male genitalia **D** female anal plate **E** fore wing and hind wing. Scale bars: 0.5 mm (**C, D**); 1 mm (**A, B, E**).

Eggs. Chorionic surface of the egg with large KCTs (knob-terminated coiled threads) or equatorial KCT (eKCT) and small KCTs or polar KCT (pKCT) (Fig. 4A). Both poles were covered with dense pKCTs. Equatorial areas were smooth (Fig. 4B) surrounded with eKCTs and micropyle (M) between eKCTs (Fig. 4C).

Distribution. Chanthaburi, Kanchanaburi, Loei, Phetchaburi, Prachuap Khiri Khan, Ratchaburi and Tak provinces (Fig. 18).

Remarks. *Afronurus cervina* was found for the first time in Ho Chi Min, Vietnam (Braasch and Soldán 1984), then reported in Ban Nam Tok (Chiang Rai province) by Braasch (1990). In this study, we found *A. cervina* at several localities along the northern to southern regions of Thailand. *Afronurus cervina* was found underneath the cobble substrate in slow running water, but can live in wide range of habitats, such as disturbed areas (as in Nakhon Nayok province) and head water streams (as in Loei province). The optimal altitude is between 24 to 527 meters. The abdominal pattern of nymph of *A. cervina* is quite similar to *A. palawanensis* (Braasch and Freitag 2008), but it can be distinguished by the markings on the anterior area of the head (Braasch and Soldán 1984).

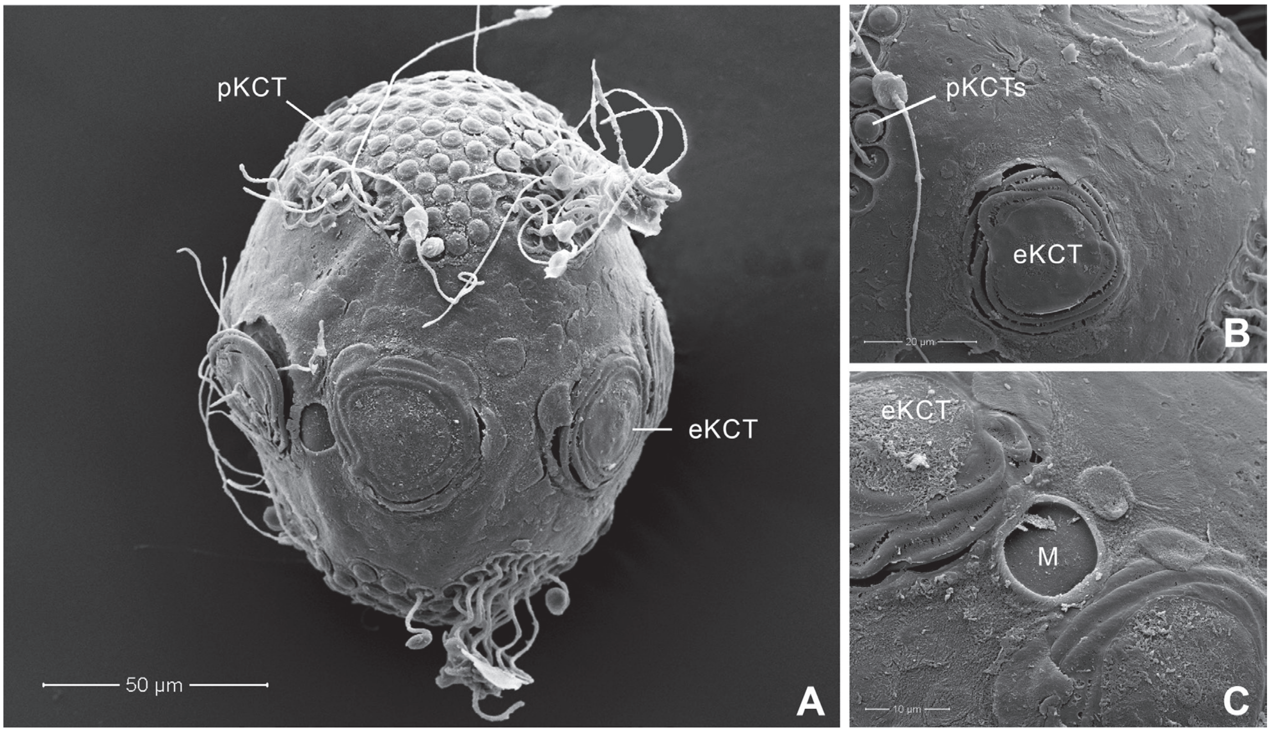

Figure 4. *Afronurus cervina* (Braasch & Soldán, 1984), SEMs of egg morphology **A** general outline of egg **B** chorion surface between polar KCT (pKCT) and equatorial KCT (eKCT) **C** micropyle (M) and enlargement of eKCTs. Scale bars: 50 µm (**A**); 20 µm (**B**); 10 µm (**C**).

Afronurus gilliesiana **(Braasch, 1990)**

Figs 5A–E, 6A–E, 7A–C, 8A–E, 17C, D

Cinygmina gilliesiana Braasch, 1990: 8, figs 13–16, original description (nymph).
Afronurus gilliesiana – Boonsoong and Braasch 2013: 86.

Material examined. 5 nymphs, Chiang Rai Prov., Khun Korn waterfall, 19°51'46.10"N, 99°39'4.70"E, 534 m, 6.V.2019, W. Anuntaya leg. (ZMKU); 4 nymphs, Chiang Rai Prov., Nang Lae Nai Waterfall, 20°3'9.50"N, 99°49'16.90"E, 529 m, 6.V.2019, W. Anuntaya leg. (ZMKU); 3 larvae Chiang Rai Prov., Pong Phrabat Waterfall, 20°0'41.80"N, 99°48'15.10"E, 470 m, 7.V.2019, W. Anuntaya leg. (ZMKU).

Description. Nymph. See Braasch (1990: 8, 10, figs 13.1–13.4, 14–16, original description).

Adult. Male subimago (in alcohol, Fig. 7A, C, E). ***Head*.** Eyes rounded, blackish on both dorsal and ventral parts, pale laterally. ***Thorax*.** Yellowish with triangular brown patch on sub-median of mesonotum. Forelegs brownish; lengths of femur, tibia, and tarsi 1.64 mm, 1.24 mm, and 0.63 mm, respectively. Midlegs brownish; lengths of femur, tibia, and tarsi 1.68 mm, 1.38 mm, and 0.72 mm, respectively. Hindlegs brownish; lengths of femur, tibia, and tarsi 1.7 mm, 1.42 mm, and 1.22 mm, respectively. Wings transparent; forewing C, Sc and RA thick and yellowish brown, other veins thinner, Sc and RA parallel along the wing, convergent at base, RS and MP forked basally, MA forked at the middle, and CuP and CuA adjacent at base; hindwings rounded, RA and MA adjacent at base of wing, MA and MP forked at the middle (Fig. 7E). ***Abdomen*.** Middle area brown

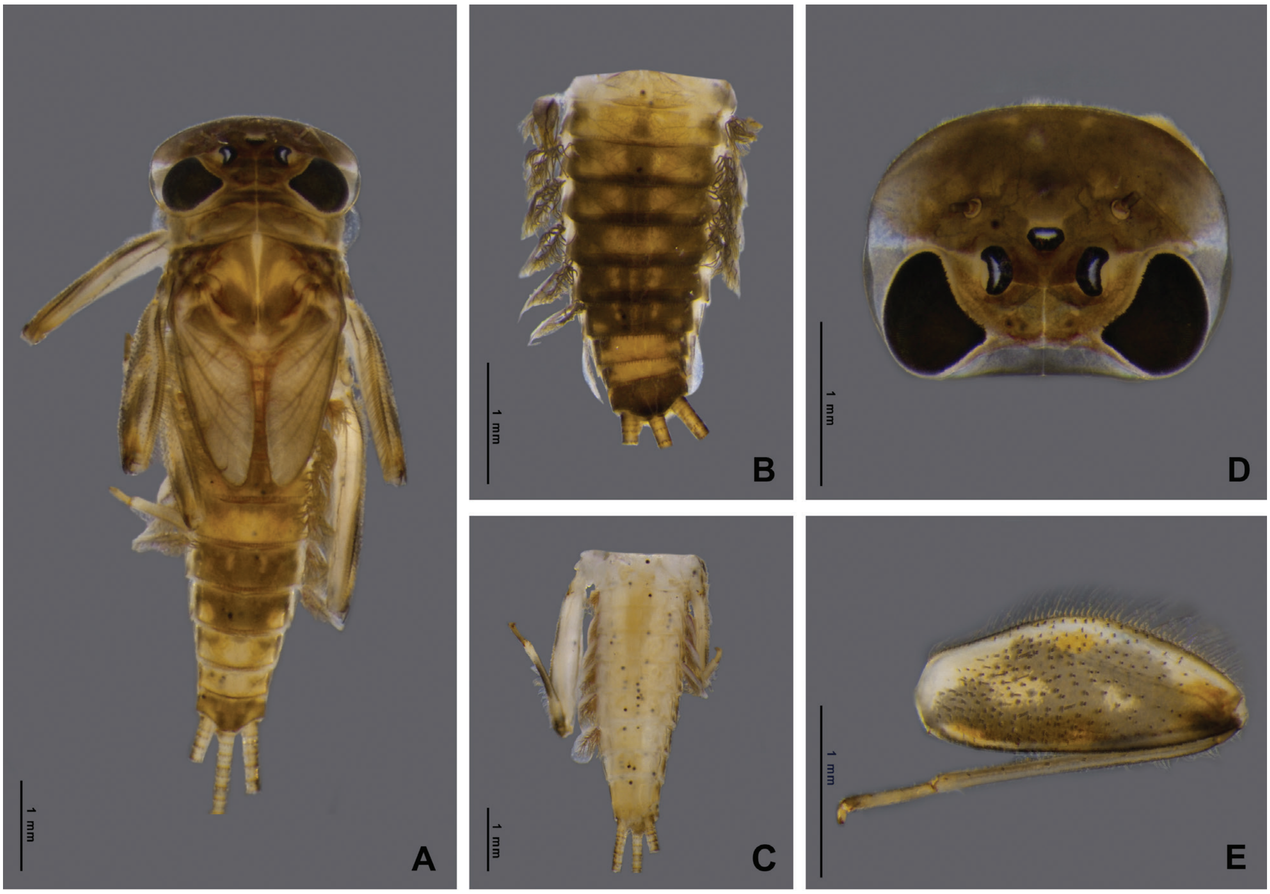

Figure 5. *Afronurus gilliesiana* (Braasch, 1990), larval morphology **A** female habitus **B** tergites I–X **C** sternites VI–X **D** head **E** hind leg. Scale bars: 1 mm.

with one pair of longitudinal yellow marks, outer margin pale yellow (Fig. 7A). ***Genitalia*:** penis bilobate, expanding laterally to the enlarged lobes, the inner part of lobes with a small cleft (Fig. 17C). Titillators very short, canine-like (Fig. 17C, D), forceps 4-segmented, segment I very small, length ratio of segment II to segment III to segment IV is 0.29: 0.12: 0.1 (Fig. 7C).

Female subimago (in alcohol, Figs 7B, D). ***Head*.** Eyes rounded with brownish dorsal part and ventral part dark brown. ***Thorax*.** Yellowish with brown patch at margin. Midlegs brownish; lengths of femur, tibia, and tarsi 1.89 mm, 1.46 mm, and 0.66 mm, respectively. Hindlegs brownish; lengths of femur, tibia, and tarsi 2.3 mm, 1.49 mm, and 0.57 mm, respectively. Wings as in male imagos. ***Abdomen*.** Tergites VII–IX, middle area pale brown with one pair of pale marks on anterior margin, tergite X pale yellow (Fig. 7B). Subanal plate trapezium-shaped and concave at tip (Fig. 7D), length 0.2 mm, width 0.5 mm.

Egg. Chorionic surface covered with pKCTs and eKCTs. Both poles densely covered with pKCTs. Equatorial and subequatorial areas with eKCTs and micropyle beside eKCTs (Fig. 8C); area between pKCTs and eKCTs with indistinct small tubercles (Fig. 8B), areas of two poles of pKTCs ~ 0.7× the size of the whole egg (Fig. 8A).

Remarks. Nymph of *Afronurus gilliesiana* is distinguishable from other species by gill shape, particularly oval-elongated gill I as well as by two large round femoral markings (Figs 5E, 6D). Anterior margin of head with four weak markings (Fig. 5D). Abdomen with marking as shown in Fig. 5A, tergites VIII and IX with pale markings (Fig. 5B) and sternites without marks (Fig. 5C). Gill V

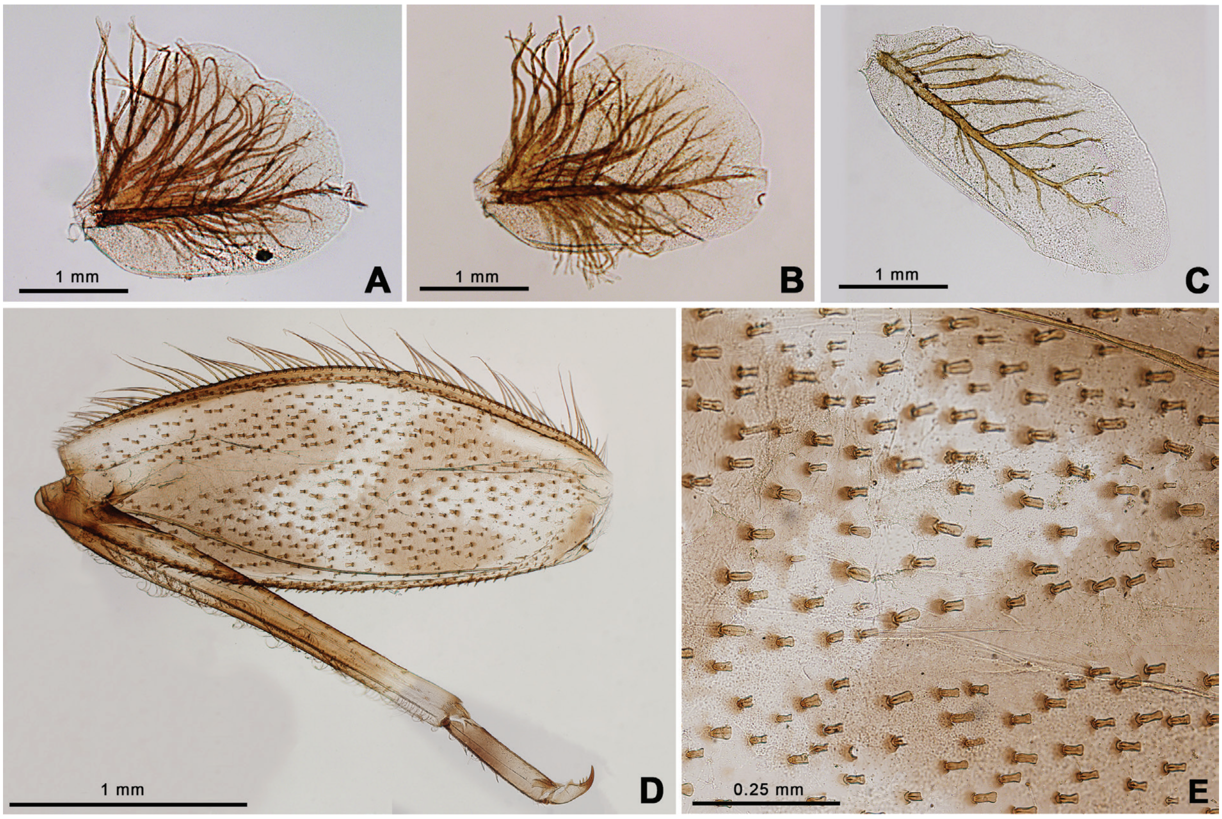

Figure 6. *Afronurus gilliesiana* (Braasch, 1990), larval morphology **A** gill V **B** gill VI **C** gill VII **D** hind leg **E** bristles on the dorsal face of the hind femur (middle part). Scale bars: 1 mm (**A–D**); 0.25 mm (**E**).

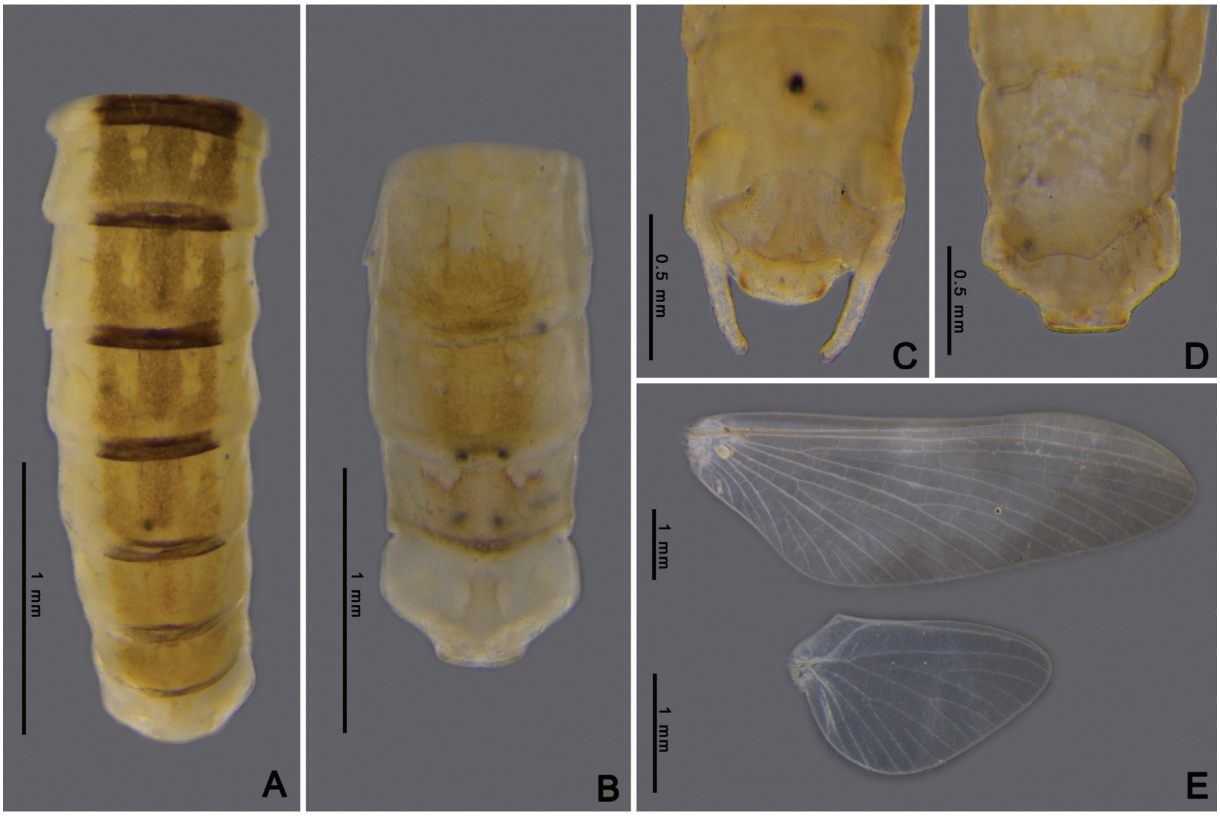

Figure 7. *Afronurus gilliesiana* (Braasch, 1990), imaginal morphology **A** male tergites IV–X **B** female tergites VII–X **C** male genitalia **D** female anal plate **E** fore wing and hind wing. Scale bars: 0.5 mm (**C, D**); 1 mm (**A, B, E**).

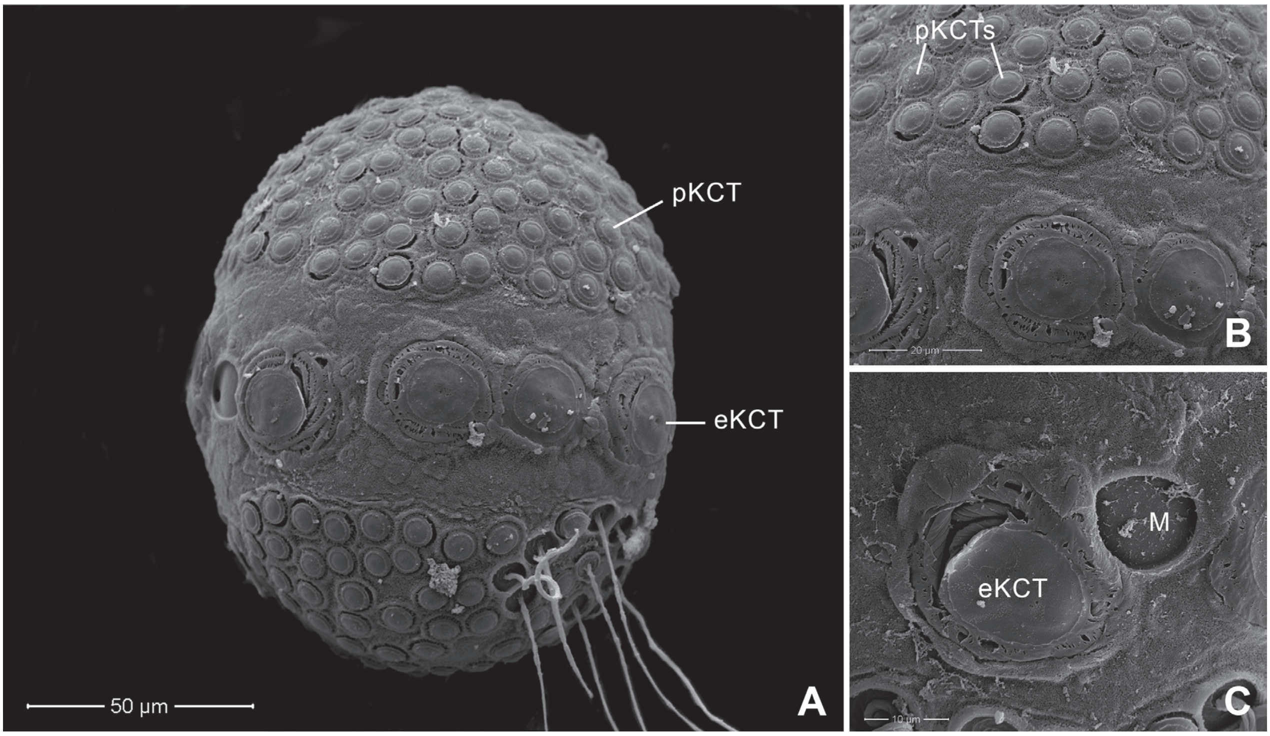

Figure 8. *Afronurus gilliesiana* (Braasch, 1990), SEMs of egg morphology **A** general outline of egg **B** chorion surface between polar KCT (pKCT) and equatorial KCT (eKCT) **C** micropyle (M) and enlargement of eKCTs. Scale bars: 50 µm (**A**); 20 µm (**B**); 10 µm (**C**).

(Fig. 6A) and gill VI (Fig. 6B) obliquely rounded, triangular, with small projection; gill VII (Fig. 6C) broad and asymmetrically oval. Bristles on dorsal face of hind femur spatulate in shape (Fig. E).

Adult male can be distinguished by its genitalia: penis bilobate, expanding into laterally enlarged lobes, the inner part of lobes with a small cleft (Fig. 17C).

Habitat. The nymph of *Afronurus gilliesiana* was reported by Braasch (1990) from Mae Sot district, Tak province in northern Thailand. In this study, *A. gilliesiana* was found restricted to three localities in Chiang Rai province. The habitats are unique with high mountain areas, waterfalls, base rock, and some areas of cobbles. The altitude is higher than 400 meters. The nymphs were found attached to the cobbles, away from the base rock with strong water falling from the waterfall. The male and female adults and eggs are described for the first time.

Distribution. Chiang Rai province (Fig. 18).

Afronurus rainulfiana (Braasch, 1990)

Figs 9A–E, 10A–E, 11A–C, 12A–C

Cinygmina rainulfiana Braasch, 1990: 8, figs 9–12, original description (male and female imago, nymph).

Afronurus rainulfiana – Boonsoong and Braasch 2013: 87.

Material examined. 3 nymphs, Thailand, Kanchanaburi Prov., Huai Kha Yeng, 14°36'20.98"N, 98°34'39.8"E, 937 m, 31.I.2019, W. Anuntaya leg. (ZMKU); 12 nymphs, Narathiwat Prov., Klong Aika Ding, 5°47'45.8988"N, 101°50'5.4996"E,

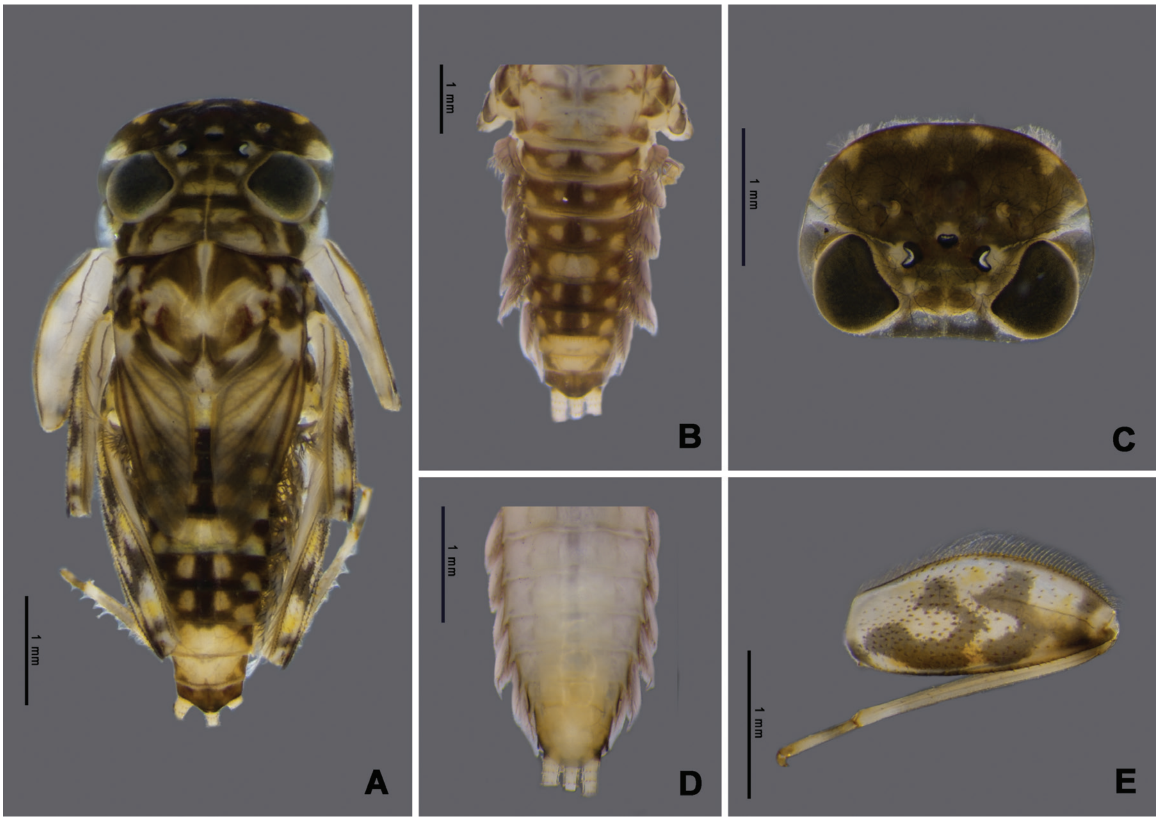

Figure 9. *Afronurus rainulfiana* (Braasch, 1990), larval morphology **A** female habitus **B** tergites I–X **D** sternites III–X **C** head **E** hind leg. Scale bars: 1 mm.

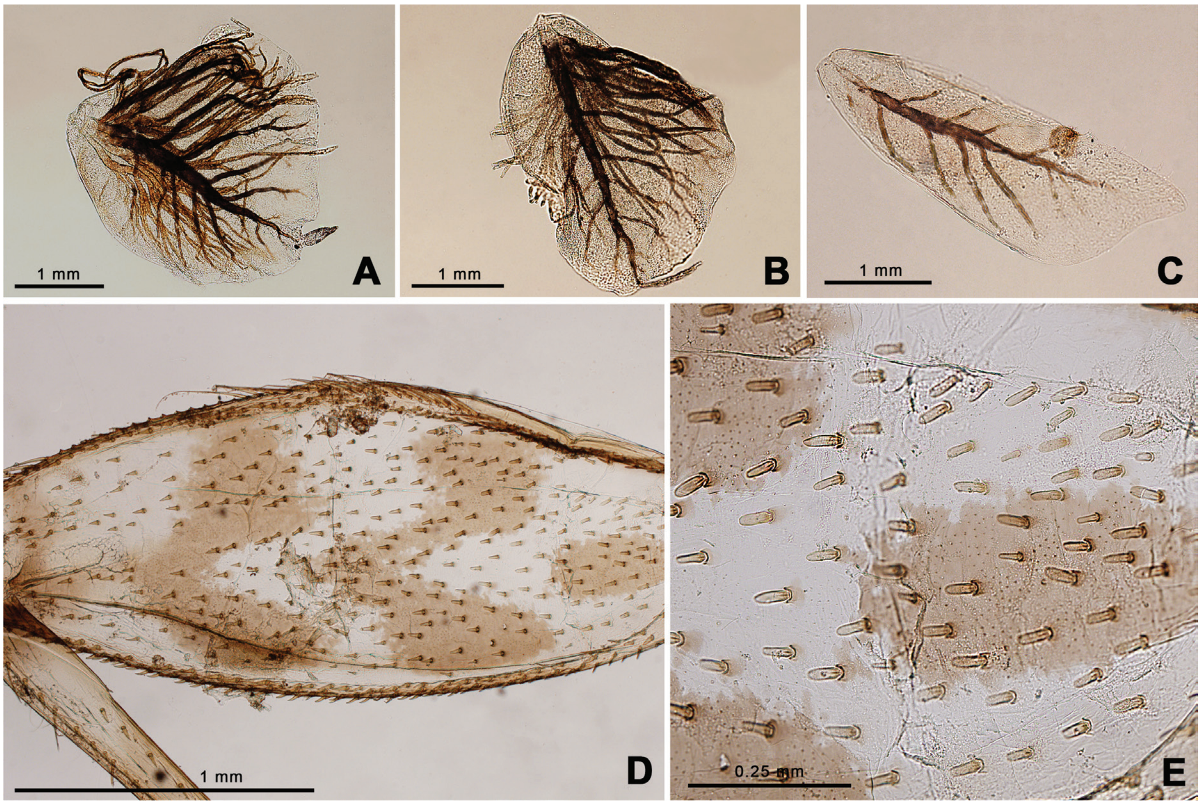

Figure 10. *Afronurus rainulfiana* (Braasch, 1990), larval morphology **A** gill V **B** gill VI **C** gill VII **D** bristles on the dorsal face of the hind femur **E** enlargement of bristles on basal part. Scale bars: 1 mm (**A–D**); 0.25 mm (**E**).

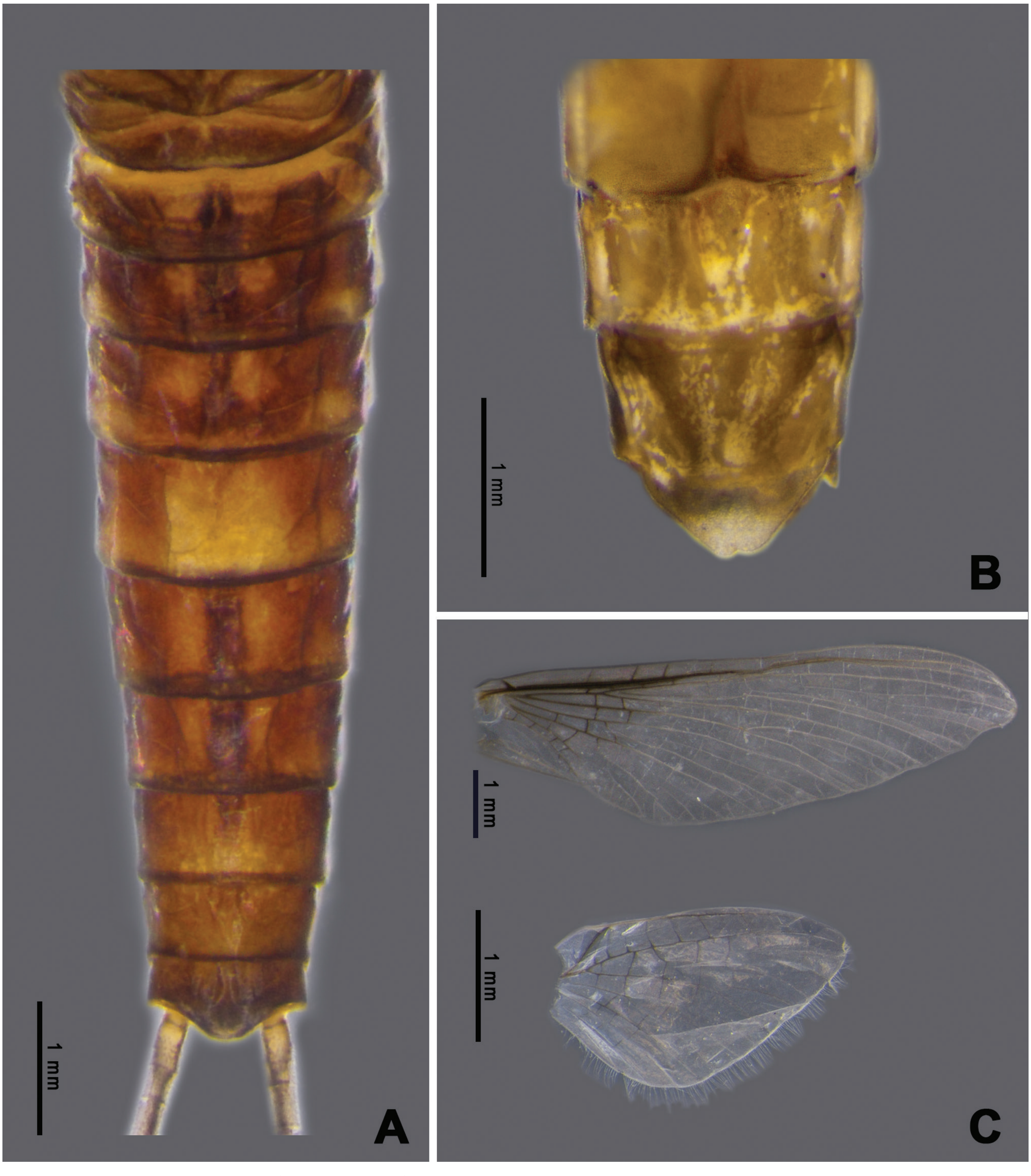

Figure 11. *Afronurus rainulfiana* (Braasch, 1990), female imaginal morphology **A** tergites I–X **B** anal plate **C** fore wing and hind wing. Scale bars: 1 mm.

56 m, 22.IV.2018, W. Anuntaya leg. (ZMKU); 1 nymph, Phetchaburi Prov., Huai Mae Kamoei, 12°58'41.4984"N, 99°34'55.401"E, 119 m, 24.II.2019, W. Anuntaya leg. (ZMKU); 4 nymphs, Ranong Prov., Klong Phon Rang, 9°53'39.4002"N, 98°38'28.8996"E, 10 m, 20.IV.2018, B. Boonsoong leg. (ZMKU); 13 nymphs, Ratchaburi Prov., Bo Klueng, 13°31'27.3612"N, 99°14'39.3606"E, 180 m, 24.XI.2018, W. Anuntaya leg. (ZMKU).

Description. Nymph. See Braasch (1990: 8, figs 9–12, 18.1–18.3).

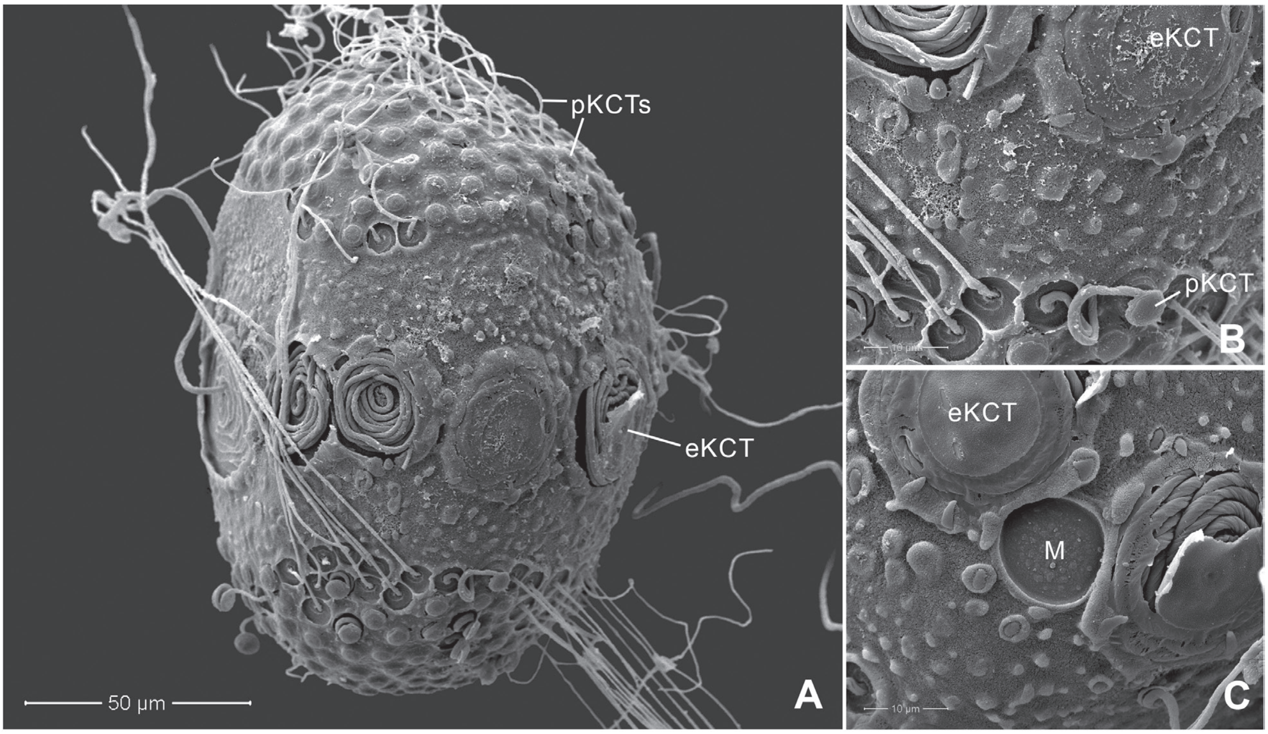

Figure 12. *Afronurus rainulfiana* (Braasch, 1990), SEMs of egg morphology **A** general outline of egg **B** chorion surface between polar KCT (pKCT) and equatorial KCT (eKCT) **C** micropyle (M) and enlargement of eKCTs. Scale bars: 50 μm (**A**); 10 μm (**B**); 10 μm (**C**).

Adult. Female imago (in alcohol, Fig. 11). ***Head*** brown with black round eyes. ***Thorax*.** Mesonotum brown with pale mark at the middle area, anterior part with dark brown heart-shaped marks. Wings transparent; forewing base area of vein thick and dark brown, RS and MP forked basally, MA forked at the middle; hindwings asymmetrical, 1.6× longer than width, RA and MA adjacent at basal of wing (Fig. 11C). ***Abdomen*.** Dorsally brown marked with yellow, tergites II-V with single pair of circular sub-median marks and another pair of circular postero-lateral marks; on tergite V the sub-median mark is fused to form a large square mark, tergites VI and VII with one pair of longitudinal marks each and tergite X pale on anterior part (Fig. 11A); subgenital plate concave at tip; subanal plate extending as triangle-shaped projection and emarginate at tip (Fig. 11B).

Egg. Chorionic surface of egg with dense pKCTs on each pole and eKCTs (Fig. 12A). Equatorial and subequatorial areas with eKCTs and micropyle beside eKCTs (Fig. 12C); area between pKCTs and eKCTs with distinct small tubercles (Fig. 12B), areas of two poles of pKTCs ~ 0.5× that of the whole egg (Fig. 12A).

Remarks. Nymph of *Afronurus rainulfiana* is distinguishable from congeners by the combination of the following characteristics: anterior margin of head with four distinct pale spots (Fig. 9C), lateral surface of the eyes with a large bright triangular spot, and area between eyes with two pairs of circular marks, each mark linked with one straight line. Abdomen tergites II-VII with two pairs of circular markings on sub-median and posterolateral areas (Fig. 9A), pair on sub-median areas fused on tergite V, all markings combined in tergites VIII and IX, and tergite X brown without markings (Fig. 9B). Sternites without markings (Fig. 9C). Gill V (Fig. 10A) and gill VI (Fig. 10B) obliquely rounded, triangular, with apical projection, gill VII narrowly lanceolate (Fig. 10C). Pattern of hind femur as shown in Figs 9E, 10D.

Bristles on the dorsal face of the hind femur both blunt and pointed (Fig. 10E). The imago can also be distinguished by the pattern on its abdomen (Fig. 11A).

Habitat. *Afronurus rainulfiana* was described only as a nymph by Braasch (1990) from Saraburi province and then subsequently by Boonsoong and Braasch (2013) in Mae Sot Distinct, Tak province. In this study, *A. rainulfiana* was found in 20 localities in six provinces. The nymphs attach to the undersides of the cobbles submerged in running water. We reported the female characteristics. The male adults of *A. rainulfiana* are still unknown.

Distribution. Kanchanaburi, Narathiwat, Phetchaburi, Ranong, Saraburi and Tak provinces (Fig. 18).

Afronurus rubromaculata You, Wu, Gui & Hsu, 1981

Figs 13A–E, 14A–E, 15A–C, 16A–E, 17E, F

Cinygmina rubromaculata You, Wu, Gui & Hsu, 1981: 4, figs 14–24 (original description, male and female).
Cinygmina rubromaculata – Wu, Chen, Cong & You, 1986: 1, 67.
Cinygmina rubromaculata – Zhou and Zheng 2003: 758, figs 7, 8, 13,17 (nymph first description).
Afronurus rubromaculatus – Braasch and Jacobus 2011: 65.
Afronurus rubromaculata – Boonsoong and Braasch 2013: 88.
Afronurus rubromaculatus – Zhang et al. 2021: 110.

Material examined. 11 nymphs, Chanthaburi Prov., Klong Phlu Lang, 12°43.207'N, 102°23.321'E, 115 m, 5.VI.2018, W. Anuntaya leg. (ZMKU); 2 nymphs, Kanchanaburi Prov., Tao Taan, 14°38'58.199"N, 98°34'55.8006"E, 116 m, 31.I.2019, W. Anuntaya leg. (ZMKU); 3 nymphs, Nan Prov., Na noi, 18°19'22.0002"N, 100°43'14.0016"E, 289 m, 5.XII.2017, B. Boonsoong leg. (ZMKU); 15 nymphs, Ratchaburi Prov., Kang Som Maew, 13°24'22.32"N, 99°6'43.74"E, 207 m, 24.XI.2018, W. Anuntaya leg. (ZMKU).

Description. Nymph. See Zhou and Zheng (2003: 757, figs 7, 8, nymph first description).

Adult. Male imago. See You et al. (1981: 28, figs 14–24, original description).

Eggs. Chorionic surface of egg with dense pKCTs on each pole and eKCTs (Fig. 16A). Equatorial and subequatorial areas with eKCTs and micropyle next to eKCTs (Fig. 16C); the area between pKCTs and eKCTs smooth (Fig. 16B), areas of two poles of pKTCs ~ 0.47× that of the whole egg (Fig. 16A).

Diagnosis. Nymph of *A. rubromaculata* is easily distinguishable from other *Afronurus* species by the following characteristics: anterior margin of head with four distinct pale yellow markings and a row of four pale dots in front of antenna bases and three pairs of pale markings between eyes (Fig. 13C), thorax with pattern as shown in Fig. 13A. Abdominal tergites II-VII with pair of pale marks on sub-median and posterolateral areas, sub-median marking of tergite V fused, large; tergites VIII and IX each with sub-median pale marking; tergite X with anterior pale area (Fig. 13B). Sternites IX and X brown (Fig. 13D). Gills V (Fig. 14A) and VI (Fig. 14B) obliquely rounded, triangular, with projection; asymmetrical gill VII (Fig. 14C). Markings of hind femur as shown in Figs 13E, 14D. Bristles on the dorsal face of the hind femur pointed (Fig. 14E).

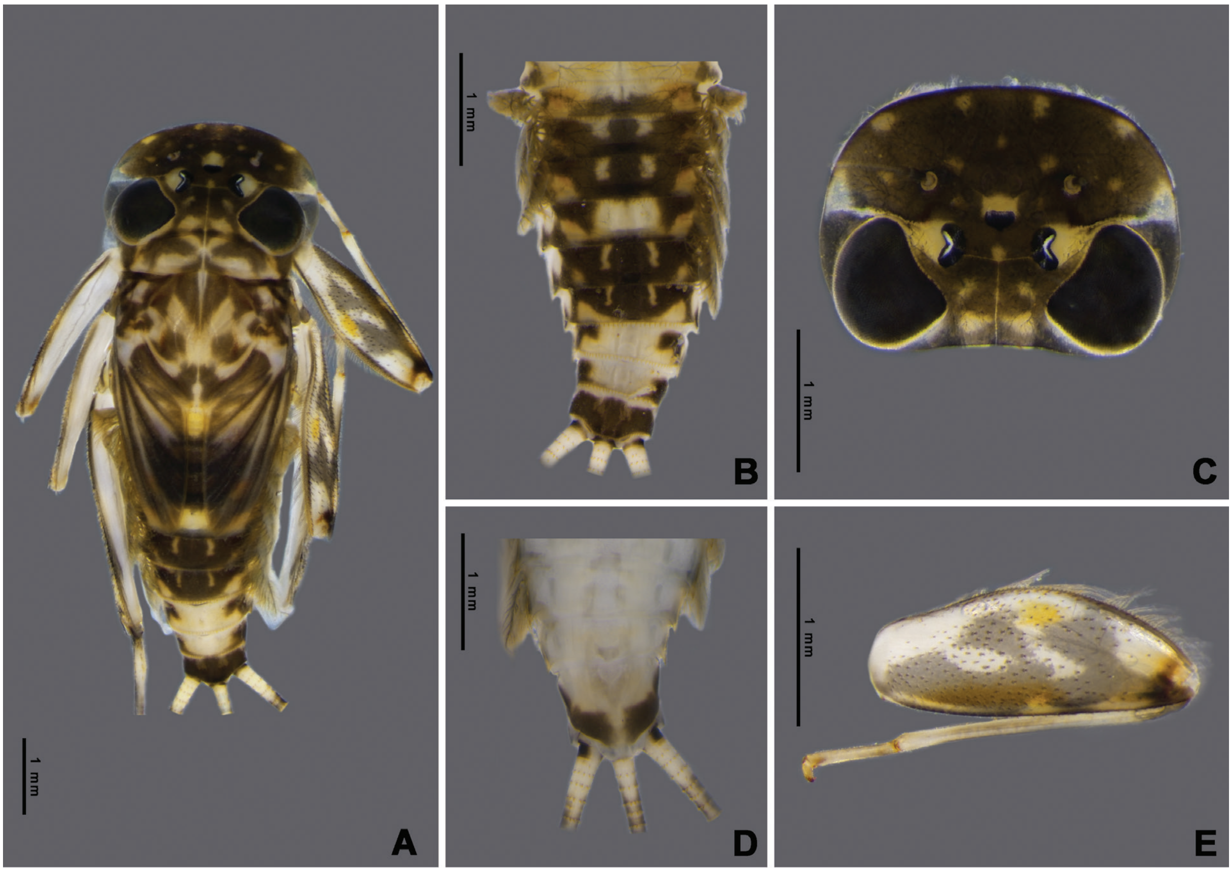

Figure 13. *Afronurus rubromaculata* (You et al., 1981), larval morphology **A** female habitus **B** tergite I–X **D** sternite VI–X **C** head **E** hind leg. Scale bars: 1 mm.

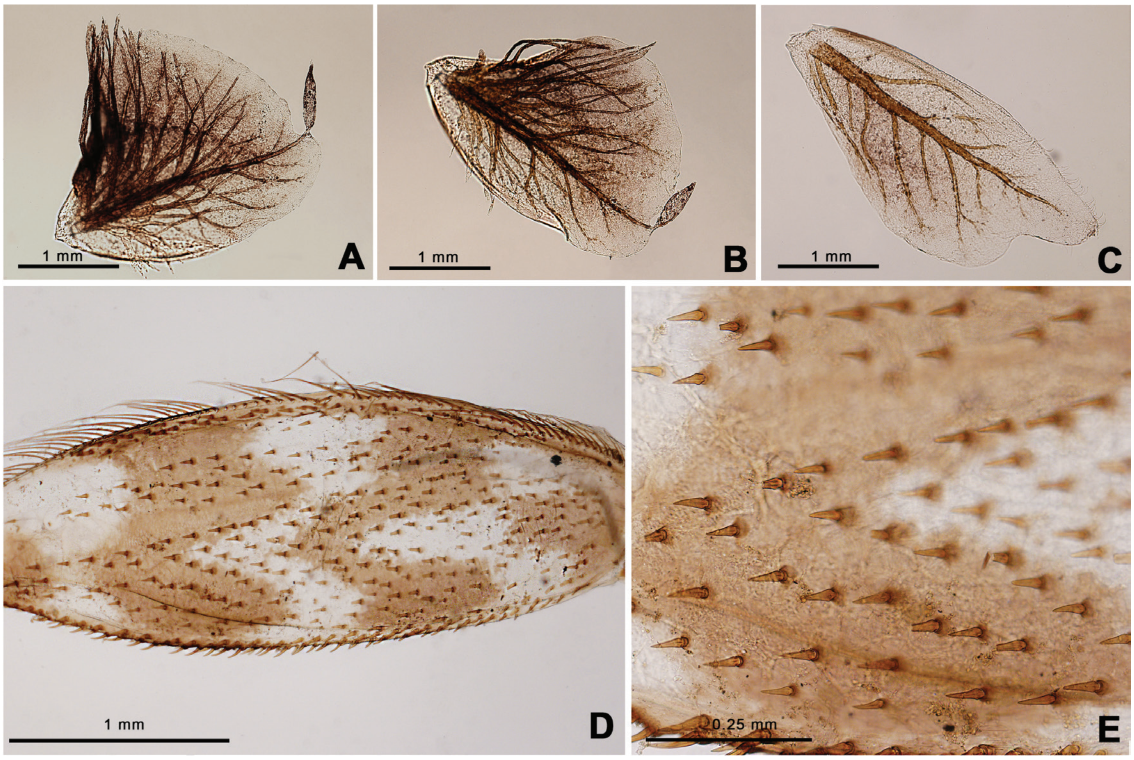

Figure 14. *Afronurus rubromaculata* (You et al., 1981), larval morphology **A** gill V **B** gill VI **C** gill VII **D** bristles on the dorsal face of the hind femur **E** enlargement of bristles on basal part. Scale bars: 1 mm (**A–D**); 0.25 mm (**E**).

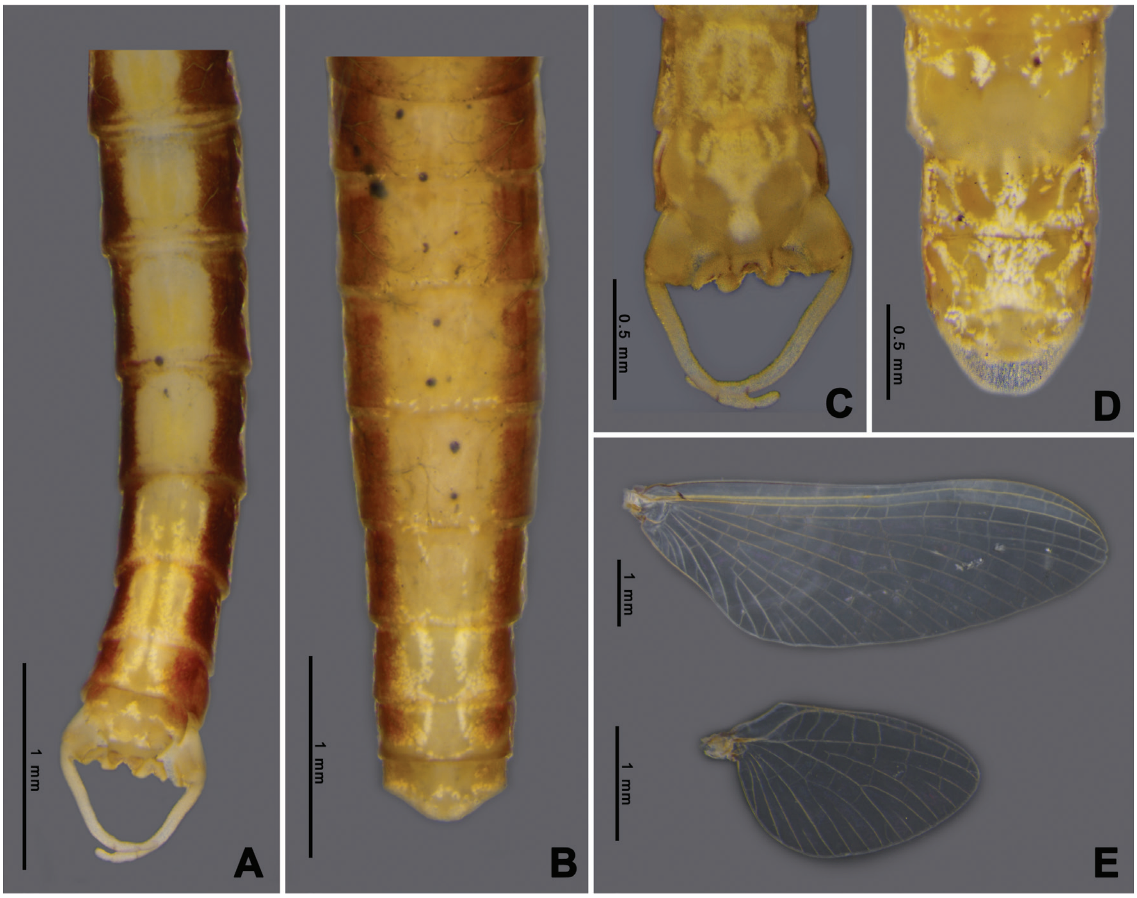

Figure 15. *Afronurus rubromaculata* (You et al., 1981), imaginal morphology **A** male tergites III–X **B** female tergites II–X **C** male genitalia **D** female anal plate **E** fore wing and hind wing. Scale bars: 0.5 mm (**C, D**); 1 mm (**A, B, E**).

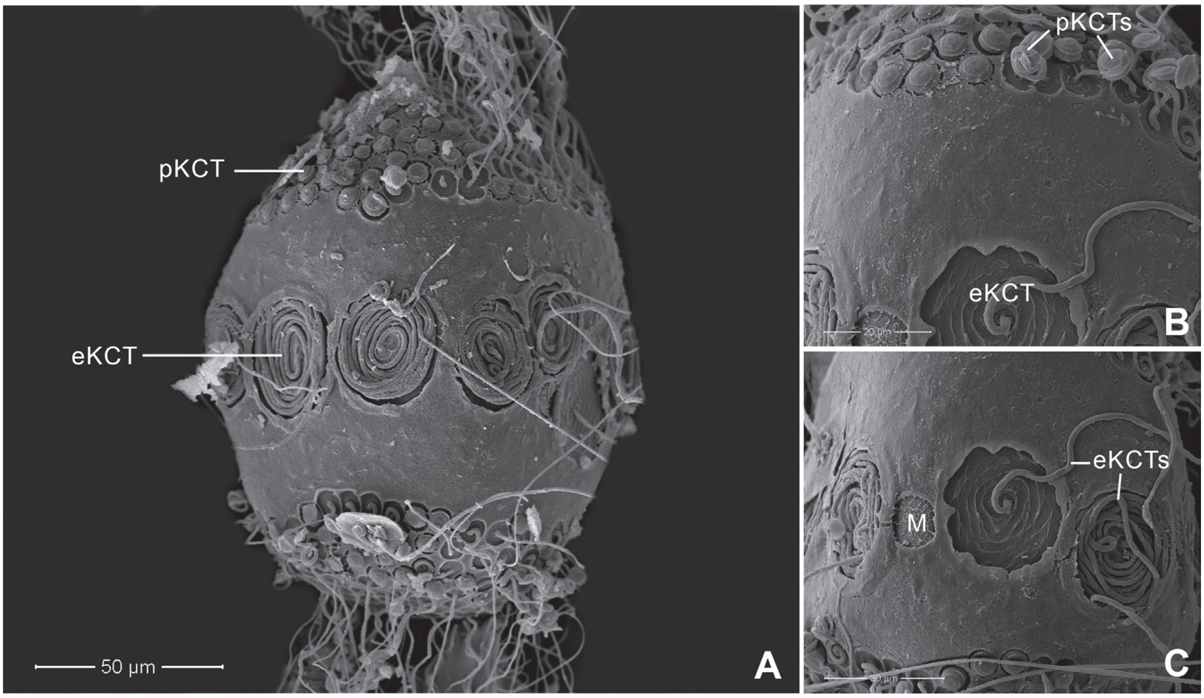

Figure 16. *Afronurus rubromaculata* (You et al. 1981), SEMs of egg morphology **A** general outline of egg **B** chorion surface between polar KCT (pKCT) and equatorial KCT (eKCT) **C** micropyle (M) and enlargement of eKCTs. Scale bars: 50 µm (**A**); 20 µm (**B**); 10 µm (**C**).

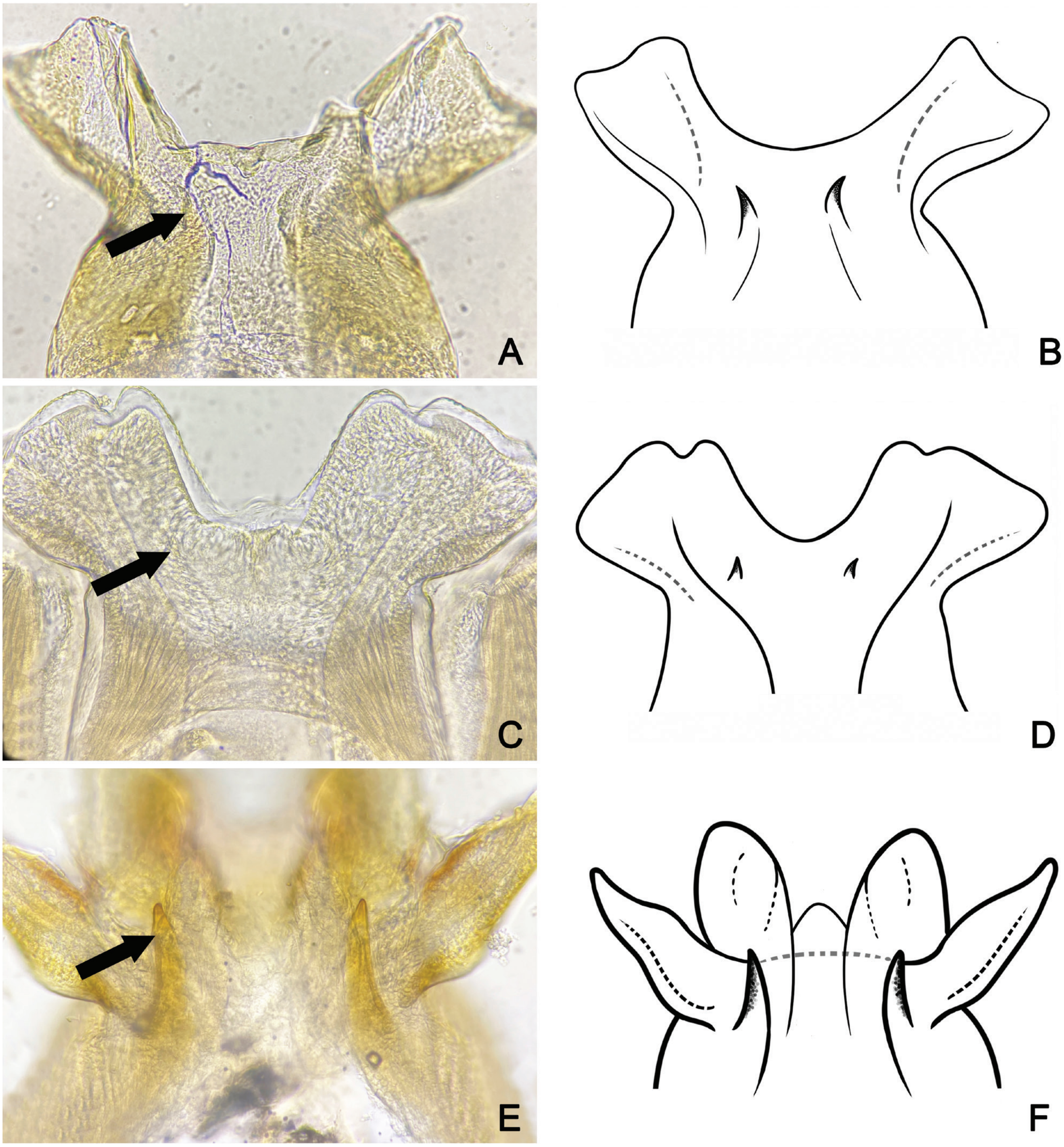

Figure 17. male genitalia **A, B** *A. cervina* **C, D** *A. gilliesiana* **E, F** *A. rubromaculata* (arrow indicates titillators).

Adult male is distinguishable by genitalia and abdominal pigmentation; genital plate emarginated, divided into two lobes, inner lobe broad. The cleft between lobes U-shaped with a small tubercle (Figs 15C, 17E, F), outer lobe canine-like. The titillators robust, canine-like. Forceps comprising four segments, segment I very short, length ratio of segment II to segment III to segment IV is 0.45: 0.14: 0.13 (Fig. 8C). Adult female with anal plate triangular, slightly truncate at tip (Fig. 15D).

Distribution. Chanthaburi, Kanchanaburi, Nan, and Ratchaburi provinces (Fig. 18).

Remarks. *Afronurus rubromaculata* is a common species in Thai streams and widely distributed (Fig.18). Suitable localities for *A. rubromaculata* appear

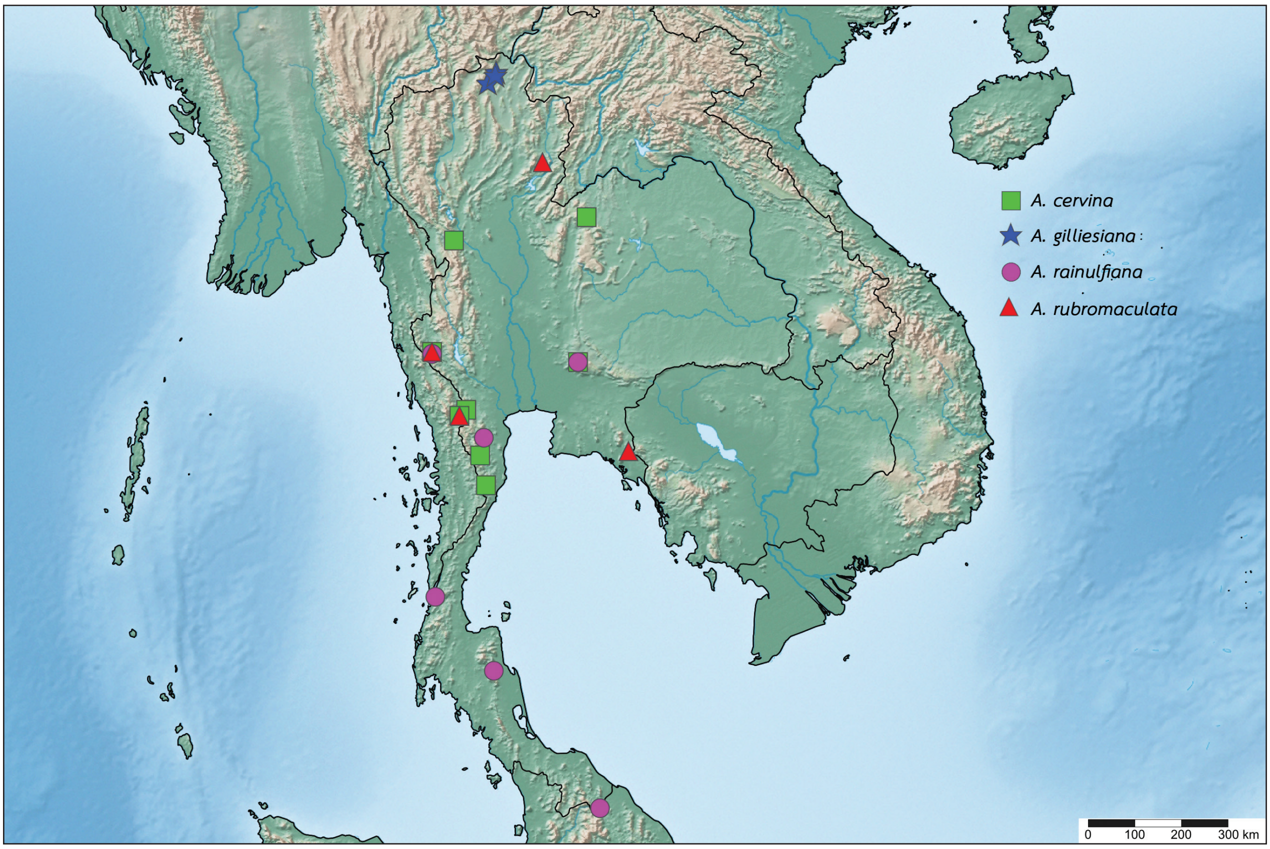

Figure 18. Distribution map of the genus *Afronurus* in Thailand.

to be with cobbles deep in running water, the area mostly covered with riparian fields. *Afronurus rubromaculata* has a unique pattern on the abdomen. She et al. (1995) described the differentiation of the spine position between *A. rubromaculata* and *A. hainanensis*, but Zhou (2013) synonymised *A. rubromaculata* and *A. hainanensis* due to the similarity of the spine on the penes that varies in size. However, in Thailand, the penial character of *A. rubromaculata* is distinct from that of other species of *Afronurus* in Thailand (Table 3). In this study, the egg morphology of *A. rubromaculata* was similar to that of a Chinese specimen, with a smooth surface in the equatorial area (Zhang et al. 2021: fig. 6E).

Molecular analysis

The Bayesian inference tree is shown in Fig. 19. The 37 samples of Thai *Afronurus* are grouped into four major clades: *Afronurus rainulfiana*, *A. cervina*, *A. gilliesiana*, and *A. rubromaculata*. Each clade is monophyletic, and strongly supported by the Bayesian posterior probabilities. The intraspecific and interspecific genetic distances are given in Table 2. The range of genetic distances within species is 3%–4%, whereas the range of genetic distances between species is 7%–30%. *Afronurus rainulfiana* is clearly supported as a monophyletic clade with the sequence of *Afronurus* sp.1 (Surat Thani Prov.) from Yanai et al. (2017). The *A. cervina* clade was divided into two sub-clades due to geography; however, the intraspecific genetic distance is 3%. The species *A. mnong* (Vietnam) was clustered with the *A. cervina* clade, with a low genetic distance (7%). By contrast, *A. meo* (Vietnam) was grouped with the *A. gillie-*

Table 2. Genetic distances (COI) of seven *Afronurus* species using the Tamura 3-parameter (Gamma).

Species	Tamura 3-parameter (Gamma) distances										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1. ***A. cervina* (TH)**	0.03										
2. ***A. gilliesiana* (TH)**	0.23	0.03									
3. *A. hyalinus* (TW)	0.22	0.23	-								
4. *A. meo* (VN)	0.19	0.18	0.24	-							
5. *A. mnong* (VN)	0.07	0.19	0.21	0.18	-						
6. *A. namnaoensis* (TH)	0.23	0.30	0.15	0.26	0.22	-					
7. ***A. rainulfiana* (TH)**	0.22	0.27	0.22	0.24	0.22	0.24	0.03				
8. ***A. rubromaculata* (TH)**	0.22	0.29	0.16	0.26	0.22	0.04	0.24	0.04			
9. *A. rubromaculata* (CN)	0.20	0.23	0.21	0.18	0.19	0.22	0.24	0.22	-		
10. *Afronurus* sp. (TH)	0.22	0.27	0.22	0.26	0.22	0.23	0.02	0.24	0.24	-	
11. *Anapos zebratus* (IT)	0.20	0.26	0.22	0.24	0.21	0.27	0.21	0.25	0.20	0.22	-

CN = China, IT = Italy, TH = Thailand, TW = Taiwan, VN = Vietnam.

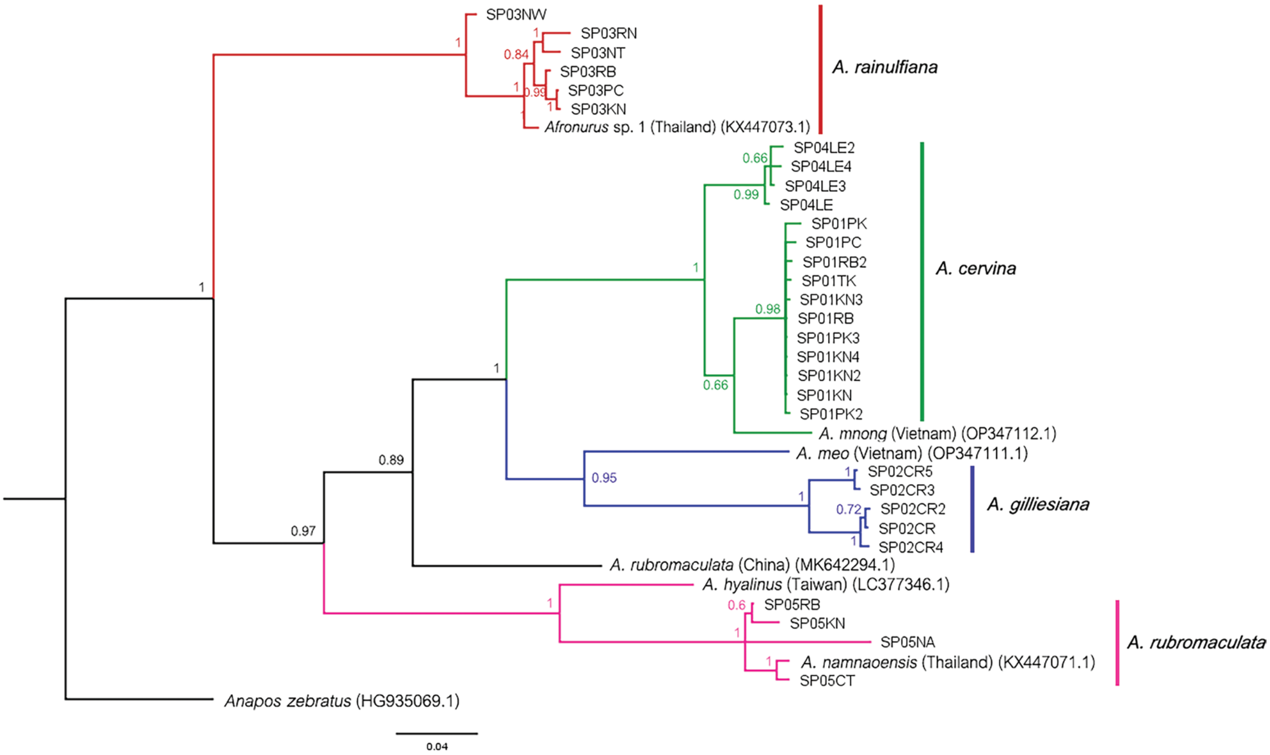

Figure 19. Bayesian inference tree of the DNA barcoding (COI) profile for 37 sequences of the genus *Afronurus* with branch probability support.

siana clade and showed a relatively high genetic distance (18%). *Afronurus hyalinus* (Taiwan) was clustered with the Thai clade of *A. rubromaculata*. Surprisingly, the *A. rubromaculata* sequence from China is in a different clade than the Thai *A. rubromaculata* sequence (genetic distance 22%), while *A. namnaoensis* was clustered in the *A. rubromaculat*a clade with low genetic distance (4%).

Table 3. Comparison of mature nymph characteristics of four Thai *Afronurus* species.

Characters	*A. cervina*	*A. gilliesiana*	*A. rainulfiana*	*A. rubromaculata*
Anterior margin of head	Without any marks[a]	2 pairs of weak marks[b]	3 pairs of bright spots[b]	3 pairs of bright spots
Abdominal pattern	Tergites I, II, VIII, IX pale along all the tergite; tergites III–VII with 2 pairs of pale marks, the pair on sub median exclamation mark-shaped, another pair on sublateral obliquely; tergite V fused; tergite X mostly dark	Tergites I, II, VIII, IX yellowish; tergites III–VII with 2 pairs of markings; 1 pair of elliptical marks on submedian and large circular mark on posterolateral area; tergite V all mark fused; tergite VII with circle marked on posterolateral; tergite X brownish	Tergites I, VIII, IX pale from median to lateral; tergites II-VII with 2 pair of circular marks on sub median and posterolateral areas; tergite V pair of marks on sub median are fused; tergite X brown with no marking	Tergites I, VIII, IX pale from median to lateral areas; tergites III–VII with 1 pair of small longitudinal marks on sub median and another pair of larger marks on posterior area; tergite V with pair of marks on sub median fused; tergite X with transverse marking on anterior area
Setae on hind femur*	B (blunted)	B (spatulated)	B (blunted)	B (blunted)
	M (blunted)	M (spatulated)	M (pointed and blunted)	M (pointed)
	D (blunted)	D (spatulated)	D (pointed)	D (pointed)
Gill VII	Leaflet, asymmetrical, expanded at tip	Leaflet, asymmetrical, 2× longer than wide	Long, end of one side of the gill expanded and pointed at tip	Long, end of gill with 2 lobes; one lobe expanded and rounded at apex
Distribution	Southeast Asia (Thailand, Vietnam)	Southeast Asia (Thailand)	Southeast Asia (Thailand)	Southeast Asia (Thailand), East Palearctic (China)

[a] Braasch and Soldán (1984); [b] Braasch (1990; definition based on fig. 21, p. 199); *B = basal area of femur; M = median area of femur; D = distal area of femur.

Key to species of the mature nymph of the genus *Afronurus* in Thailand

1 Head without any dorsal markings **2**
– Head with markings dorsally **3**
2 Gill I with sharply pointed apex .... ***A. namnaoensis***
– Gill I up-turned, banana-shaped .... ***A. cervina***
3 Head with indistinct spots dorsally .... ***A. dama***
– Head with distinct spots dorsally **4**
4 Head with 2 pairs of pale dots dorsally (Fig. 5D) .... ***A. gilliesiana***
– Head with 3 pairs of bright spots dorsally **5**
5 Gill VII unsymmetrically ovaloid, obtusely pointed apically (Fig. 14C) .... ***A. rubromaculatus***
– Gill VII narrowly lanceolate (Fig. 10C) .... ***A. rainulfiana***

Discussion

In this study, four species belonging to the genus *Afronurus* were found in Thailand: *A. cervina*, *A. gilliesiana*, *A. rainulfiana*, and *A. rubromaculata*. The identifications were based on a combination of morphology, ootaxonomy, and molecular analyses. When compared to the previous studies by Braasch (1990) and Boonsoong and Braasch (2013), who reported six species of *Afronurus* in Thailand, two species (*A. dama* and *A. namnaoensis*) of *Afronurus* were not found in this study because the specific habitats of their nymphs were not sampled. *Afronurus dama* was recorded by Braasch (1990) from Nam Tok Ban Du, Chiang Rai province, and only adult specimens were found; Boonsoong and Braasch (2013) referred to the distribution of *A. namnaoensis* from Chaiyaphum, Petchabun, Mae Hong Son, and Chiang Mai provinces, where it was found in high mountain ranges that block dispersion and could be the cause of the absence of this species at our sampling points.

Taken together, the analysis results indicate that *A. rainulfiana* is a common species that is most widely distributed in all regions of Thailand. We found this species to be clearly distinguished from other species based on nymph, imago, and egg morphologies and molecular analysis. The results of molecular analysis showed a more distant relationship between *A. rainulfiana* and other groups, in agreement with the morphological characteristics. For *A. gilliesiana*, the abdominal pattern resembling that of *A. cervina* (Table 3) was also supported by the Bayesian inference tree. In addition, the tree indicated a division of the *A. cervina* clade into two subclades: the first clade includes *A. cervina* from Loei province (northeastern) and the second clade is from the western region of Thailand.

The intraspecific distances of the Thai *Afronurus* species are low (ranging from 2.8 to 4%), which is lower than the cut-off of 4% (Hebert et al. 2003; Ball et al. 2005; Zhou et al. 2010). The genetic distance data obtained in this study indicates a distance of the different species between 22-30% for the Thai *Afronurus* species. The molecular result was useful for establishing that *A. rainulfiana* was clearly monophyletic but on the contrary, the fact that Thai *A. rubromaculata* clusters with *A. hyalinus* and *A. namnaoensis* but is separated from *A. rubromaculata* from China still needs further study.

Egg characteristics have also proved useful to identify Thai *Afronurus* species (Table 4), as the morphology of the eggs in African and Asian species indicated differences in the size of the large equatorial KCTs (Belfiore et al. 2003). Kang and Yang (1994) also reported differences in equatorial and subequatorial areas of the chorion from Taiwan species.

Table 4. Comparison of adult and egg characteristics of four Thai *Afronurus* species.

Characters	*A. cervina*	*A. gilliesiana*	*A. rainulfiana*	*A. rubromaculata*
Abdominal pattern	Middle area brown with yellow patch along the margin, tergites III- VIII with 1 pair thick lines, each tergite with straight line	Middle area brown with 1 pair of longitudinal yellow marks, outer margin pale yellow	Dorsum brown with yellow marks; tergites II-V with 1 pair of circular marks on sub median and another pair of circular marks on posterolateral; on tergite V the mark on sub median is fused to large square shape; tergites VI, VII with 1 pair of longitudinal marks; tergite VIII, IX mostly pale; tergite X pale on anterior part only	Middle area pale yellow with a pair of oval yellow marked, outer margin brownish
Genitalia	Emarginated to forked lobes, the outer ends stronger than the inner, between each lobe cone-shaped tubercle	Bilobed, expanded laterally into enlarged lobes, terminal lobe with 3 serrations, cleft between lobe U-shaped	N/A	Emarginate, each plate divided into 2 lobes, inner lobe broad, outer lobe canine-like, cleft between lobes U-shaped with one serration[c]
Terminal segment of female	Subanal plate tongue-shaped, slightly truncate at tip[a]	Subanal plate extended as trapezium shape, concave at tip	Subgenital plate concave Subanal plate extended as triangle shape and emarginated at tip	Subanal plate extended, rounded at tip
Chorionic surface	Smooth	Smooth	Scattered small tubercles	Smooth
Polar KTCs covering area	0.54×	0.7×	0.5×	0.47×

[a] Braasch and Soldán (1984), [b] You et al. (1981: definition based on fig. 19, p. 4).

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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

Anuntaya Wongyam: Writing - Original Draft, Investigation, Methodology, Data curation; Michel Sartori: Conceptualization, Visualization, Validation, Writing - Review & Editing; Boonsatien Boonsoong: Conceptualization, Validation, Writing - Review & Editing, Project administration, Funding acquisition.

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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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Research Article

Illustrated key to the European genera of Opiinae (Hymenoptera, Braconidae), with the description of two new Palaearctic genera and two new species

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Abstract

An illustrated key to the European genera of the subfamily Opiinae (Hymenoptera, Braconidae) is presented and two new genera are described and illustrated: *Cavopius* **gen. nov.** (type species: *Opius* (*Agnopius*) *daghoides* Zaykov & Fischer, 1983) from West and East Palaearctic regions and *Pseudosteres* **gen. nov.** (type species: *Biosteres adanaensis* Fischer & Beyarslan, 2005) from West Palaearctic region. Two new species are described and illustrated: *Cephaloplites gijswijti* **sp. nov.** from Greece and *Cavopius depressorius* **sp. nov.** from S. Korea. *Opius* (*Hypocynodus*) *kilisanus* Fischer & Beyarslan, 2005 is a new synonym of *Cephaloplites mocsaryi* Szépligeti, 1897. The following new combinations are proposed: *Cavopius daghestanicus* (Telenga, 1950), **comb. nov.**, *C. daghoides* (Zaykov & Fischer, 1983), **comb. nov.**, *Pseudosteres adanaensis* (Fischer & Beyarslan, 2005), **comb. nov.**, *P. arenaceus* (Jakimavičius, 1986), **comb. nov.**, *P. christenseni* (Papp, 1982), **comb. nov.**, *P. pseudarenaceus* (Fischer & Beyarslan, 2005), **comb. nov.**, and *P. riphaeus* (Tobias, 1986), **comb. nov.** Keys to species are provided for *Cavopius* **gen. nov.**, *Cephaloplites* Szépligeti, 1897, and *Pseudosteres* **gen. nov.**

Key words: Bulgaria, *Cavopius*, Greece, Korea, new combination, new genus, new synonym, *Pseudosteres*, Turkey

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Introduction

Opiinae is a large subfamily of the family Braconidae with ca 2,000 valid species and 39 genera according to Yu et al. (2016). It is a common group containing generally small (body length 2–5 mm) parasitoid wasps of mainly mining or fruit-infesting dipterous larvae. The subfamily has a worldwide distribution and its species have been reviewed by Fischer (1972, 1977, 1986, 1987). Wharton (e.g., 1987, 1988, 1997) published important updates and some additions for the existing keys to the genera of Opiinae, but the number of genera and the limits of some genera remain a matter of discussion, especially of *Opius* Wesmael, 1835 and of *Eurytenes* Foerster, 1863. The host of subgenera as used by Fischer (e.g., 1972) is mainly based on one character only and some specimens can be assigned to three subgenera with the key by Fischer (1972) because of intermediate conditions. In Li et al. (2013) most of the subgenera used by Fischer in his revisions were synonymised, but *Phaedrotoma* Foerster, 1863 was recognised as a valid genus for the species with symmetrical mandibles and excluded from the genus *Opius* Wesmael (following van Achterberg (1997, 2004) and van Achterberg and Salvo (1997)). However, this proved problematic because of intermediate specimens, even belonging to the same species and, therefore, *Phaedrotoma* is here synonymised with *Opius*.

Among the large collection of Opiinae in Naturalis Biodiversity Center (Leiden) two new genera were discovered and a new species of the rare genus *Cephaloplites* Szépligeti. The new taxa are described, keyed, and illustrated below and an illustrated key to the genera is provided. This paper is part of the revision of the European species of the subfamily Opiinae.

In this paper the criterium for recognition as a separate (new) genus is the possession of a set of presumably derived characters. The results of molecular research published in Li et al. (2013) support, at least partly, the choices made as far as taxa were included but also show that the inclusion of *Phaedrotoma* in *Opius* makes the latter genus more polyphyletic. Unfortunately, *Opius* s.l. lacks a set of derived characters, but we do not yet have enough knowledge of the phylogeny of the Opiinae to solve this problem.

Materials and methods

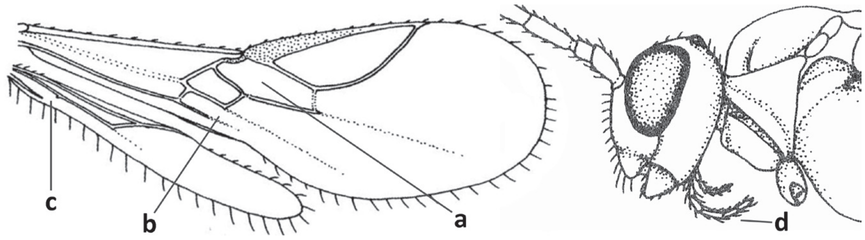
The specimens were either collected in a Malaise trap or collected by using a sweep net. The Malaise trap specimens were chemically treated with a mixture of xylene + alcohol 96% and amylacetate, respectively (AXA-method; van Achterberg 2009). For identification of the subfamily Opiinae, see van Achterberg (1990, 1993, and 1997); for references to the Opiinae, see Yu et al. (2016).

Morphological terminology follows van Achterberg (1988, 1993), including the abbreviations for the wing venation. Measurements are taken as indicated by van Achterberg (1988): for the length and the width of a body part the maximum length and width is taken, unless otherwise indicated. The length of the mesosoma is measured from the anterior border of the mesoscutum to the apex of the propodeum and of the tergite I from the posterior border of the adductor to the medio-posterior margin of the tergite.

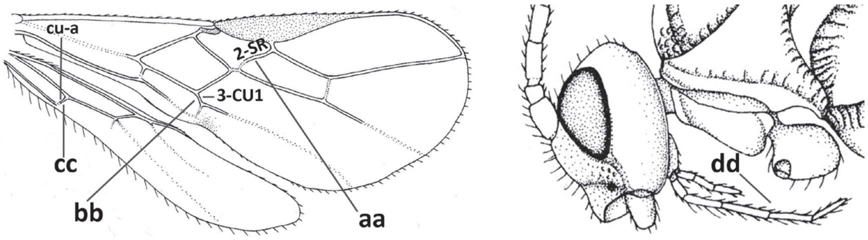
Observations and descriptions were made either under an Olympus SZX11 stereomicroscope. Photographic images were taken with a Canon 5Ds 50.6-megapixel camera combined with a Canon MP-E 65 mm f/2.8 1–5× Macro lens, Laowa Macro Twin flash KX-800 and an electronic WeMacro Z-stepper rail. The photos were stacked with Helicon Focus 7 software. The type specimens are deposited in the Naturalis collection (**RMNH**) at Leiden. **NMW** and **ZISP** stand for Naturhistorisches Museum at Vienna and Zoological Institute at St. Petersburg, respectively.

Key to European genera of Opiinae

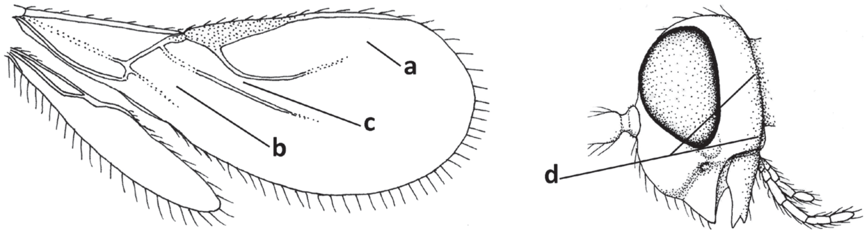
1 Vein 2-SR of fore wing absent (a); first subdiscal cell of fore wing open apically (b); vein cu-a of hind wing absent (c); segments of maxillary palp usually shorter (d); [clypeus wide, short, and impressed; hind wing narrow]..**2**



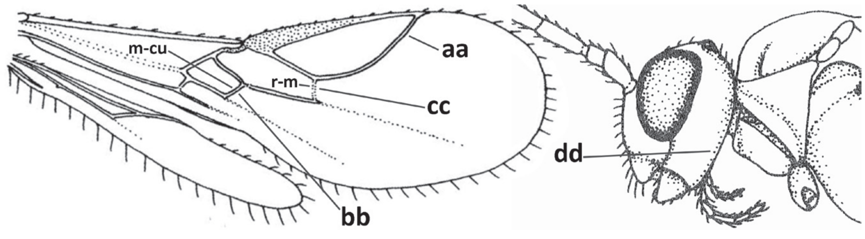
– Vein 2-SR of fore wing present (aa), rarely absent; first subdiscal cell of fore wing at least partly closed by vein 3-CU1 apically (bb); vein cu-a of hind wing nearly always present (cc); segments of maxillary palp often elongate (dd) **3**



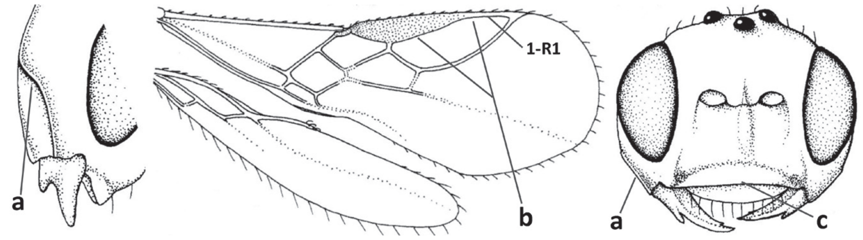
2 Marginal cell of fore wing open apically (a) and long; veins m-cu (b) and r-m (c) of fore wing absent; occipital carina absent laterally (d); [metasomal tergites I and III more or less coriaceous or rugulose; tergites IV–VI largely retracted]..***Indiopius* Fischer, 1966**



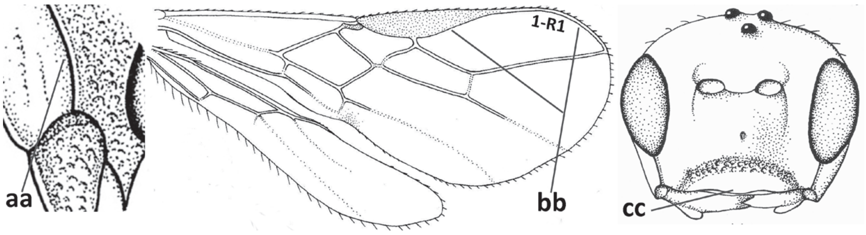
– Marginal cell of fore wing closed apically (aa) and shortened; veins m-cu (bb) and r-m (cc) of fore wing present; occipital carina present laterally (dd) .... ***Pokomandya* Fischer, 1959**



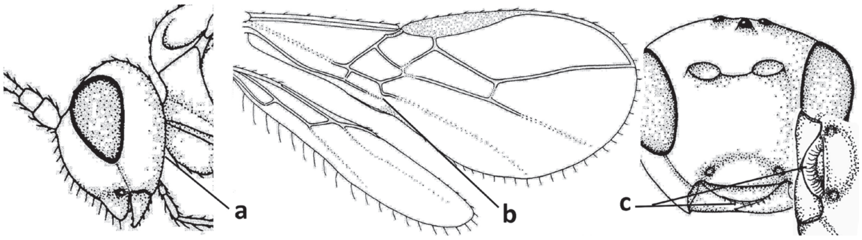
3 Medio-laterally occipital carina near level of middle of eye strongly curved, resulting in an oblique part of carina or crest (a); vein 1-R1 of fore wing shorter than pterostigma (b); clypeus flat and short (c)................................ .... ***Hoplocrotaphus* Telenga, 1950**



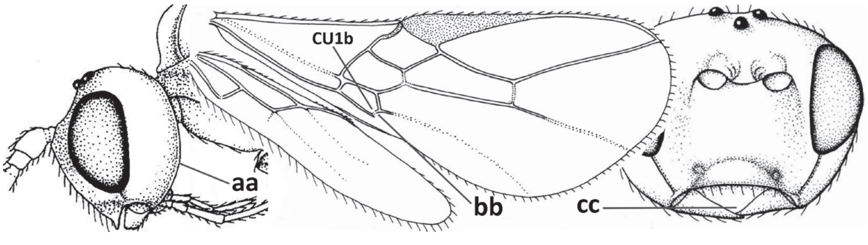
– Medio-laterally occipital carina straight or slightly curved (aa) or carina absent; vein 1-R1 of fore wing usually as long as or longer than pterostigma (bb); if shorter then clypeus more or less convex and longer (cc).............**4**



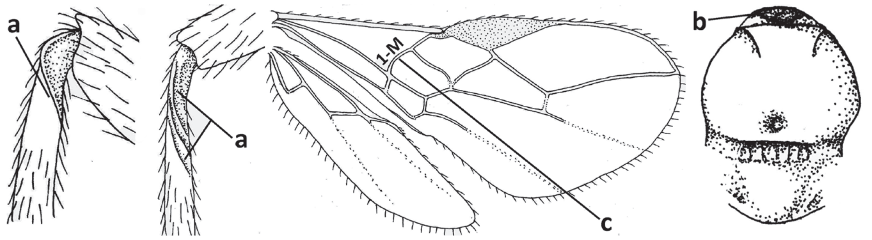
4 Occipital carina completely absent (a) **and** vein CU1b of fore wing absent (b); convex labrum with long curved setae forming a comb ventrally (observe in oblique view and mandibles at least partly open: c); [body black; malar suture slightly longer than basal width of mandible; anterior tentorial pits close to apical margin of clypeus; ovipositor sheath shorter than apical height of metasoma]........................... ***Desmiostoma* Foerster, 1863**



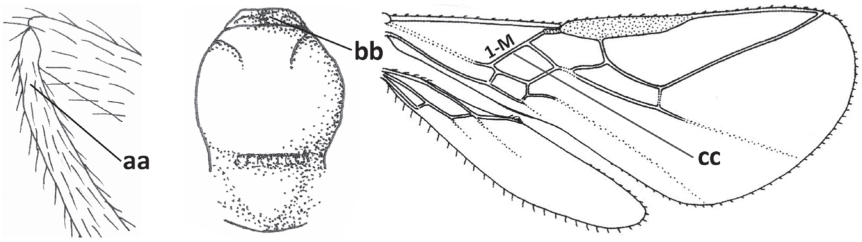
– Occipital carina present laterally (aa); if rarely absent then vein CU1b of fore wing present (bb); labrum flattened and without comb of setae ventrally (cc) **5**



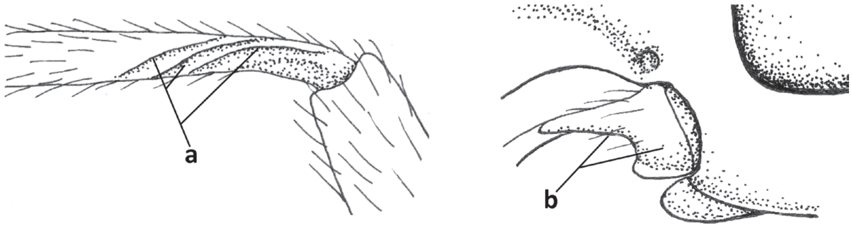
5 Inner side of hind tibia with 1 carina or 3 or 4 carinulae baso-laterally in a glabrous area (a); pronope slit-like, round or elliptical, often large (occupying most of medio-dorsal part of pronotum) and deep (b); vein 1-M of fore wing more or less curved (c); [propodeum often coarsely (reticulate-) rugose and with more or less curved transverse carina dorso-laterally; dorsope absent or slightly impressed because of strongly developed dorsal carinae; hypostomal flange usually large below base of mandible] **6**



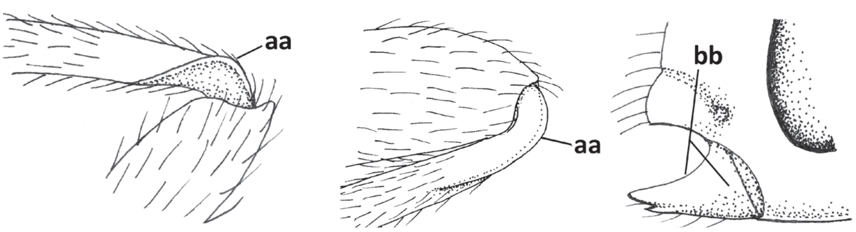
– Inner side of hind tibia without carina or carinulae baso-laterally and largely setose (aa); pronope usually round and smaller or pronotum only with shallow transverse groove (bb); vein 1-M of fore wing usually straight (cc) **7**



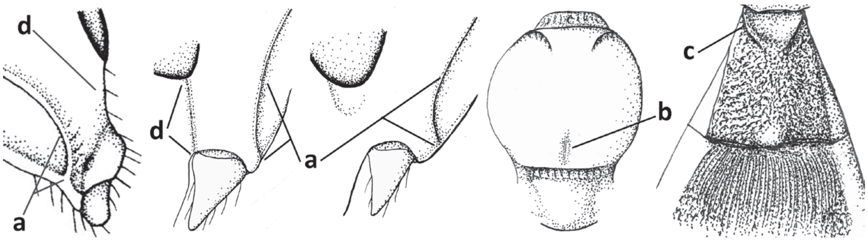
6 Inner side of hind tibia with 3 or 4 fine carinulae (a; rarely 5 or 6 carinulae); mandible distinctly asymmetrical, more narrowed submedially (b) and widened ventro-basally, but less so in *O. pactus*)... ....***Opiognathus* Fischer, 1972**



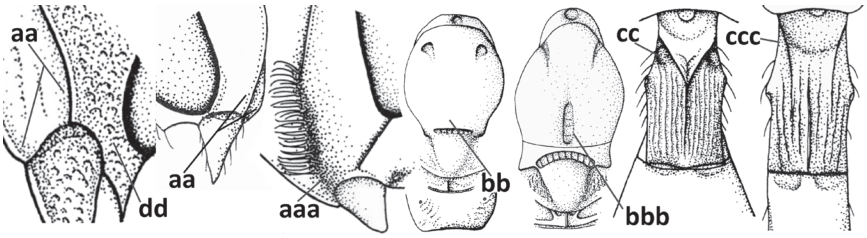
– Inner side of hind tibia with one rather lamelliform carina (aa); mandible nearly symmetrical, gradually narrowed submedially (bb) and usually slightly widened ventro-basally .... ***Utetes* Foerster, 1863 s.str.**



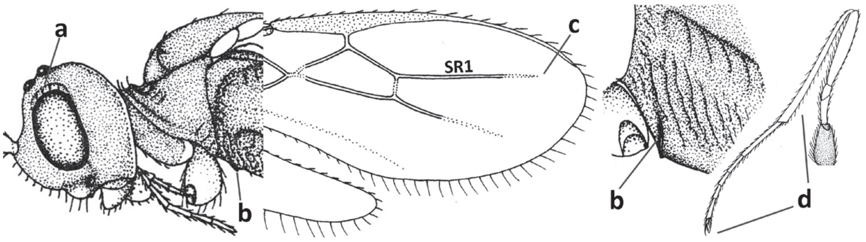
7 Occipital carina above mandibular base curved and (just) meeting hypostomal carina (a); medio-posterior depression of mesoscutum present (b), only rarely absent or obsolescent; dorsope absent (c); malar space comparatively long (d); [if dorsope present and medio-posterior depression of mesoscutum absent, see *Atormus* van Achterberg] ....***Apodesmia* Foerster, 1863**



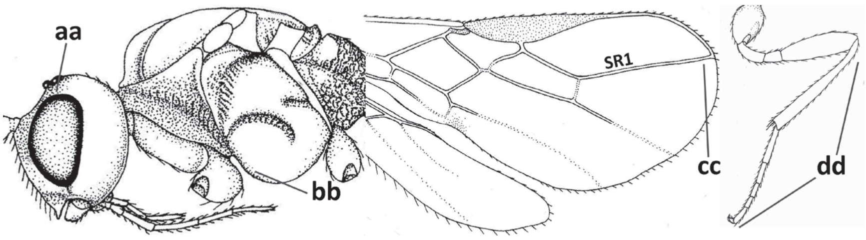
– Occipital carina not or slightly curved ventrally and remain removed from hypostomal carina (aa) or occipital carina absent laterally (aaa); medio-posterior depression of mesoscutum absent (bb) or present (bbb); dorsope present (cc) or absent (ccc); if absent then malar space usually short (dd)................ **8**



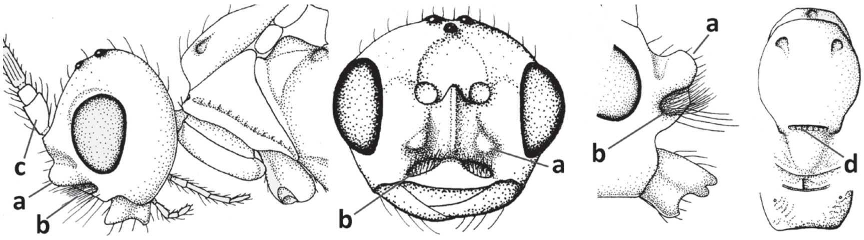
8 Crenulate depression above eye present (a); prepectal carina more or less developed ventrally (b); vein SR1 of fore wing reduced apically, resulting in an open marginal cell (c); hind tibia and tarsus, and tarsal claws very slender (d); [medio-posteriorly scutellum with continuation of lateral elevated area; malar suture absent or obsolescent] ....***Ademon* Haliday, 1833**



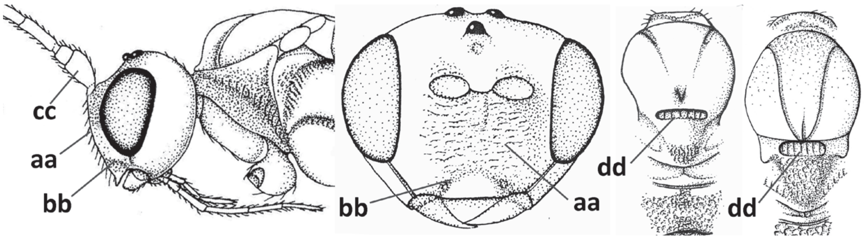
– Crenulate depression above eye absent (aa); prepectal carina absent ventrally (bb); vein SR1 of fore wing completely sclerotised, reaching margin of wing and resulting in a closed marginal cell (cc); hind tibia and tarsus, and tarsal claws medium-sized (dd)..**9**



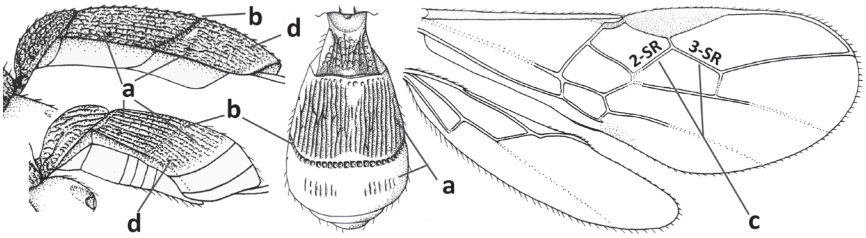
9 Face with pair of tubercles below antennal sockets (a); epistomal suture with pair of large oblique and long pubescent depressions below facial tubercles (b); scape strongly compressed (c); scutellar sulcus narrow (d); [mandible with wide basal tooth] .... ***Cephaloplites* Szépligeti, 1897**



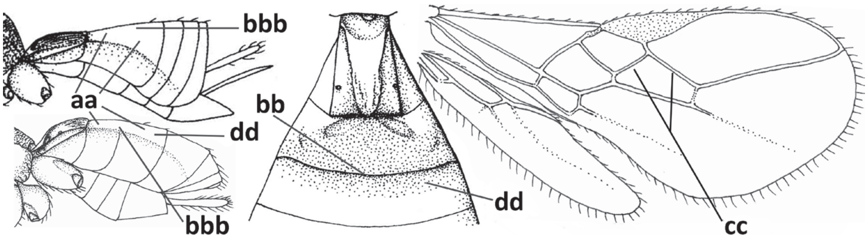
– Face without tubercles (aa); epistomal suture without large depressions (bb); scape at most weakly compressed (cc); scutellar sulcus usually wider (dd)..**10**



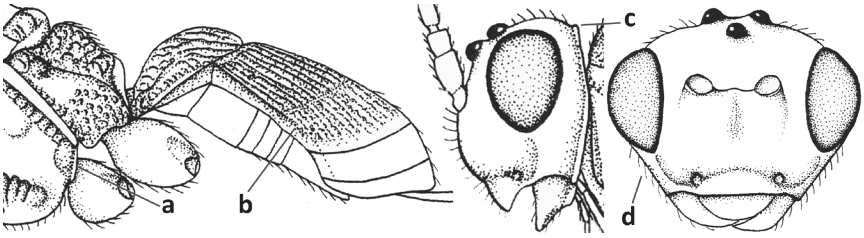
10 Metasomal tergite II distinctly (1.3–1.9×) longer than tergite III and differentiated (a); tergite II bordered posteriorly by a curved second metasomal suture (b); vein 3-SR of fore wing approx. as long as vein 2-SR or slightly longer (c); tergite II longitudinally striate or rugose (d) **11**



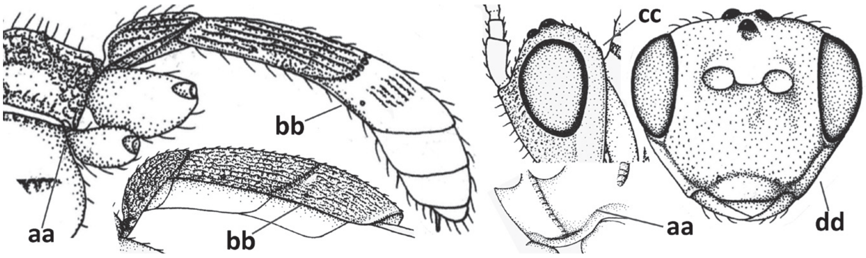
– Tergite II 0.7–1.1× as long as tergite III (aa), but frequently tergite II hardly or not differentiated; second metasomal suture nearly straight (bb) or absent (bbb); **if** metasoma shortened then vein 3-SR of fore wing distinctly longer than vein 2-SR (cc; *Opius agnesae*) and tergite II smooth or granulate (dd); [if metasoma shortened and propodeum with transverse carina subbasally, see *Coleopioides* van Achterberg & Li] **13**



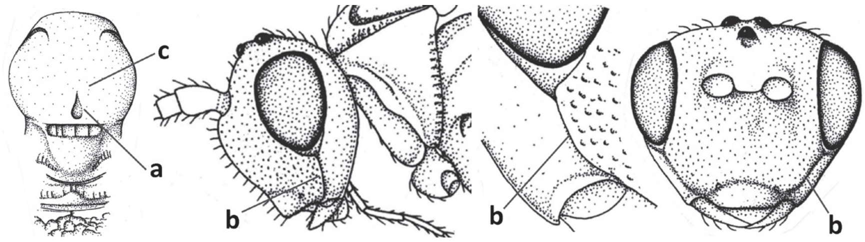
11 Around base of middle coxa with a circular carina (a); tergite III without sharp lateral crease (b); occipital carina present latero-dorsally and rather protruding in lateral view (c); head less elongate in anterior view (d); [dorsal carinae of tergite I united basally and medially forming a median carina; mesoscutum smooth laterally] ....***Bitomoides* van Achterberg, 2004**



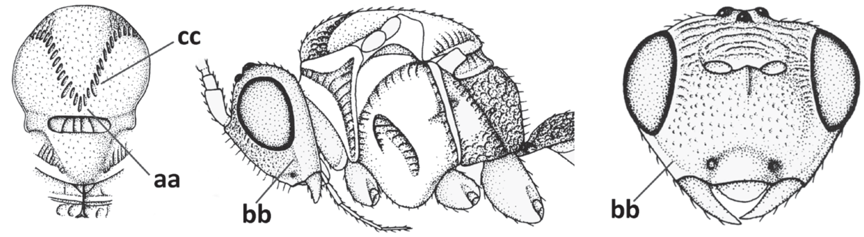
– Around base of middle coxa without a circular carina (aa); tergite III with a sharp lateral crease (bb); occipital carina absent latero-dorsally or if present usually less protruding in lateral view (cc); head more elongate in anterior view (dd); [mesoscutum finely crenulate laterally; dorsal carinae of tergite I usually separated basally and medially without a median carina] **12**



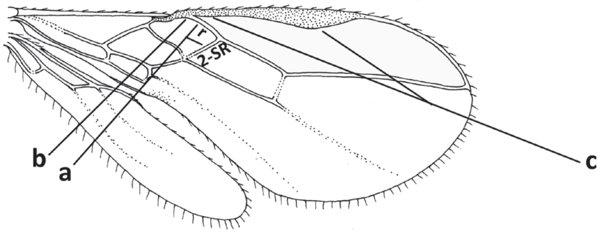
12 Medio-posterior depression of mesoscutum present (a); malar suture complete and distinctly impressed (b); posterior 1/2 of notauli entirely or largely absent (c)..***Orientopius* Fischer, 1966**

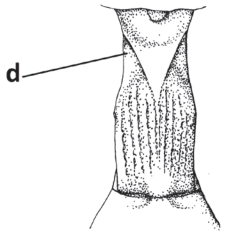


– Medio-posterior depression of mesoscutum absent (aa); malar suture absent or obsolescent (bb); posterior 1/2 of notauli complete (cc); [laterope distinct] .... ***Coleopius* Fischer, 1964**

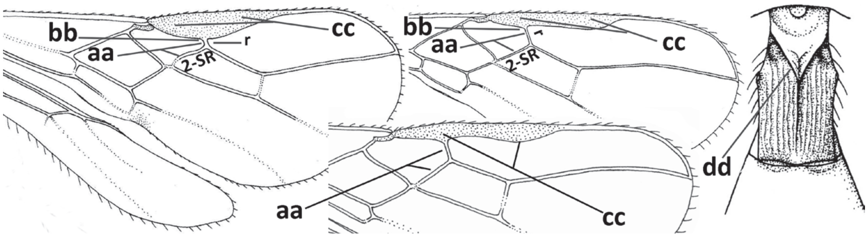


13 Vein r of fore wing approx. as long as vein 2-SR (a), slightly curved, not angled with vein 3-SR and issued near base of pterostigma (b); basal third of pterostigma much narrower than apical third (c); dorsal carinae of metasomal tergite I largely obsolescent (d); [dorsope often minute or obsolescent; spiracles of tergite I situated near middle of notum; malar suture distinct]... ***Eurytenes*** **Foerster, 1863 s.str.**

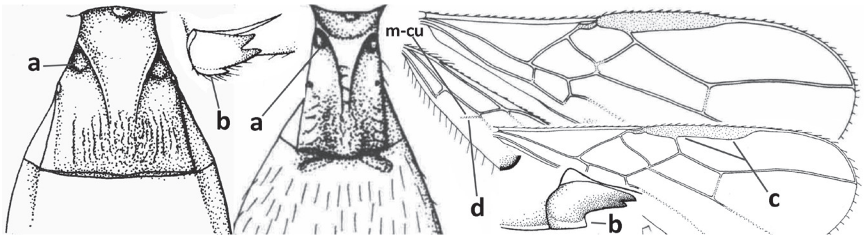




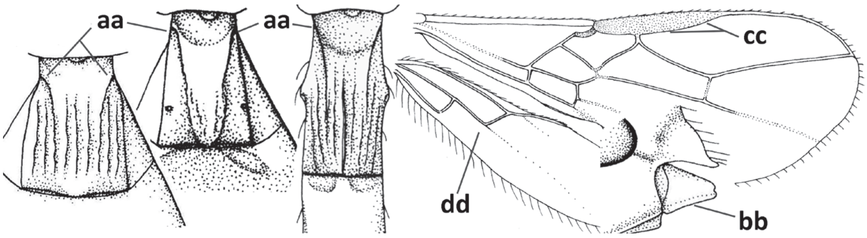
– Vein r of fore wing much shorter than vein 2-SR (aa), straight, more or less angled with vein 3-SR and issued distinctly removed from base of pterostigma (bb); basal 1/3 of pterostigma approx. as wide as apical 1/3 or slightly narrower (cc); dorsal carinae of tergite I usually distinctly developed (dd)..**14**



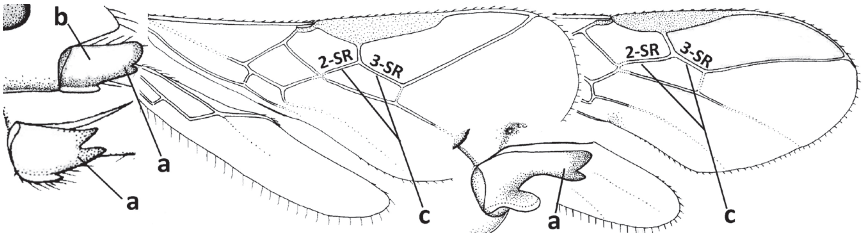
14 Dorsope present (a); if with small dorsope then mandible with a short lamella ventro-basally (b) or pterostigma slightly widened subapically (c); vein m-cu of hind wing usually present, at least as a partly pigmented trace (d); [medio-posterior depression of mesoscutum present, rarely absent or obsolescent; antenna often longer than 1.3× length of fore wing] **15**



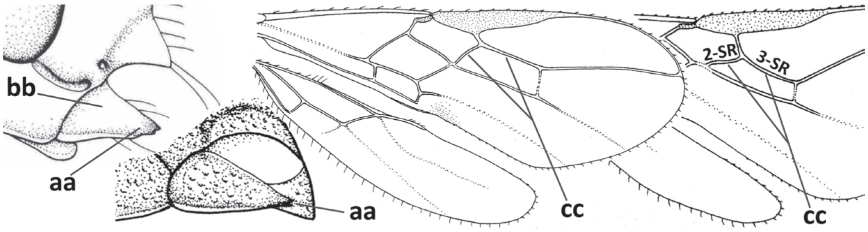
– Dorsope absent (aa); mandible usually without a short lamella ventro-basally or with a narrow carina along most of mandible ventrally (bb); pterostigma narrowed subapically (cc); vein m-cu of hind wing often absent (dd), but present in *Diachasma* and *Bathystomus*; [antenna usually less than 1.3× length of fore wing]...**21**



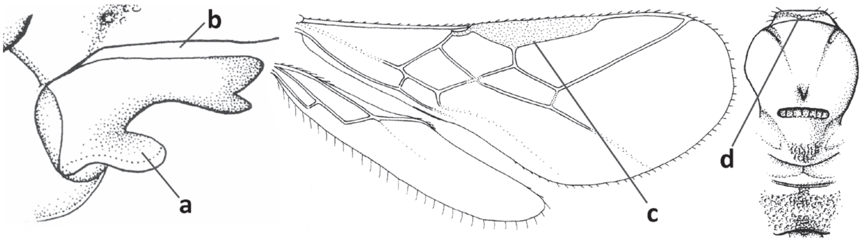
15 Mandible not or slightly twisted medially and its second tooth distinctly visible in lateral view (a), wide apically and its outer side convex (b); vein 3-SR of fore wing 1.3× as long as vein 2-SR or less (b), but 1.2–1.6× in *Pseudosteres* **16**



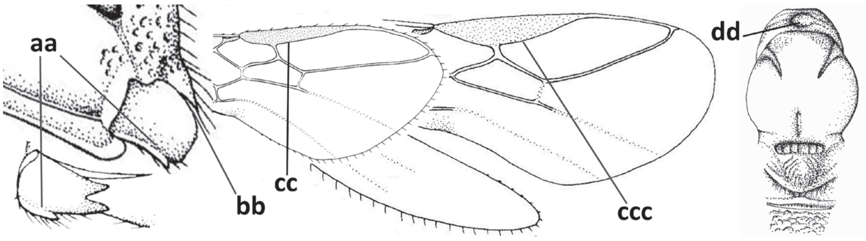
– Mandible more or less twisted medially and its second tooth hardly or not visible in lateral view (aa), triangular and its outer side often flattened or weakly convex (bb), but convex in *Xynobius* and *Atormus*; vein 3-SR of fore wing 1.4–2.5× longer than vein 2-SR (cc) **18**



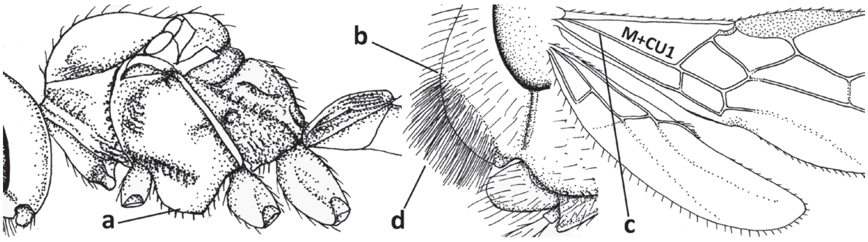
16 Mandibles asymmetrical, with a large ventro-basal tooth or lobe (a); hypoclypeal depression medium-sized to narrow (b), rarely absent; pterostigma wide elliptical or elongate triangular (c); pronope absent or nearly so (d); [vein 3-SR of fore wing 1.2–1.6× longer than vein 2-SR; vein m-cu of fore wing sometimes curved] .... ***Pseudosteres* gen. nov.**



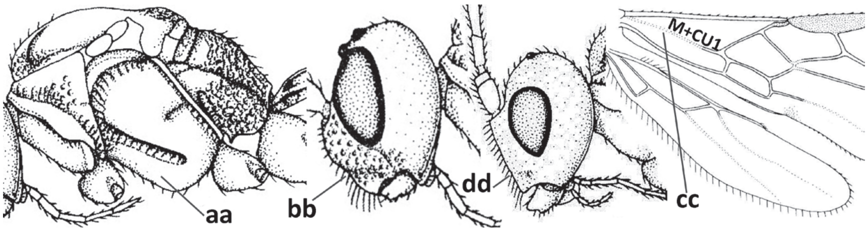
– Mandible symmetrical, without ventro-basal tooth, at most with an acute basal lamella (aa); hypoclypeal depression absent (bb) or narrow; shape of pterostigma variable (cc), **if** comparatively wide elliptical or triangular (ccc) then usually pronope large (dd); [vein m-cu of fore wing straight] **17**



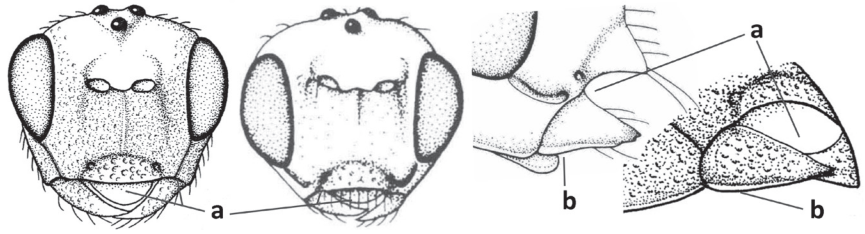
17 Mesopleuron narrowed ventrally (a); epistomal suture absent (b); basal 1/2 of vein M+CU1 of fore wing entirely or largely sclerotised (c); clypeus with dense long setae, and convex (d); [mandible without distinct ventro-basal carina, but sometimes weakly developed] .... ***Chilotrichia* Foerster, 1863**



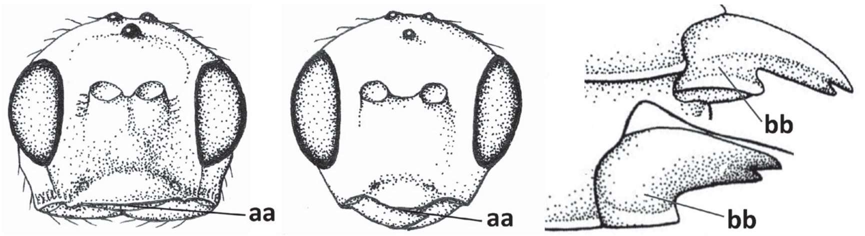
– Mesopleuron wider and rounded ventrally (aa); epistomal suture present (bb); if shallow then basal 1/2 of vein M+CU1 of fore wing largely unsclerotised (cc); clypeus with less dense and shorter setae, if setae longer and denser then clypeus flattened (dd) .... ***Biosteres* Foerster, 1863**



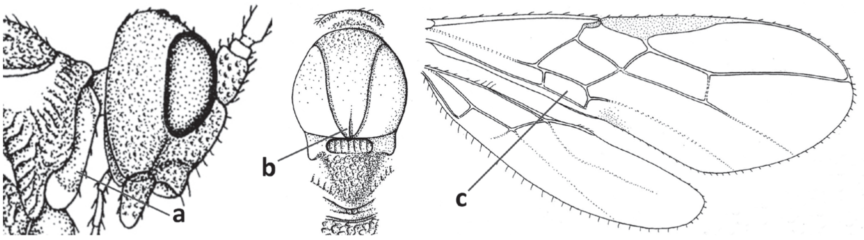
18 Hypoclypeal depression present, large, and medially ventral margin of clypeus near upper level of condyles of mandibles ("subcyclostome condition"; a); mandible symmetrical basally (b) and outer side convex; [malar suture deep] **19**



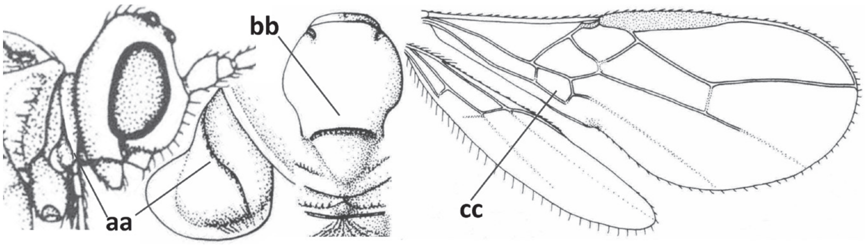
– Hypoclypeal depression absent or narrow, and medially ventral margin of clypeus more or less below upper level of condyles of mandibles ("mouth closed" or nearly so; aa); mandible often widened ventro-basally (asymmetrical: bb) and outer side usually flattened or weakly convex **20**



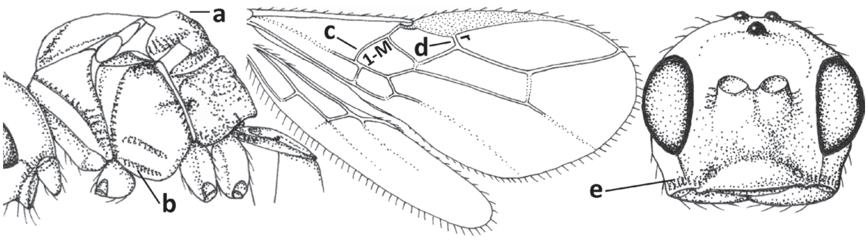
19 Propleuron without fine oblique carina subapically (a); medio-posterior depression of mesoscutum present (b); first subdiscal cell of fore wing subparallel-sided (c)..***Xynobius*** **Foerster, 1863**



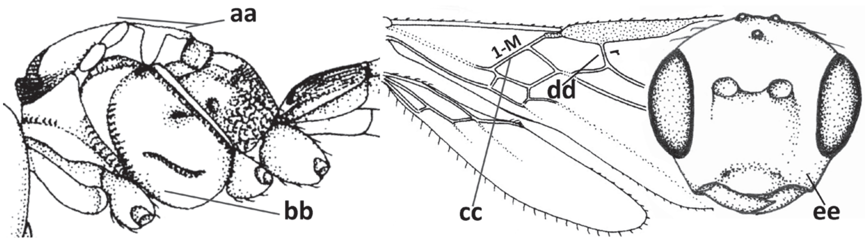
– Propleuron with (oblique or transverse) carina subapically (aa); medio-posterior depression of mesoscutum absent (bb); first subdiscal cell of fore wing widened distally (cc); [metasomal tergite III evenly setose, but sometimes sparsely so; hypostomal flange protruding ventrally] ....
....***Atormus*** **van Achterberg, 1997**



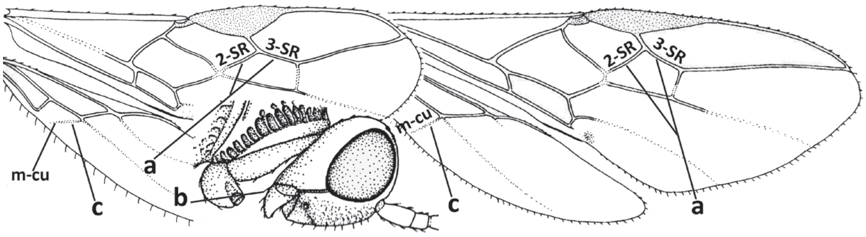
20 Scutellum elevated above level of mesoscutum (a); below precoxal sulcus with a second finely sculptured sulcus (= sternaulus: b), but sometimes only medially superficially impressed; vein 1-M of fore wing curved (c) and vein r short (d); malar space crenulate ventrally (e); [vein m-cu of fore wing strongly converging posteriorly to vein 1-M or subparallel].........................
....***Biophthora*** **Foerster, 1863**



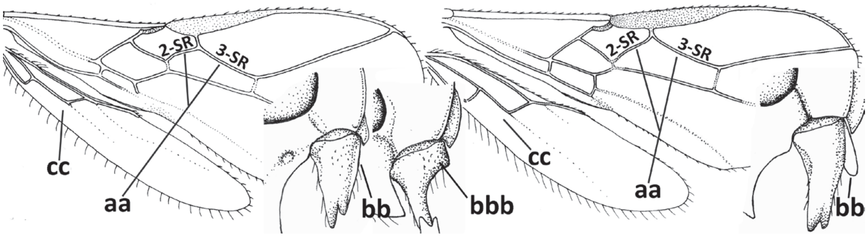
– Scutellum hardly or not elevated above mesoscutum (aa); sternaulus absent (bb), at most with one groove (= precoxal sulcus) present; vein 1-M of fore wing straight (cc) or nearly so and vein r medium-sized to long (dd); malar space smooth ventrally (ee)....................***Opiostomus*** **Fischer, 1972**



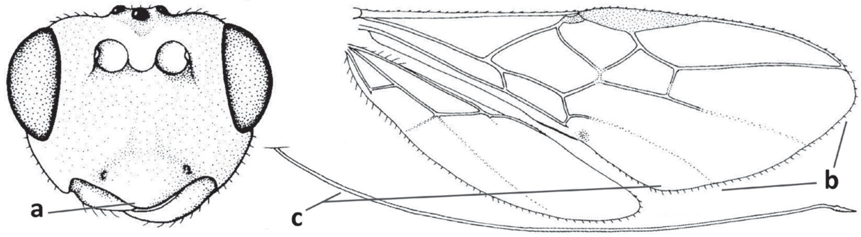
21 Vein 3-SR of fore wing 0.8–1.3× vein 2-SR (a); if rarely 1.3–1.5× then basal 1/2 of mandible symmetrical (b); i.e., without a ventral carina or tooth) and vein m-cu of hind wing present (at least as a distinct partly pigmented trace: c); length of fore wing usually more than 3 mm **22**



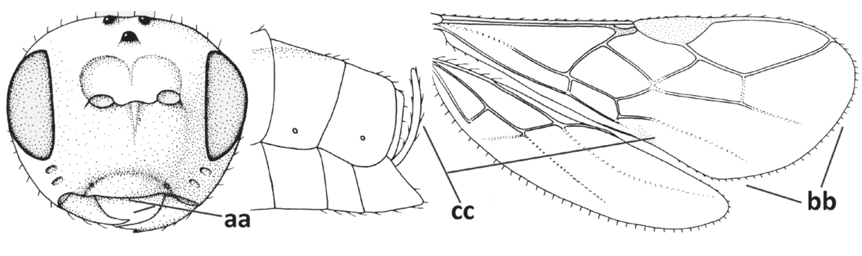
– Vein 3-SR of fore wing 1.3× vein 2-SR or longer (aa); if 1.0–1.5× then basal 1/2 of mandible with a narrow ventral carina (bb) or baso-ventrally widened (asymmetrical: bbb) and/or vein m-cu of hind wing absent (cc) or obsolescent; length of fore wing usually less than 3 mm **25**



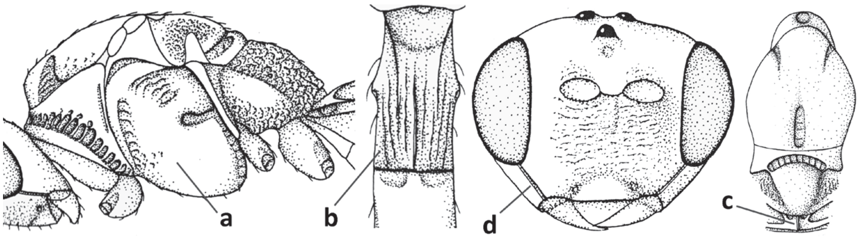
22 Ventral margin of clypeus roundly protruding and without distinct hypoclypeal depression, at most with a narrow slit (a); fore wing pointed apically (b); ovipositor sheath long (c), usually as long as fore wing or longer; parasitoids of Tephritidae........................***Diachasmimorpha* Viereck, 1913**



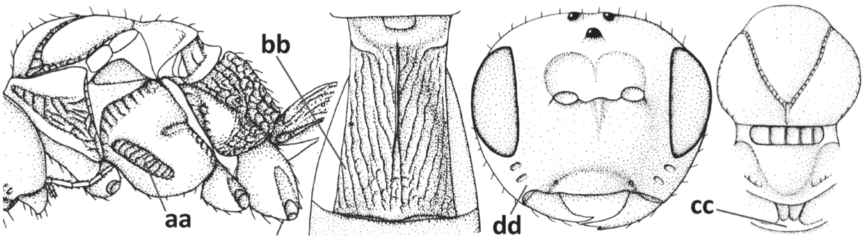
– Ventral margin of clypeus almost straight or slightly concave, and with a distinct hypoclypeal depression (aa); fore wing broadly rounded apically (bb); ovipositor sheath short (cc), approx. as long as apical height of metasoma or concealed; parasitoids of other dipterous hosts **23**



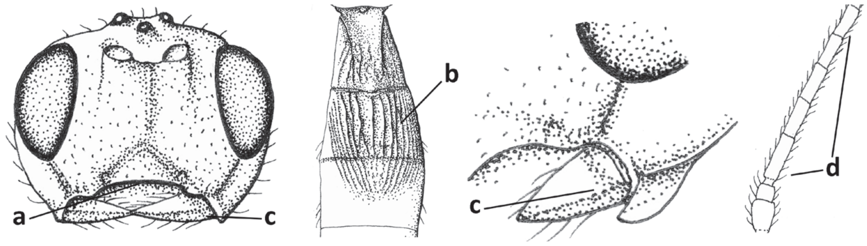
23 Precoxal sulcus absent or largely so (a); metasomal tergite I 2.0–2.5× longer than its apical width (b); metanotum with median carina posteriorly (c); malar suture complete and oblique (d); [metasomal tergites II and III of ♀ distinctly compressed; tergite II smooth] .... ***Bathystomus* Foerster, 1863**



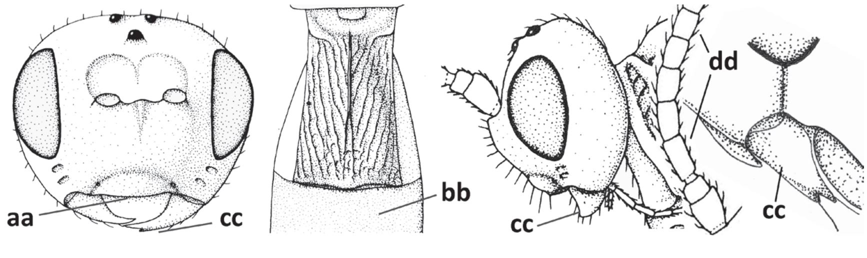
– Precoxal sulcus present and often wide on anterior 1/2 of mesopleuron (aa); length of tergite I 1.0–1.5× its apical width (bb); metanotum without median carina posteriorly or weakly developed (cc); malar suture absent or obsolescent (dd); [tergites II and III of ♀ not compressed] **24**



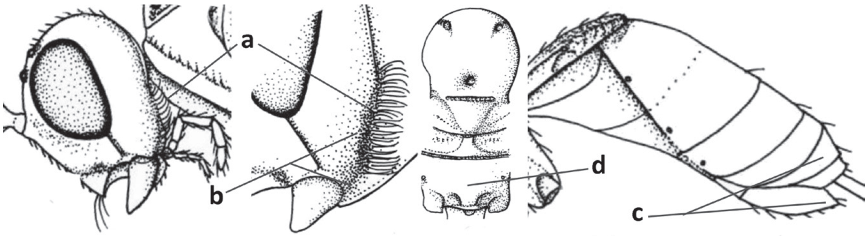
24 Clypeus concave medio-ventrally (a); tergite II of ♀ with coarse striation (b); mandible strongly twisted basally (c); antennal segments slender (d); parasitoids of Psilidae; [mesosoma ~ 1.8× longer than high in lateral view; metanotum finely crenulate posteriorly; eyes diverging ventrally] .... ***Atoreuteus* Foerster, 1863**



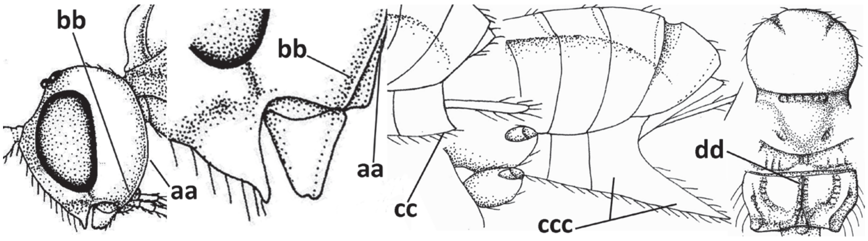
– Clypeus straight or protruding medio-ventrally (aa); tergite II of ♀ smooth or with very fine sculpture (bb); mandible not twisted basally (cc); antennal segments robust (dd); parasitoids of Anthomyiidae .... ***Diachasma* Foerster, 1863**



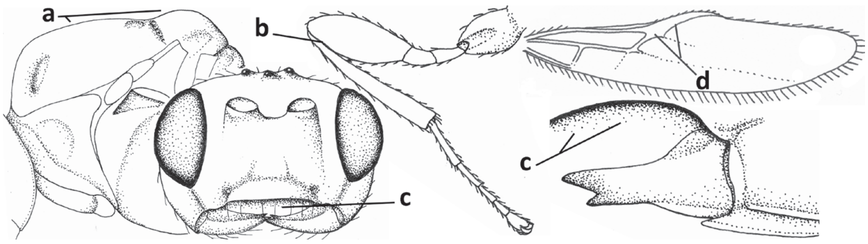
25 Ventral 1/2 of occiput with conspicuous and more or less curved setae (a); occipital carina widely absent ventrally (b); hypopygium of ♀ acute apically, nearly as long as apical height of metasoma or somewhat shorter (c) and propodeum largely smooth (d) .... ***Cavopius* gen. nov.**



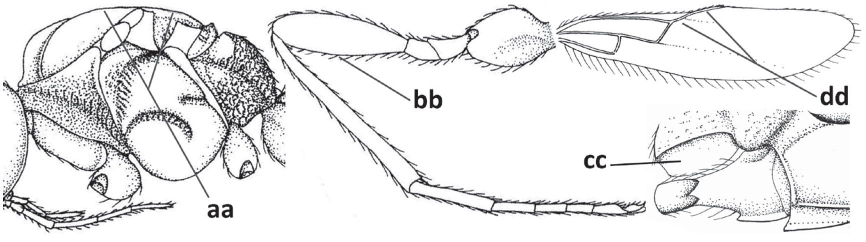
– Ventral 1/2 of occiput glabrous (aa); occipital carina present (bb), at most narrowly absent ventrally; hypopygium of ♀ usually obtuse apically (cc), if acute then acute part longer than basal part (ccc) and/or anteriorly propodeum with medio-longitudinal carina (dd) and/or partly sculptured **26**



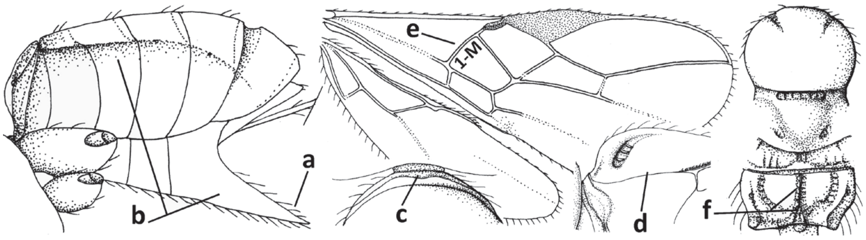
26 Scutellum distinctly protruding above level of mesoscutum (a); hind femur very robust, 2–3× longer than wide (b); labrum slanted backwards, leaving a large space below clypeus (c); medio-anterior veins of hind wing of ♂ strongly widened (d); [hypopygium of ♀ 0.3–0.5× as long as metasoma and apically usually more or less protruding; dorsal face of propodeum strongly transverse; hind wing narrow] ....***Psyttoma* van Achterberg & Li, 2012**



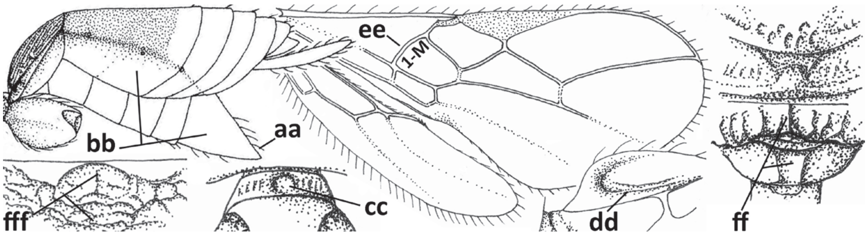
– Scutellum at level of mesoscutum (aa); hind femur more slender, 3–5× longer than wide (bb); labrum normal, without large space below clypeus (cc); medio-anterior veins of hind wing of ♂ narrow (dd).........................**27**



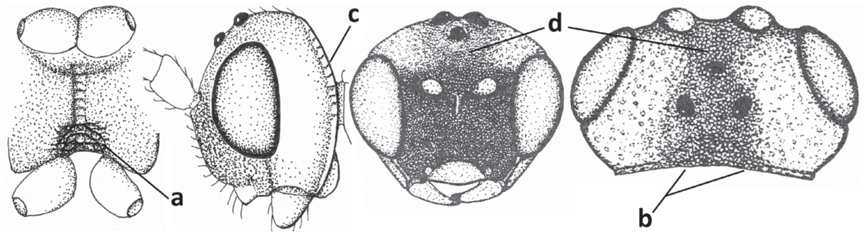
27 Hypopygium of ♀ more narrowed apically (a), acute, 0.3–0.5× as long as metasoma (b); pronope absent or small in dorsal view (c); lateral carina of mesoscutum absent in front of tegula (d); vein 1-M of fore wing straight or nearly so (e); propodeum often with medium-sized medio-longitudinal carina connected to narrow triangular areola (f), but largely obscured by sculpture in *P. cyclogaster*; [tergite II (if differentiated) distinctly shorter than tergite III or of similar length; hind wing and hypoclypeal depression wide; antenna of ♀ 1.3–1.6× as long as fore wing] ....***Psyttalia* Walker, 1860**



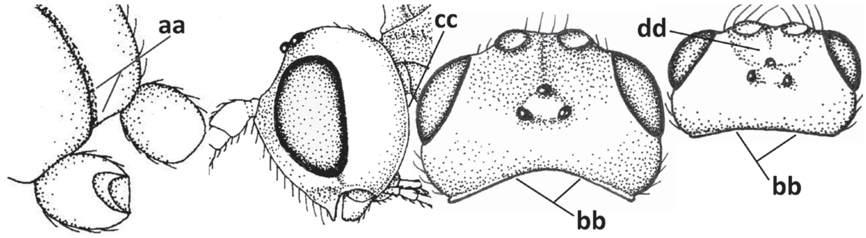
– Hypopygium of ♀ less narrowed apically (aa) and usually shorter than 0.3× length of metasoma (bb); pronope usually large to medium-sized (cc); **if** absent then in front of tegula with fine lateral carina of tegula (dd; sometimes area aciculate) **or** vein 1-M of fore wing curved (ee); medio-longitudinal carina of propodeum usually absent, **if** present then short and either no or wide areola (ff), rarely carina hardly developed (fff); [metasomal tergite II approx. as long as tergite III]...**28**



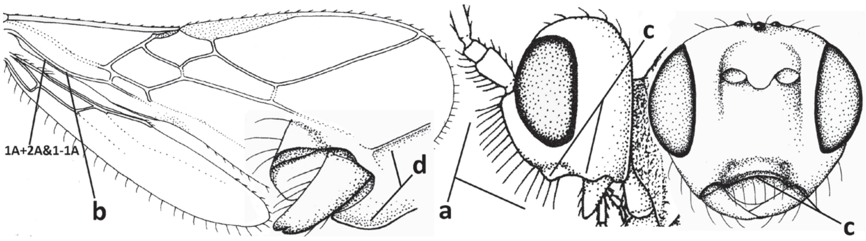
28 Medio-posterior triangular area of mesosternum coarsely reticulate (a); occipital carina complete medio-dorsally or nearly so (b) and distinctly lamelliform dorso-laterally (c); frons distinctly granulate (d)....***Neopius* Gahan, 1917**



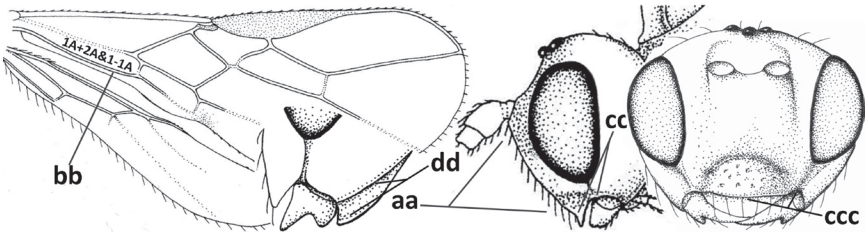
– Mesosternum largely smooth medio-posteriorly (aa); occipital carina widely interrupted medio-dorsally (bb); **if** more or less complete dorsally then occipital carina narrow lateral (cc) and/or frons smooth (dd) **29**



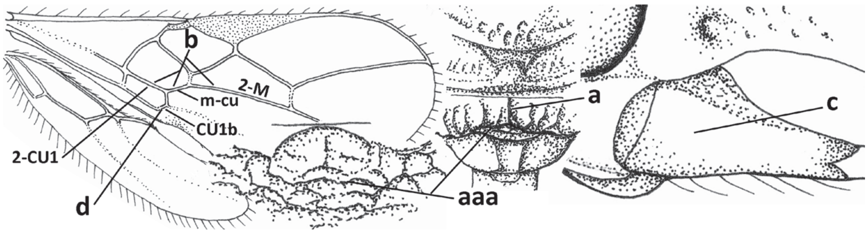
29 Face conspicuously long and densely setose medio-dorsally (a); vein 1A+2A & 1-1A of fore wing distinctly bent and reaching posterior border of wing (b); thick ventral margin of clypeus distinctly concave medio-apically (c; medially distinctly above upper level of mandibular condyles); occipital carina fine and remaining far removed from hypostomal carina ventrally (d); [metasoma (except dark brown tergite I) orange-yellow]........................ ....***Eutrichopsis* Foerster, 1863**



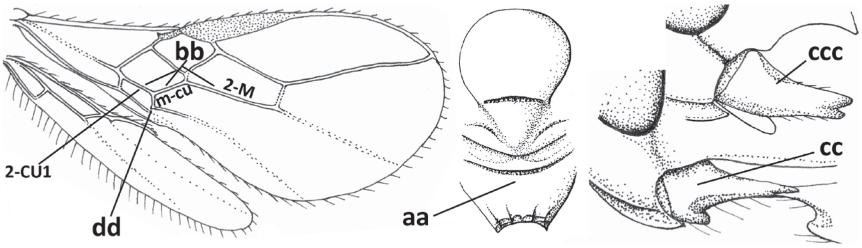
– Face without conspicuous setosity (aa); vein 1A+2A & 1-1A of fore wing straight or nearly so and remaining removed from posterior border of wing (bb); ventral margin of clypeus below upper level of mandibular condyles (cc) or approx. same level (ccc)) and usually thin; occipital carina usually more developed and closer to hypostomal carina ventrally (dd)...**30**



30 Medio-longitudinal carina of propodeum present anteriorly (a), if very short and reduced or obscured by coarse reticulation, then with transverse elements of rugosity subanteriorly (aaa); vein m-cu of fore wing gradually merging into 2-CU1 and sublinear with vein 2-M (b); mandible symmetrical or nearly so (c); vein CU1b of fore wing distinct and medium-sized to small (d); [hypoclypeal depression medium-sized to large; medio-posterior depression of mesoscutum absent and precoxal sulcus distinctly crenulate] .... ***Rhogadopsis* Brèthes, 1913**



– Medio-longitudinal carina of propodeum absent and area smooth (aa), **if** rugose then without distinct transverse elements; vein m-cu of fore wing angled with veins 2-CU1 and vein 2-M (bb); shape of mandible varies from more or less asymmetrical and strongly narrowed medially (cc) to symmetrical and slightly narrowed medially (ccc); vein CU1b of fore wing short or absent (dd) ....***Opius* Wesmael, 1835 s.l.**



Taxonomy

***Cavopius* gen. nov.**
https://zoobank.org/D9D463B6-14A6-4338-9E8F-20DEDD152578
Figs 1–33

Type species. *Opius* (*Agnopius*) *daghoides* Zaykov & Fischer, 1983.

Etymology. From *cavus* (Latin for hollow) and the generic name *Opius* Wesmael, because of the long and curved setae make a kind of cave at the back of the head (Fig. 13). Gender: masculine.

Diagnosis. Antenna with 26–37 segments and 1.1–1.2× as long as fore wing (latter unknown of *C. daghestanicus*); ventral half of occiput with medium-sized to large area of long conspicuous and usually curved setae (Figs 13, 17, 25, 31); occipital carina absent ventrally (from lower level of eye downwards; Fig. 31) and dorsally; face without tubercles; scapus, fore coxa and trochanter at most weakly compressed; epistomal suture without large depressions (Fig. 30); inner side of antennal sockets not protruding (Figs 16, 30); hypoclypeal depression large; labrum visible, smooth; clypeus straight ventrally (Fig. 30); mandible robust, gradually widened basally and with ventro-basal carina, its dorsal tooth robust (Figs 13, 31); malar suture deep and long (Figs 30, 31); pronotum short and subvertical anteriorly and pronope deep and rather large; notauli largely absent on mesoscutal disc (Figs 14, 26); mesoscutum with medio-posterior depression; scutellar sulcus narrow to medium-sized (Figs 14, 26); precoxal sulcus absent; mesopleuron with transverse carina below anterior subalar depression in type species (Fig. 22); postpectal carina absent medio-ventrally (Fig. 25); vein 1-SR of fore wing 0.3–0.5× as long as vein 1-M; vein 3-SR of fore wing 1.9–2.1× longer than vein 2-SR; hind tibia without basal carina; laterope large (Fig. 22); dorsope absent; tergites II and III smooth and of subequal length or tergite II somewhat shorter, tergite II with pair of oblique depressions basally; epipleuron of tergite III similarly sclerotised as its notum laterally and largely gently folded under notum; second metasomal suture largely absent (Figs 1, 22, 27); tergite IV clearly visible (Figs 22, 27); ovipositor sheath far protruding, its setose part 0.6–1.2× as long as fore wing; hypopygium medium-sized and acute apically (Figs 22, 28).

Distribution. Palaearctic: three species.

Figure 1. *Cavopius depressorius* sp. nov., holotype, ♀, S. Korea (Hudong-Li), habitus lateral.

Key to species of the genus *Cavopius* gen. nov.

1 Setose part of ovipositor sheath 0.6–0.7× as long as fore wing (Fig. 22); vein 1-M of fore wing ~ 2× as long as vein 1-SR (Fig. 11); face laterally yellowish brown .... ***C. daghoides* (Zaykov & Fischer, 1983)**

– Setose part of ovipositor sheath 0.9–1.2× as long as fore wing (Fig. 1); vein 1-M of fore wing 3–4× as long as vein 1-SR (Figs 2, 24); face laterally black or dark reddish brown (Figs 7, 30)... **2**

2 Setose part of ovipositor sheath ~ 1.2× as long as fore wing (Fig. 1); metasomal tergites IV–VI broadly depressed and membranous antero-medially (Figs 1, 27, 28); vein 1-M of fore wing 3× as long as vein 1-SR (Fig. 24); antenna of ♀ with ~ 33 segments; curved setae of ventral 1/2 of occiput conspicuous (Figs 26, 31) .... ***C. depressorius* sp. nov.**

– Setose part of ovipositor sheath ~ 0.9× as long as fore wing; tergites IV–VI flat and evenly sclerotised; vein 1-M of fore wing 4× as long as vein 1-SR (Fig. 2); antenna of ♀ with ~ 28 segments; curved setae of ventral 1/2 of occiput less conspicuous (Fig. 9) .... ***C. daghestanicus* (Telenga, 1950)**

***Cavopius daghestanicus* (Telenga, 1950), comb. nov.**
Figs 2–10

Opius daghestanicus Telenga, 1950: 306; Fischer 1961: 7, 1971: 60.

Opius (*Misophthora*) *daghestanicus*; Fischer 1972: 228, 241; Papp 1981: 64.
Opius (*Agnopius*) *daghestanicus*; Fischer 1982: 22, 1983: 15–17 (redescription).
Opius (*Allotypus*) *daghestanicus*; Tobias and Jakimavičius 1986: 55 (transl. 79); Tobias 1998: 596.

Type material. ***Holotype***, ♀ (ZISP), “Dagestan, Hodshan-Maha, 28. vi.[19]26, g. Rubov”, “*Opius daghestanicus* sp. n., N. Telenga det.”, “Zoological Institute St. Petersburg, INS_HYM_ 0002787”.

Diagnosis. Antenna of ♀ with approximately 28 segments; face laterally black or dark brown; ventral half of occiput less conspicuously setose (Fig. 9); vein 1-M of fore wing 4× as long as vein 1-SR (Fig. 2); metasomal tergite I rather narrowed posteriorly (Fig. 5); metasomal tergites IV–VI flat and evenly sclerotised; setose part of ovipositor sheath approx. 0.9× as long as fore wing.

Biology. Unknown.

Distribution. Moldova, Russia (N. Caucasus, Siberia).

Notes. Photographs of the damaged holotype were very kindly supplied by Konstantin Samartsev (ZISP), showing clearly the more or less curved occipital setae (Fig. 9) which were overlooked by Fischer (1983).

Cavopius daghoides **(Zaykov & Fischer, 1983), comb. nov.**

Figs 11–23

Opius (*Agnopius*) *daghoides* Zaykov & Fischer, 1983: 41–44.
Phaedrotoma daghoides; Jiménez-Peydró and Peris-Felipo 2011: 475.

Type material. ***Holotype***, ♀ (RMNH), “Bulgaria, ex coll. Zaykov, RMNH Leiden 1991”, “Rhodopi, Konush [= village near Plovdiv at foothills of Rhodope Mts, c. 190 m], 3.vi.1975, A. Zaykov”, “♀ *Opius daghoides* n. sp., Holotype, det. Fischer, 1982 / Holotype Fischer & Zaykov”, “*Opius* (*Misophthora*) sp. n., ♀, det. Papp J., 1981 / aff. *daghestanicus* Tel., 26-art. ”, “19”.

Diagnosis. Antenna with 26 segments (♀); face laterally yellowish brown; curved setae of ventral half of occiput conspicuous (Figs 13, 22); vein 1-M of fore wing ~ 2× as long as vein 1-SR (Fig. 11); setose part of ovipositor sheath 0.6–0.7× as long as fore wing (Fig. 22)

Biology. Unknown.

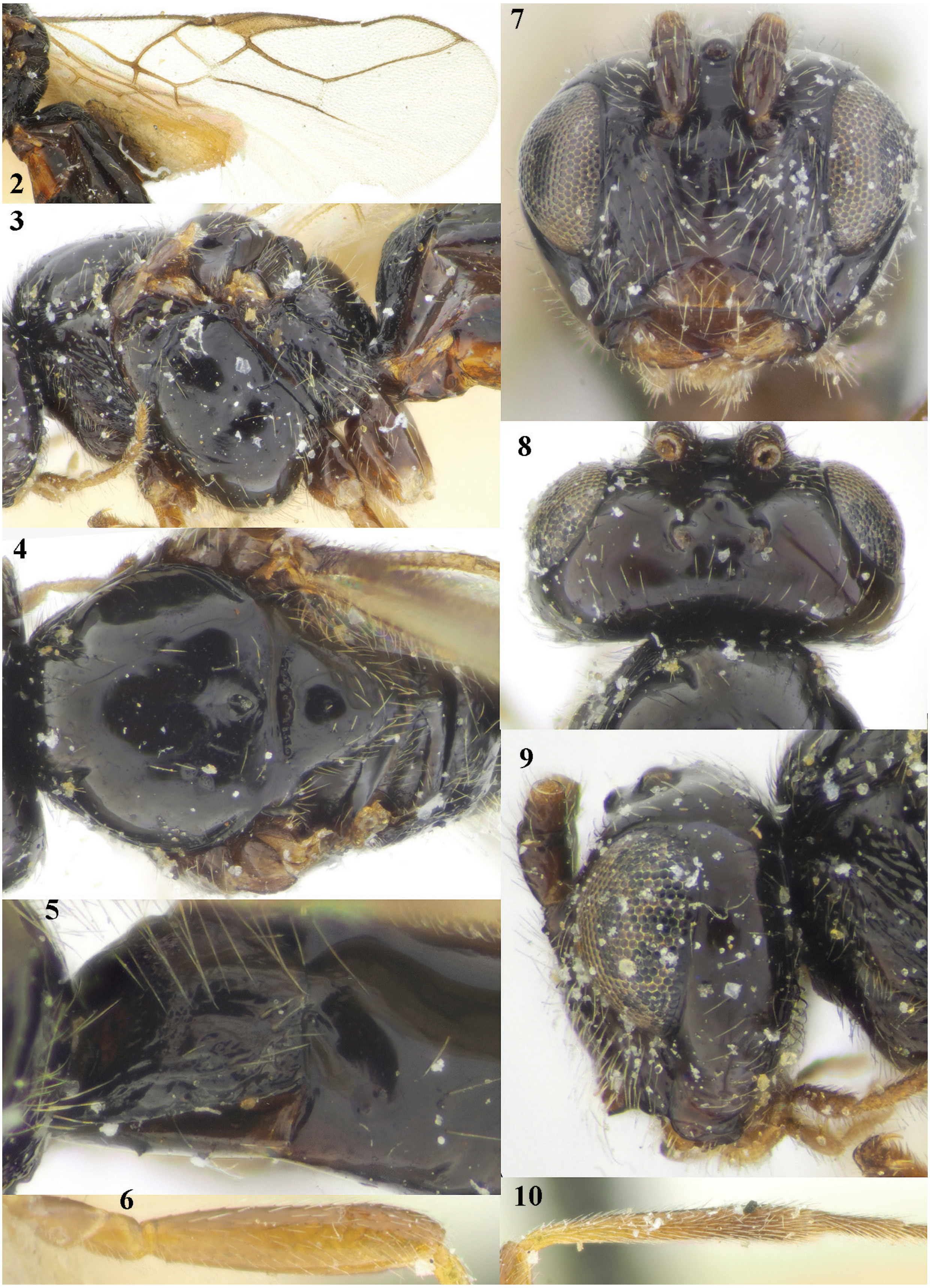
Distribution. Bulgaria, Spain.

Cavopius depressorius **sp. nov.**

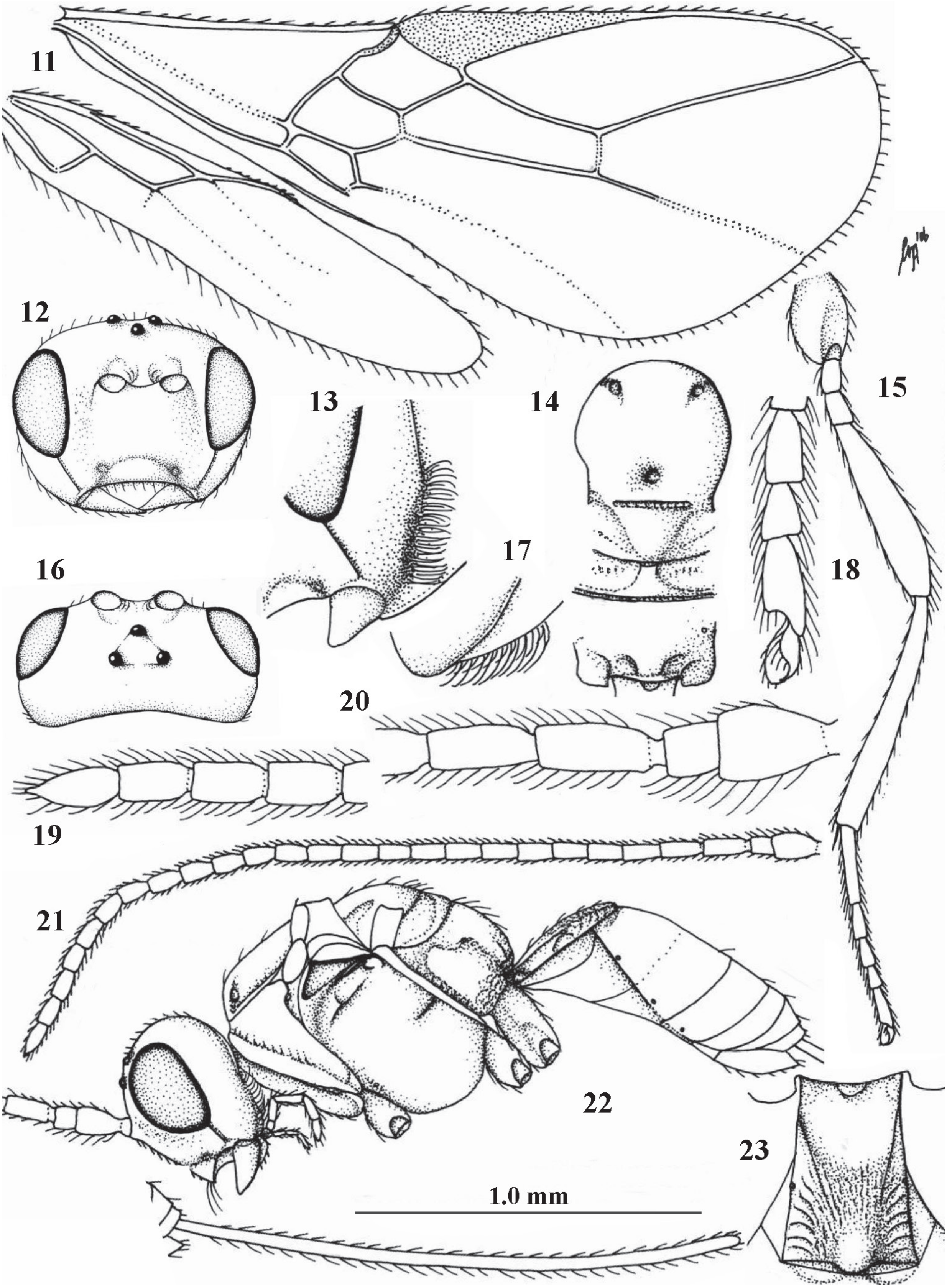
https://zoobank.org/204BFF47-4F5C-4860-BCB1-3A3670348578
Figs 1, 24–33

Type material. ***Holotype***, ♀ (RMNH), “S. Korea: Kangwondo, Cuncheon Nammyon, Hudong-Li, Mal[aise] tr[ap] in half shadow at forest edge, 25.v.–14. vi.2003, P. Tripotin, RMNH”.

Diagnosis. Antenna with 37 segments (♀); curved setae of ventral half of occiput conspicuous (Figs 25, 31); face laterally black or dark reddish brown



Figures 2–10. *Cavopius daghestanicus* (Telenga), comb. nov., holotype, ♀, Russia (Dagestan) **2** wings **3** mesosoma lateral **4** mesosoma dorsal **5** metasomal tergite I latero-dorsal **6** hind femur lateral **7** head anterior **8** head dorsal **9** head lateral **10** hind tibia lateral. Photographs by K. Samartsev.



Figures 11–23. *Cavopius daghoides* (Zaykov & Fischer), comb. nov., holotype, ♀, Bulgaria (Konush) **11** wings **12** head anterior **13** ventral half of occiput and mandible lateral **14** mesosoma dorsal **15** hind leg **16** head dorsal **17** ventral half of occiput latero-posterior **18** outer hind tarsal claw **19** apex of antenna **20** base of antenna **21** antenna **22** habitus lateral **23** metasomal tergite I dorsal. Scale bar: 1.0× (**11, 12, 14–16, 21, 22**); 1.5× (**23**); 1.6× (**13, 17**); 2.5× (**18–20**).

(Fig. 30); vein 1-M of fore wing 3× as long as vein 1-SR (Fig. 24); tergites IV–VI broadly depressed and membranous antero-medially (Figs 1, 27, 28); setose part of ovipositor sheath ~ 1.2× as long as fore wing (Fig. 1).

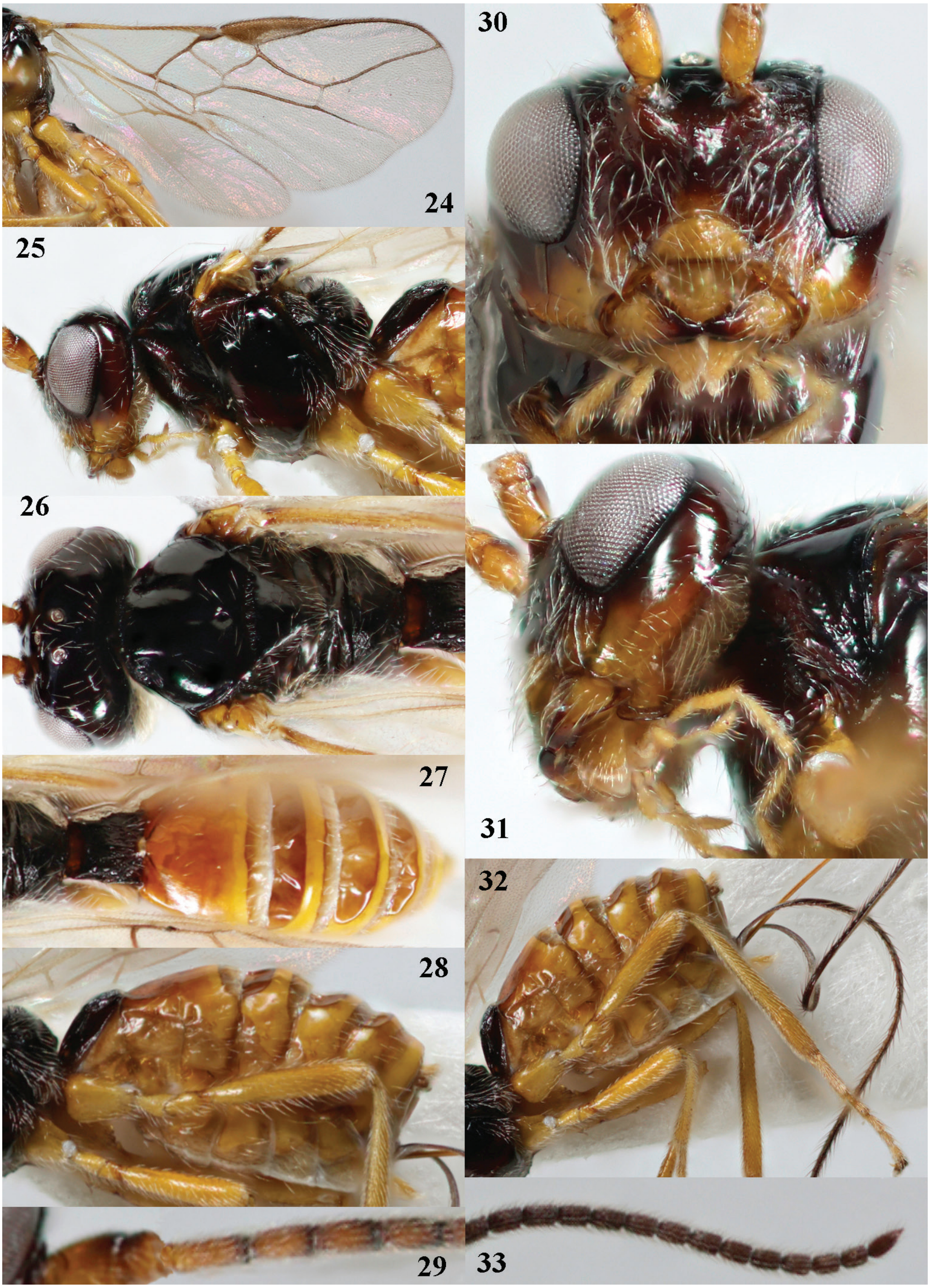
Description. Holotype, ♀, length of body 3.2 mm, of fore wing 3.4 mm.

***Head*.** Antenna with 37 segments and 1.2× as long as fore wing; third segment 1.2× longer than fourth segment, length of third, fourth and penultimate segments 1.5×, 1.2× and 1.4× their width, respectively (Figs 29, 33); width of head 2.1× its median length in dorsal view, smooth dorsally and posteriorly; behind stemmaticum with indistinct depression; vertex flattened and sparsely setose; OOL: diameter of ocellus: POL= 3:1:2 (Fig. 26); anterior half of frons shallowly depressed and smooth, its posterior half smooth and laterally setose (Fig. 26); face largely smooth, shiny and with conspicuous, long setae (Fig. 30); clypeus distinctly convex, semi-circular, largely smooth (except punctulation because of very long setae) and its ventral margin thin and straight, width of clypeus 2.8× its maximum height and 0.5× minimum width of face; hypoclypeal depression large and deep (Fig. 30); eye in dorsal view 1.4× longer than temple and temple behind eye subparallel-sided (Fig. 26); occipital carina distinct but dorsally and ventrally (behind malar space) absent (Fig. 31); temple and malar space smooth; length of malar space 1.1× basal width of mandible and 0.4× height of eye; malar suture deep and complete; mandible rather twisted apically but upper tooth robust, basally symmetrical or nearly so, basal half with ventral carina (Figs 30, 31); length of maxillary palp 1.1× height of head; labial palp segments robust.

***Mesosoma*.** Length of mesosoma 1.2× its height (Fig. 25); laterally pronotum smooth but anteriorly and posteriorly with crenulate groove; dorsal pronope round and rather large; propleuron weakly and evenly convex, shiny and smooth (Fig. 31); mesopleuron sparsely punctate; postpectal carina absent; precoxal sulcus absent and its area smooth; pleural sulcus smooth; mesosternal sulcus narrow and finely crenulate; metapleuron smooth and long setose (Fig. 25); mesoscutum steeply raised above pronotum, shiny and smooth except anteriorly; notauli only impressed in anterior 1/3 of mesoscutum, rather deep and anteriorly crenulate (Fig. 26); medio-posterior depression of mesoscutum deep, round and medium-sized; transverse suture of mesoscutum present; scutellar sulcus deep and broad medially, with eight carinae and medially 0.3 × as long as scutellum (Fig. 26); scutellum largely smooth, punctulate, weakly convex, without subposterior depression; side of scutellum smooth (Fig. 26); propodeum largely smooth and lacking carinae, but medio-posteriorly somewhat elevated, rugose and antero-laterally punctate, latero-posteriorly with lamella (Figs 25, 26).

***Wings*.** Fore wing (Fig. 25): pterostigma elongate triangular, 4.5× as long as its maximum width and gradually merging into vein 1-R1; vein M+CU1 weakly curved and only sclerotised in distal quarter; vein r-m present; 1-R1 ending just before wing apex; r:2-SR:3-SR:r-m:SR1 = 5:21:42:11:72; vein r slightly widened, arising before middle of pterostigma and 2-SR sinuate; m-cu postfurcal and nearly straight, gradually merging into 2-CU1; cu-a postfurcal and vertical; 1-CU1 slightly widened; CU1b medium-sized (Fig. 24). Hind wing: M+CU:1-M:1r-m = 22:20:10; cu-a slightly curved; m-cu absent.

***Legs*.** Hind femur, tibia and basitarsus 5.3×, 8.7× and 4.8× as long as wide, respectively (Fig. 32); hind femur with dense, long setae.



Figures 24–33. *Cavopius depressorius* sp. nov., holotype, ♀, S. Korea (Hudong-Li) **24** wings **25** head and mesosoma lateral **26** head and mesosoma dorsal **27** metasoma dorsal **28** metasoma lateral **29** base of antenna **30** head anterior **31** head lateral **32** hind leg lateral **33** apex of antenna.

***Metasoma*.** Tergite I 1.1× as long as wide apically and slightly widened apically, its surface convex medially and largely rugulose-punctate, dorsal carinae rather weakly developed and nearly up to apex of tergite (Fig. 27); tergite II and following tergites smooth; second suture absent dorsally; apex of tergites III–VI and antero-medially tergites IV–VI membranous and slightly sclerotised (more or less depressed in dead specimen; Figs 27, 28); setose part of ovipositor sheath 1.21× as long as fore wing, 8.1× tergite I and 3.6× as long as hind tibia; hypopygium acute ventro-apically and approximately as long as tergite I (Fig. 28).

***Colour*.** Black; temple chestnut brown; mandible largely, clypeus, malar space largely, palpi and legs pale yellow; tergite II and following tergites brownish yellow, but membranous parts more or less brown; antenna (but ventro-basally yellowish) dark brown; pterostigma and veins brown; ovipositor sheath dark brown; wing membrane subhyaline (Fig. 24).

Distribution. Korea.

Biology. Unknown.

Etymology. Named after the broadly depressed metasomal tergites IV–VI (Figs 27, 28); "*depressus*" is Latin for "pressed down, low".

Pseudosteres gen. nov.

https://zoobank.org/CCA60A06-1470-46E9-BB50-0C26C5BD7ABB
Figs 34–44

Type species. *Biosteres adanaensis* Fischer & Beyarslan, 2005.

Etymology. From "*pseudos*" (Greek for "fallacy") and the generic name *Biosteres* Foerster, because it is similar to *Biosteres*, but differs considerably as indicated below. Gender: masculine.

Diagnosis. Hypoclypeal depression usually medium-sized, and medially ventral margin of clypeus above upper level of condyles of mandibles, but depression absent in *P. riphaeus* and narrow in *P. adanaensis* (Fig. 35); mandible with a large ventral tooth and its outer side convex (Fig. 40), mandible not twisted apically and second tooth clearly visible; notauli largely absent posteriorly (Fig. 36); medio-posterior depression of mesoscutum present; scutellum punctate medio-posteriorly; precoxal sulcus either absent, as a smooth and narrow suture or depressed and distinctly crenulate; precoxal sulcus without a second sculptured sulcus below; vein m-cu of fore wing slightly converging to vein 1-M posteriorly (Fig. 34) or parallel; vein r more or less angled with vein 3-SR of fore wing; vein 3-SR of fore wing 1.2–1.6× longer than vein 2-SR; vein m-cu of fore wing antefurcal or interstitial; pterostigma elliptical (Fig. 34) or elongate triangular; hind tibia without oblique carina basally; dorsope present (Fig. 41); hypopygium of ♀ truncate.

Distribution. Palaearctic: five species.

Notes. Most species are similar to the genus *Biosteres* Foerster, 1863, but differ by having a large ventro-basal tooth or lobe and in part of species also by the presence of a medium-sized hypoclypeal depression. Members of *Opiostomus* Fischer have also the mandibles basally widened and the dorsope developed, but the mandible is distinctly twisted medially, the second submarginal is much longer and the scutellum is smooth posteriorly (but punctate in *O. leptostigma* (Wesmael)).

Key to species of the genus *Pseudosteres* gen. nov.

1 Antenna of ♀ with ~ 44 segments; vein r of fore wing emitted medially from pterostigma; hypoclypeal depression absent; [vein 3-SR of fore wing ~ 1.2× vein 2-SR; precoxal sulcus distinctly sculptured; scutellum convex and finely rugulose; vein r from middle of pterostigma; mesoscutum with pair of brownish stripes; metasoma (except tergite I) reddish brown; head densely setose dorsally; notauli absent on disc; mesoscutum punctate medially; propodeum coriaceous-rugulose]; Iran, Russia .... ***P. riphaeus*** **(Tobias, 1986)**

– Antenna of ♀ with 20–33 segments; vein r of fore wing emitted before middle from pterostigma (Fig. 34); hypoclypeal depression present, medium-sized, but narrow in *P. adanaensis* (Fig. 35) **2**

2 Antenna of ♀ with ~ 33 segments (of ♂ with 32 (according to label of holotype, but in description 35) segments; length of body 2.5–3.0 mm and of fore wing ~ 3.0 mm; vein 1-R1 almost reaching apex of fore wing; vein m-cu of fore wing curved; [vein 3-SR of fore wing ~ 1.6× longer than vein 2-SR; ventro-basal lobe of mandible obtuse and distinctly protruding outwards; medio-posterior depression of mesoscutum large; precoxal sulcus distinctly crenulate; clypeus 5× wider than high; orbita of head largely yellow; length of hind femur (♂) ~ 3× longer than wide; vein m-cu of fore wing antefurcal; setose part of ovipositor sheath as long as metasomal tergite I]; England, Georgia, Greece, Ukraine (Crimea), and Asian part of Turkey ... .***P. christenseni*** **(Papp, 1982)**

– Antenna of ♀ with 20–25 segments (♂ unknown); length of body 1.5–2.1 mm and of fore wing 1.7–2.3 mm; vein 1-R1 of fore wing remaining distinctly removed from apex of wing (Fig. 34); vein m-cu of fore wing straight; [pronotum, hind coxa and metasomal tergite II orange brown; setose part of ovipositor sheath 0.05–0.08× as long as fore wing and 0.5–0.7× as long as tergite I; vein r of fore wing 0.5–0.6× as long as width of pterostigma; vein CU1b of fore wing absent] **3**

3 Clypeus slightly sinuate ventrally, largely strongly shiny and comparatively weakly widened medially (Fig. 35); vein m-cu of fore wing interstitial (Fig. 34); medio-posterior depression of mesoscutum triangular (Fig. 36); scutellum black; Asian Turkey.. ....***P. adanaensis*** **(Fischer & Beyarslan, 2005)**

– Clypeus straight ventrally, weakly shiny and distinctly widened medially; vein m-cu of fore wing antefurcal; medio-posterior depression of mesoscutum narrow elliptical; scutellum orange brown or chestnut brown **4**

4 Vein SR1 of fore wing ~ 2.5× as long as vein 3-SR; lateral lobes of mesoscutum largely yellowish brown; [tergite I medio-posteriorly weakly striate; scutellar sulcus with distinct carinae; mesopleuron dorsally, mesoscutum, prothorax yellowish brown and metasomal tergite I black]; Ukraine... .... ***P. arenaceus*** **(Jakimavičius, 1986)**

– Vein SR1 of fore wing ~ 3.5× as long as vein 3-SR; lateral lobes of mesoscutum black; [tergite I medio-posteriorly striate; scutellar sulcus crenulate; mesopleuron largely (except dorsally) and tergite I blackish or dark brown]; Asian Turkey ....***P. pseudarenaceus*** **(Fischer & Beyarslan, 2005)**

Pseudosteres adanaensis (Fischer & Beyarslan, 2005), comb. nov.

Figs 34–44

Biosteres (*Biosteres*) *adanaensis* Fischer & Beyarslan, 2005: 380–382; Beyarslan and Fischer 2013: 404.

Type material. ***Holotype***, ♀ (NMW), "[Turkey], Adana-Balcali, 9. iv. 1985, A. Beyarslan", "Holotype, ♀, *Biosteres adanaensis* sp. n., M. Fischer det. 2003".

Diagnosis. Antenna of ♀ with > 25 segments (♂ unknown); hypoclypeal depression narrow; clypeus slightly sinuate ventrally, largely strongly shiny and comparatively weakly widened medially (Fig. 35); vein m-cu of fore wing interstitial (Fig. 34); medio-posterior depression of mesoscutum triangular (Fig. 36); scutellum black; precoxal sulcus absent (Fig. 44); pterostigma wide elliptical (Fig. 34); vein 1-R1 of fore wing 0.6–0.7× as long as pterostigma, remaining distinctly removed from apex of wing; vein r of fore wing 0.5–0.6× as long as width of pterostigma; vein CU1b of fore wing absent; vein m-cu of fore wing interstitial (Fig. 34); vein 3-SR of fore wing 1.3× longer than vein 2-SR; pronotum, hind coxa and metasomal tergite II orange brown; setose part of ovipositor sheath 0.05–0.08× as long as fore wing and 0.5–0.7× as long as tergite I.

Biology. Unknown.

Distribution. Turkey (Asian part).

Pseudosteres arenaceus (Jakimavičius, 1986), comb. nov.

Opius (*Allotypus*) *arenaceus* Jakimavičius (in Tobias & Jakimavičius), 1986: 63; Fischer 1991: 152–154 (redescription); Beyarslan et al. 2017: 329 [holotype, ♀ (ZISP) not examined].

Biology. Unknown.

Distribution. Ukraine.

Notes. According to Fischer and Beyarslan (2005) *O. arenaceus* is closely related to *Opius pseudarenaceus* Fischer & Beyarslan from Asian Turkey which belongs to *Pseudosteres* gen. nov. and, therefore, *O. arenaceus* is provisionally transferred to this genus.

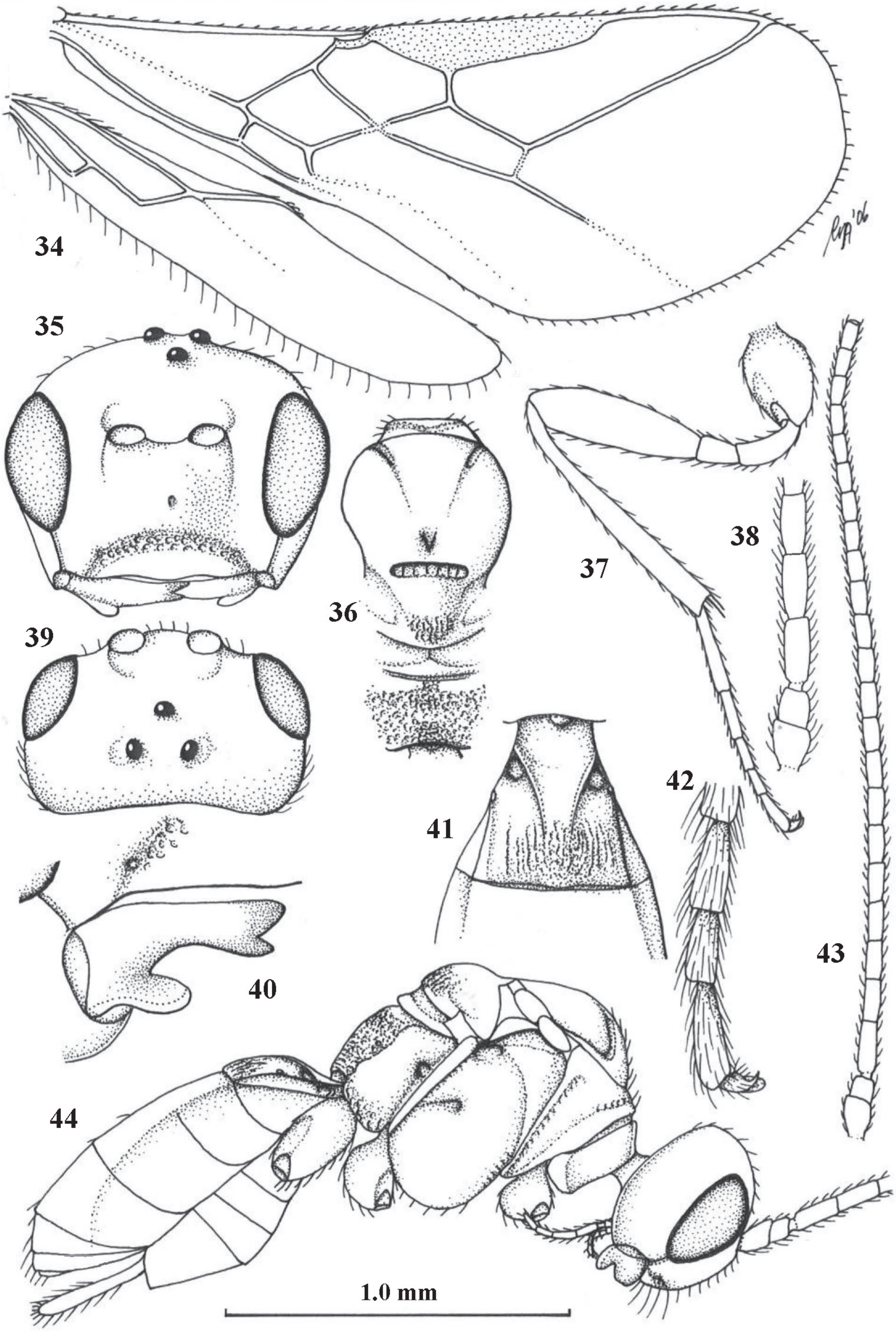
Pseudosteres christenseni (Papp, 1982), comb. nov.

Opius (*Xynobius*) *christenseni* Papp, 1982: 185; Fischer 1986: 618–620 (redescription); Tobias and Jakimavičius 1986: 29; Beyarslan and Fischer 2013: 447.

Type material. ***Holotype***, ♂ (MTMA), "Greece, Peloponnese, Monemvasia", "15. iv. 1978, J. Papp", "Holotypus ♂ *Opius* (*Xynobius*) *christenseni* sp. n., Papp, J., 1980% / ant. 32-art., "Hym. Typ. No. 2844, Museum Budapest".

Biology. Unknown.

Distribution. England, Greece, Ukraine, Georgia, and Asian Turkey.



Figures 34–44. *Pseudosteres adanaensis* (Fischer & Beyarslan), comb. nov., holotype, ♀ Turkey (Adana-Balcali) **34** wings **35** head anterior **36** mesosoma dorsal **37** hind leg **38** base of antenna **39** head dorsal **40** mandible lateral **41** metasomal tergite I dorsal **42** outer hind claw lateral **43** antenna **44** habitus lateral. Scale bar: 1.0× (**34, 36, 37, 43, 44**); 1.3× (**35, 39**); 1.5× (**41**); 2.5× (**38, 40, 42**).

Notes. As indicated on the label the holotype has 32 antennal segments, but according to the original description and the redescription the holotype male should have 35 antennal segments.

Pseudosteres pseudarenaceus **(Fischer & Beyarslan, 2005), comb. nov.**

Opius (*Allotypus*) *pseudarenaceus* Fischer & Beyarslan, 2005: 407–409; Beyarslan and Fischer 2013: 416.

Type material. ***Holotype***, ♀ (NMW), "Turkey, Adana-Balcali, 4. vi. [19]80, [A.] Beyarslan", "Holotype, ♀, *Opius* (*Allotypus*) *pseudarenaceus* sp. n., det. M. Fischer, 2003".

Biology. Unknown.

Distribution. Turkey (Asian part).

Notes. If *Opius arenaceus* indeed has a distinct dorsope, then *P. pseudarenaceus* and *P. arenaceus* are very similar and *P. pseudarenaceus* may be only a colour variety of the latter. The differences given by Fischer and Beyarslan (2005) are of very limited value: mesosoma 1.3× as long as high (1.4× in *P. arenaceus*), scutellar sulcus crenulate (with only three carinae), tergite I striate (rugose) and mesopleuron largely black (red).

Pseudosteres riphaeus **(Tobias, 1986), comb. nov.**

Opius riphaeus Tobias, 1986: 11, 12, 23 [holotype, ♀ (ZISP) not examined].

Opius (*Opiostomus*) *riphaeus*; Fischer, 1991: 180–182; Ameri et al. 2014: 7; Gadallah et al. 2016: 22.

Biology. Unknown.

Distribution. Iran, Russia (Asian part: Central Ural).

Cephaloplites **Szépligeti, 1897**

Figs 45–70

Cephaloplites Szépligeti, 1897: 600; Fischer 1972: 475–477. Type species (by monotypy): *Cephaloplites mocsaryi* Szépligeti, 1897.

Diagnosis. Antenna comparatively short, approximately as long as fore wing; scapus, fore coxa and trochanter distinctly compressed; face with pair of facial tubercles below antennal sockets more or less developed (Figs 45, 51, 53, 58, 67); epistomal suture with pair of large oblique and long pubescent tentorial depressions below tubercles (Figs 51, 59, 63, 68); clypeus narrow laterally, triangular and flattened (Fig. 59); occipital carina widely absent medio-dorsally and ventrally remaining far removed from hypostomal carina; hypoclypeal depression nearly absent to medium-sized (Figs 53, 58, 68); malar suture partly present (Fig. 51) or absent (Figs 58, 67); mandible strongly widened basally (Figs 63, 64, 67, 69) and more or less twisted apically; crenulate depression above eye absent;

pronope rather large and round (Fig. 61); prepectal and postpectal carinae absent (Fig. 53); notauli reduced to a pair of droplet-shaped isolated depressions (Fig. 61); medio-posterior depression of mesoscutum absent; scutellar sulcus narrow (Fig. 61); pterostigma narrowed subapically (Figs 46, 56); vein 3-SR of fore wing distinctly longer than vein 2-SR (Figs 46, 56); vein m-cu of hind wing weakly developed (Figs 46, 56); vein SR1 of fore wing completely sclerotised, reaching margin of wing and resulting in a closed marginal cell (Fig. 46); medio-posteriorly scutellum without elevated area; metasomal tergite I without dorsope; tergite II smooth and approximately as long as tergite III; ovipositor sheath hardly or not protruding beyond apex of metasoma (Fig. 67).

Biology. Parasitoids of Agromyzidae (Fischer 1964).

Distribution. Palaearctic: three species.

Key to species of the genus *Cephaloplites* Szépligeti

1 Head black; pair of tubercles of face indistinctly developed, only as a pair of small bumps, invisible in dorsal view of head and slightly visible in lateral view (Fig. 70); setose tentorial depression close to apical margin of clypeus and comparatively wide (Fig. 68); mandible with distinct ventral carina (Fig. 69)...................................... ***C. tadzhicus* Tobias & Saidov, 1995**

– Head largely reddish or brownish yellow (Fig. 45); pair of tubercles of face distinctly developed, distinctly visible in dorsal view of head (Fig. 62) and in lateral view (Figs 45, 67); setose tentorial depression either more removed from apical margin of clypeus (Fig. 51) or distinctly narrower (Fig. 59); ventral carina of mandible at most slightly developed (Fig. 64) **2**

2 Setose tentorial depression smaller and closer to base of mandible (Fig. 59); mesoscutum, scutellum and metasomal tergite III largely brownish yellow; head completely brownish yellow or largely so; coxae and trochanters brownish yellow or largely so; metasomal tergite I 1.1× as long as wide apically (Fig. 65); malar suture absent (Fig. 67)...................... ***C. mocsaryi* Szépligeti, 1897**

– Setose tentorial depression larger and further removed from base of mandible (Fig. 51); mesoscutum, scutellum and tergite III blackish; head partly black; coxae and trochanters dark brown or black; tergite I 1.3× longer than wide apically (Fig. 49); malar suture distinct (Fig. 51) .... ***C. gijswijti* sp. nov.**

Cephaloplites gijswijti sp. nov.

https://zoobank.org/AB164E12-6837-486D-A5B2-45DB1E2FFBDB

Figs 45–55

Type material. ***Holotype***, ♂ (RMNH), "Ellas [= Greece], Pelepon[nesos], prov. Korinthia, M.J. Gijswijt", "Nemea, 20.iv.1989".

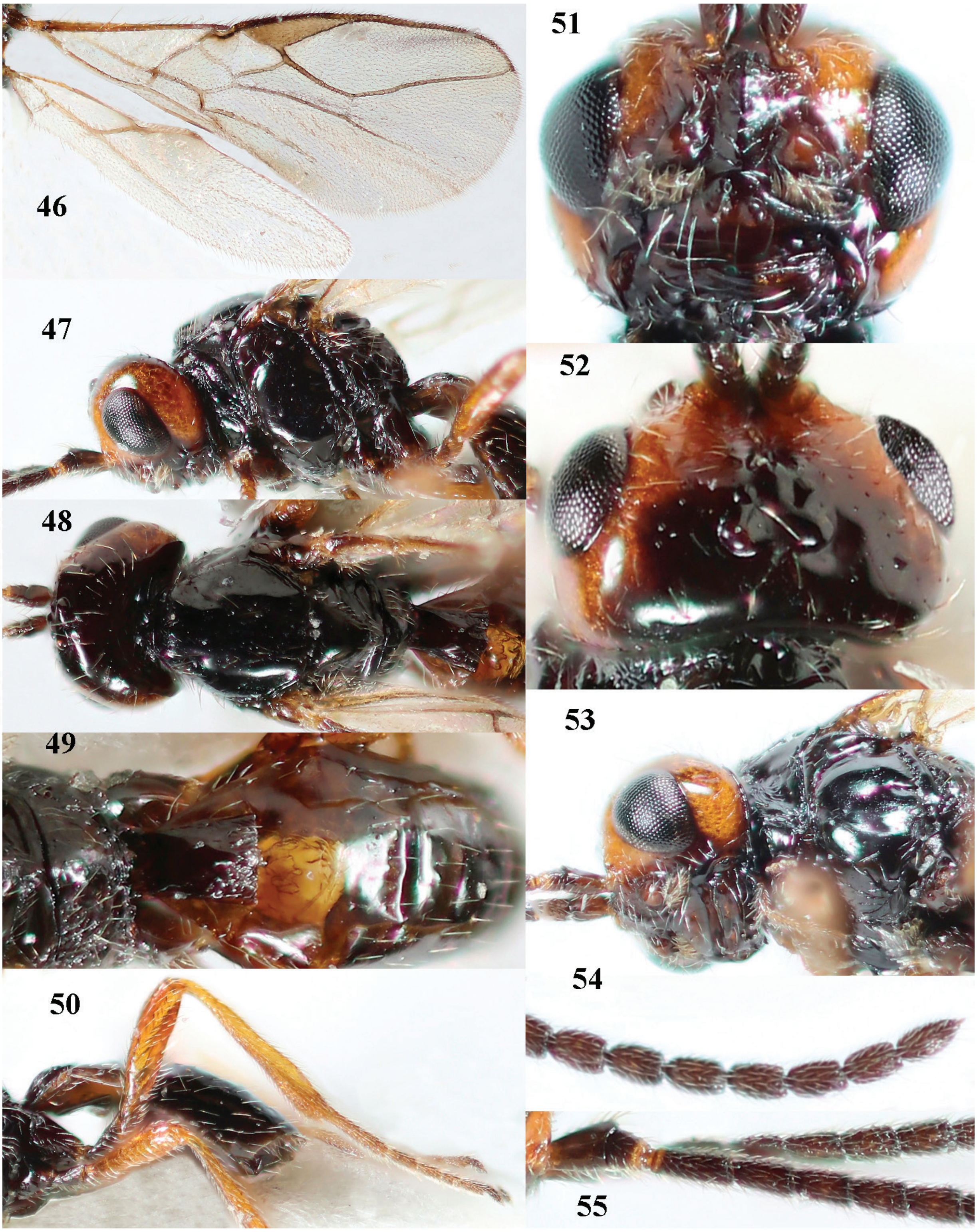
Diagnosis. Pair of tubercles of face distinctly developed, distinctly visible in dorsal view of head (Fig. 52) and in lateral view (Fig. 45); setose tentorial depression distinctly removed from apical margin of clypeus and large (Fig. 51); malar suture distinct (Fig. 51); head largely reddish or brownish yellow in lateral view (Fig. 45) and partly black in dorsal view; ventral carina of mandible at most slightly developed (Fig. 51); tergite I ~ 1.3× longer than wide apically (Fig. 49); mesoscutum, scutellum and tergite III blackish; coxae and trochanters dark brown or black.

Description. Holotype, ♂, length of body 1.7 mm, of fore wing 2.0 mm.

***Head*.** Antenna with 25 segments and as long as fore wing; third segment 1.3× longer than fourth segment, length of third, fourth and penultimate segments 3.0×, 2.1× and 1.7× their width, respectively, and apical segment with minute spine (Figs 54, 55); width of head 2× its median length in dorsal view, mainly smooth dorsally and posteriorly; behind stemmaticum without distinct depression; vertex convex and sparsely setose; OOL: diameter of ocellus: POL = 7:3:4 (Fig. 52); frons shallowly depressed medially and mainly smooth, medio-posteriorly with groove (Fig. 52; eye in dorsal view 1.1× longer than temple and temple behind eye subparallel-sided (Fig. 52); face with pair of distinctly protruding convex and smooth tubercles (Figs 45, 53), visible in dorsal view of head (Fig. 52); long setose tentorial depression comparatively large and distinctly removed from base of mandible (Fig. 51); clypeus flat, triangular, smooth, shiny and its ventral margin thin and straight, width of clypeus 3.8× its maximum height and 0.8× minimum width of face; hypoclypeal depression medium-sized and deep (Fig. 51); occipital carina distinct but dorsally absent (Fig. 52); temple and malar space smooth; length of malar space 0.6× basal width of mandible and 0.2× height of eye; malar suture distinct, narrow and complete (Fig. 51); mandible weakly twisted apically, upper tooth slender, basally asymmetrical because of wide ventral lobe and no distinct ventral carina (Fig. 51); length of maxillary palp 0.7× height of head.

***Mesosoma*.** Length of mesosoma 1.3× its height (Fig. 47); laterally pronotum smooth except some crenulae and rugulae anteriorly; dorsal pronope round (Fig. 52), rather large and oblique; propleuron flattened, shiny and smooth; mesopleuron smooth and shiny; postpectal carinae absent; precoxal sulcus absent and area flat and smooth; pleural sulcus finely crenulate; mesosternal sulcus deep and moderately crenulate; metapleuron smooth but ventrally rugose (Fig. 47); mesoscutum steeply raised above pronotum, shiny and

Figure 45. *Cephaloplites gijswijti* sp. nov., holotype, ♂, Greece (Nemea), habitus lateral.



Figures 46–55. *Cephaloplites gijswijti* sp. nov., holotype, ♂, Greece (Nemea) **46** wings **47** head and mesosoma lateral **48** head and mesosoma dorsal **49** metasoma dorsal **50** hind leg lateral **51** head anterior **52** head dorsal **53** head ventro-lateral **54** apex of antenna **55** base of antenna.

smooth anteriorly; notauli as pair of droplet-shaped and isolated depressions of mesoscutum, rather deep and anteriorly crenulate; transverse suture of mesoscutum absent; scutellar sulcus shallow and narrow, medially 0.1× as long as scutellum (Fig. 48); scutellum smooth, weakly convex, without subposterior depression; side of scutellum smooth; propodeum largely smooth, no carinae, but medio-posteriorly somewhat elevate, punctate and with some transverse striae but no lamella.

***Wings*.** Fore wing (Fig. 46): pterostigma triangular, distally elongate, 3.6× longer than its maximum width and gradually merging into vein 1-R1; vein M+CU1 weakly curved and unsclerotised; vein r-m present; 1-R1 ending just before wing apex; r:2-SR:3-SR:r-m:SR1 = 3:16:22:7:49; veins r and 3-SR slightly widened, vein r arising before middle of pterostigma and 2-SR slightly sinuate; m-cu postfurcal and straight, angled with 2-CU1; cu-a postfurcal and vertical; 1-CU1 very short and widened; CU1b rather short (Fig. 46). Hind wing: M+CU:1-M:1r-m = 7:8:5; cu-a straight; m-cu faintly indicated.

***Legs*.** Hind femur, tibia, and basitarsus 3.3×, 7.7×, and 3.0× as long as wide, respectively (Fig. 50); hind femur shiny and with long setae.

***Metasoma*.** Tergite **I** 1.3× longer than its apical width and slightly widened apically, its surface convex medially and largely smooth (only some rugulae posteriorly), dorsal carinae weakly developed and nearly up to apex of tergite (Fig. 49); tergite **II** and following tergites smooth; second suture absent dorsally.

***Colour*.** Black; temple, frons largely and face laterally reddish yellow; remainder of face, clypeus, malar space, antenna and tergite **II** dark brown; palpi brown; coxae and trochanters black or dark brown, remainder of legs brownish yellow; pterostigma and veins brown; wing membrane subhyaline (Fig. 46).

Biology. Unknown.

Distribution. Greece.

Etymology. Named after the aimable collector of the holotype, Martinus Johannes (Theo) Gijswijt (10.xi.1927–27.v.2015), who was one of the major specialists of European Chalcidoidea.

Cephaloplites mocsaryi Szépligeti, 1897
Figs 56–67

Cephaloplites mocsaryi Szépligeti, 1897: 600–601; Fischer 1972: 476–477; Papp 2004: 157 (type lost).

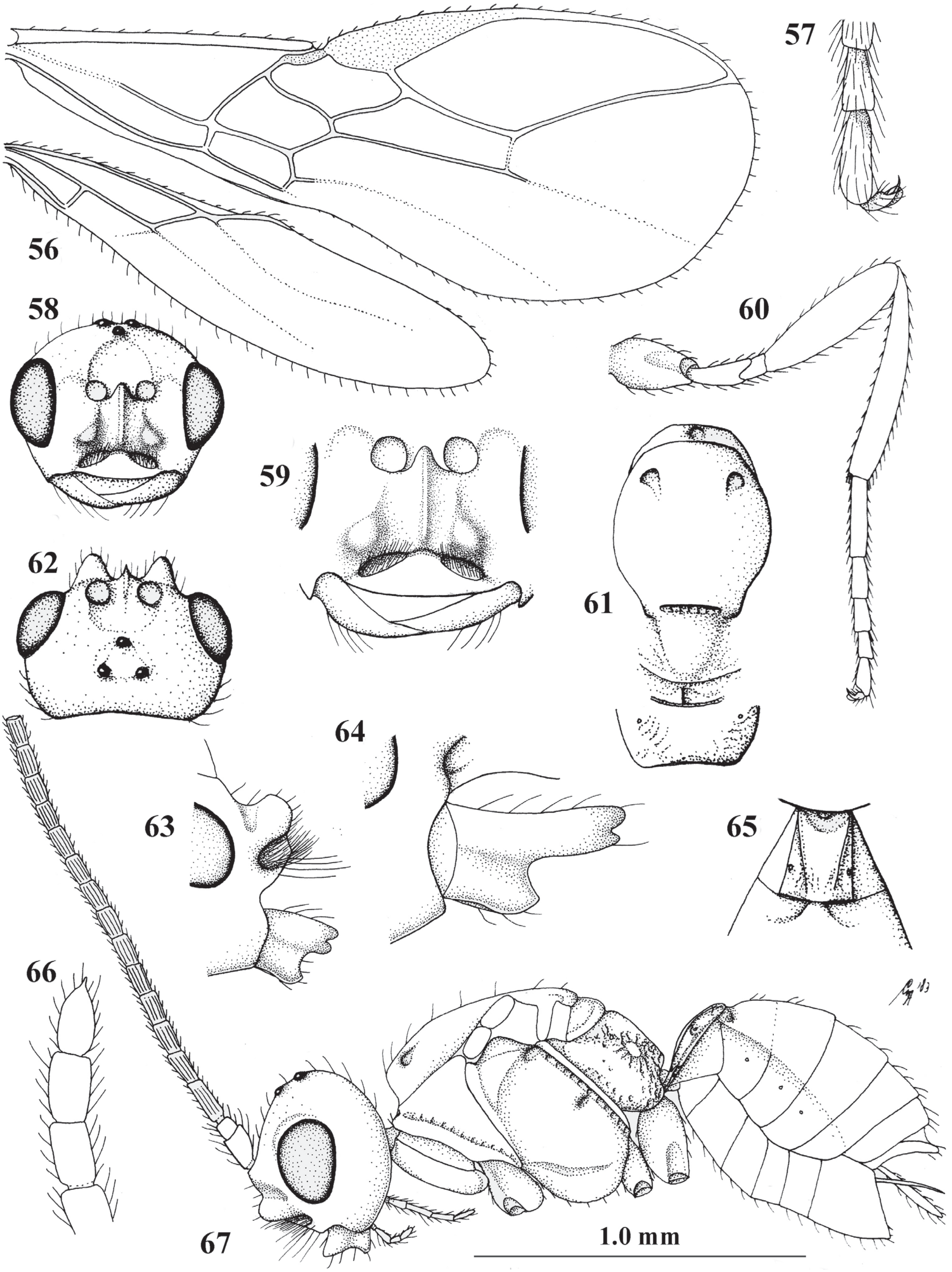
Opius (*Hypocynodus*) *kilisanus* Fischer & Beyarslan, 2005: 403–405; Beyarslan and Fischer 2013: 421. Syn. nov.

Type material. *Holotype* of *C. mocsaryi* (♀ from Hungary, Budapest, Zugliget) is lost. ***Holotype*** of *O. kilisanus*, ♂ (NMW), “[Turkey], Hatay-Kilis, 6. v. 1985, A. Beyarslan”.

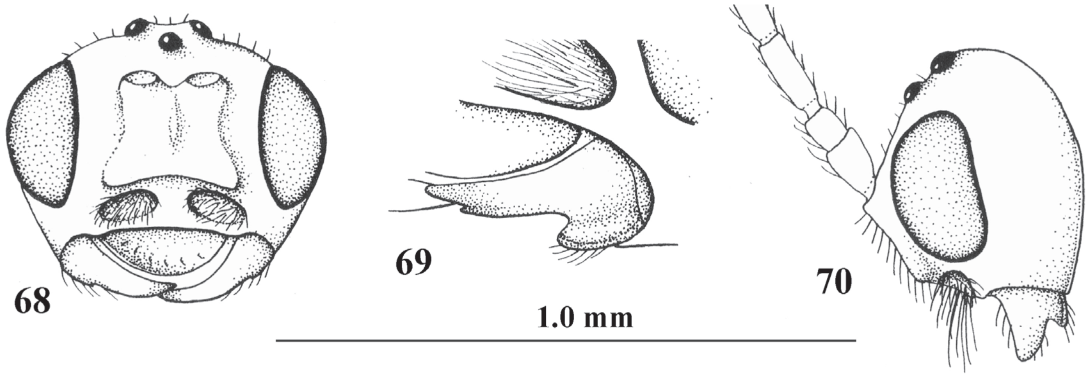
Biology. Parasitoids of Agromyzidae: *Agromyza woerzi* Groschke (Fischer 1964).

Distribution. Czech Republic, Germany, Hungary, and Turkey (Asian part).

Notes. The holotype of *O. kilisanus* has the mandible, malar space, temple ventrally, bases of hind and middle coxae and of trochantelli, mesosoma (except mesoscutum, scutellum, dorsal part of pronotum and mesopleuron) and metasoma (except second and most of tergite **III**) blackish, the temple somewhat less rounded and narrowed than figured for the female and the antenna with 26 segments. The differences are most likely clinal and considered to fall within the species limits of *C. mocsaryi*.



Figures 56–67. *Cephaloplites mocsaryi* Szépligeti, ♀, Hungary (Budapest), but apex of antenna of Germany (Stuttgart) **56** wings **57** outer hind claw lateral **58** head anterior **59** face anterior **60** hind leg **61** mesosoma dorsal **62** head dorsal **63** face and mandible lateral **64** mandible lateral **65** metasomal tergite I dorsal **46** apex of antenna **67** habitus lateral. Scale bar: 1.0× (**56, 58–62, 65, 67**); 1.5× (**63, 64**); 2.5× (**57, 66**).



Figures 68–70. *Cephaloplites tadzhicus* Tobias & Saidov, holotype, ♂, Tajikistan **68** head anterior **69** mandible lateral **70** head lateral. Scale bar: 1.0×, but detail of mandible 1.5×.

Cephaloplites tadzhicus **Tobias & Saidov, 1995**
Figs 68–70

Cephaloplites tadzhicus Tobias & Saidov, 1995: 683–684.

Type material. ***Holotype***, ♂ (ZISP), "[Tajikistan], Vysje, Pos. Anzov, 2000 m, Gissarsk. Chr., 26. vi. [1]965, Tobias", "Holotypus *Cephaloplites tadzhicus* Tobias & Zaidov sp. n.". Three male paratypes (ZISP) with same label data not examined.

Biology. Unknown.

Distribution. Central Asia: Tajikistan.

Notes. The venation of *C. tadzhicus* is similar to that of the type species (cf. Fig. 56), the protuberances of the face are small (Fig. 70; not visible in dorsal view), the ♂ antenna with 28 segments, the metasoma dark brown as the remainder of the body, coxae, trochanters and trochantelli, the apex of the hind tibia and all tarsi infuscate to rather dark brown.

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Additional information

Conflict of interest

The author has 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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Review Article

Peruvian nudibranchs (Mollusca, Gastropoda, Heterobranchia): an updated literature review-based list of species

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Abstract

Nudibranchs, as a group, have received limited attention in terms of scientific study along the coastline of Peru. Here, an updated and comprehensive list of nudibranch species found in the Peruvian sea is presented, compiled through an extensive review of relevant literature. This compilation encompasses a total of 31 species, classified into two suborders, 10 superfamilies, 20 families, and 28 genera. With respect to the biogeographic provinces along the Peruvian coast, 23 species inhabit the Warm Temperate Southeastern Pacific province, 18 species occur in the Tropical Eastern Pacific province, and 10 species are found in both provinces, crossing the transitional zone between them. In terms of distribution patterns, two species exhibit a cosmopolitan distribution (*Glaucus atlanticus* and *Fiona pinnata*), while two species display a circumtropical distribution (*Cephalopyge trematoides* and *Phylliroe bucephala*). One species exhibits a bipolar distribution in the Eastern Pacific and possesses an amphi-South American distribution (*Rostanga pulchra*). Additionally, six species exhibit an amphi-South American distribution (*Rostanga pulchra*, *Diaulula punctuolata*, *Doto uva*, *Tyrinna evelinae*, *Tyrinna delicata*, and *Doris fontainii*), and two species are endemic to Peru (*Corambe mancorensis* and *Felimare sechurana*). This study provides comprehensive information on biogeographical aspects, geographical distributions, and taxonomic updates within the nudibranch species documented in Peru. Furthermore, we discuss the status of species listed in previous literature that have not been confirmed by collections, referring to them as potentially occurring species.

Key words: biogeography, bibliographic compilation, geographic distribution, Nudibranchia, Peru, sea slug, taxonomy

Introduction

Nudibranchia Cuvier, 1817 (Subclass Heterobranchia, Infraclass Euthyneura, Superorder Nudipleura) represents an order of exclusively marine gastropod mollusks, distinguished by the absence of shells in the adult stage (Behrens et al. 2005; Wägele and Klussmann-Kolb 2005). These remarkable organisms exhibit striking aposematic body colorations, making them frequent targets of underwater photography. Approximately 3000 species of nudibranchs have been described worldwide inhabiting both cold and tropical regions (Shields 2009; Almada et al. 2016), primarily in shallow waters ranging from 0 to 30 m in depth (Wägele and Klussmann-Kolb 2005). However, novel species discoveries have expanded our knowledge of nudibranchs in deeper habitats (Valdés 2001a, 2001b; Gosliner et al. 2008). The ecological importance of nudibranchs stems from their role in controlling populations of cnidarians and poriferans, from which they acquire and incorporate toxins for their own defense (Greenwood 2009; Faulkner and Ghiselin 1983). Furthermore, recent research has elucidated symbiotic associations between nudibranchs and bacteria, wherein the bacteria provide essential nutrients to the hosts (Zhukova et al. 2022). Certain species have been found to synthesize secondary metabolites with high biotechnological potential (Pereira et al. 2012; Dean and Prinsep 2017). Additionally, nudibranch species possess the potential to serve as environmental indicators, as they are sensitive to ocean stressors such as coastal pollution (Caballer et al. 2008).

The most recent inventory of aquatic mollusks in Peru, as documented by Ramírez et al. (2003), recorded a total of 1018 marine species, primarily comprising gastropods and bivalves, with only a limited number of nudibranch species reported. In fact, the Peruvian sea is recognized as one of the impoverished regions worldwide in terms of nudibranch diversity (Schrödl 1997, 2002, 2003; Schrödl and Hooker 2014). In comparison, other regions such as the Caribbean Sea, the Tropical Eastern Pacific, the Indian Ocean, the Mediterranean Sea (Sachidhanandam et al. 2000; Valdés et al. 2006; Chavanich et al. 2013; Ah Shee Tee et al. 2019; Furfaro et al. 2020; Londoño-Cruz 2021), as well as other South American countries including Brazil, Chile, and Venezuela (Fischer and Cervera 2005b; Ardila et al. 2007; Aldea et al. 2011; Padula et al. 2011; Alvim and Pimenta 2013; Gutiérrez et al. 2015; Araya and Valdés 2016; Londoño-Cruz 2021) exhibit considerably higher richness of nudibranch species.

The earliest records of nudibranchs in Peru can be attributed to d’Orbigny (1835–1846) and later to Dall (1909). Following a significant period without the discovery of new species, Millen et al. (1994) reported the presence of *Okenia luna* in Peruvian and Chilean waters. Subsequently, the first list of Peruvian aquatic mollusks was published, which included some nudibranch species (Álamo and Valdivieso 1997), and two years later, another list was published (Paredes et al. 1999), in which Sandra Millen was acknowledged for the preliminary list of species belonging to the infraclass Opisthobranchia (a taxonomic category that has since been abandoned and deprecated; see Jörger et al. 2010; Schrödl et al. 2011; Wägele et al. 2014). In 2003, an updated inventory of Peruvian aquatic mollusks was published (Ramírez et al. 2003), which included the nudibranch species reported up to that time. Several years later, four new species were reported on the northern coast of Peru, *Felimida baumanni*, *Doriopsilla janaina*, *Kynaria cynara*, and *Cuthona* sp. (Nakamura 2006), with the species *Corambe mancorensis* identified as endemic (Martynov et al. 2011), and the species *Spurilla neapolitana* (later corrected as *Spurilla braziliana*) (Uribe and Pacheco 2012). Subsequently, four additional nudibranch species were documented for the Peruvian coast (Uribe et al. 2013), and another study focused on the species shared with Chile (Schrödl and Hooker 2014). *Felimare sechurana* was identified as an endemic species in the transition zone of the northern coast (Hoover et al. 2017) and, additionally, two new species of planktonic nudibranchs were described (Quesquen 2017).

We must emphasize that certain species have been listed in previous publications as occurring in Peru without sufficient evidence, such as assumptions of geographic continuity (e.g., *Cadlina sparsa;* Álamo and Valdivieso 1997), reliance on personal communications only (e.g., *Polycera* cf. *alabe*; Paredes et al. 1999; Uribe et al. 2013), and misinterpretations (e.g., *Gargamella immaculata* and *Thecacera darwini*; Nakamura 2006), which has created problems as these listings have persisted in the literature. To distinguish them from the confirmed species, the term "potentially occurring" is used hereafter. However, such statuses can be revised in the future, as exemplified by the species *Rostanga pulchra*, which was initially predicted for Peruvian waters for many years until its confirmation (Schrödl and Hooker 2014).

El Niño-Southern Oscillation (ENSO) warm events have been observed to induce southward displacement of tropical species (Velez and Zeballos 1985; Paredes et al. 1998) while cold events tend to enhance the intensity of the Humboldt Current, resulting in the northward transport of larvae. Specifically, the northward transport of larvae by the Humboldt Current or the southward transport facilitated by warm ENSO events may introduce Magellanic or tropical species, respectively, into Peruvian waters, thereby influencing distribution ranges. In addition, the susceptibility of nudibranchs to temperature fluctuations, particularly during their larval stages (Leatherman 2019) due to the aragonite-based internal structure found in several species (Ehrlich 2010), their small body size, limited populations (Nybakken 1978), and the sensitivity of the Humboldt Current Ecosystem to oceanic stressors (Echevin et al. 2012) such as warming and acidification (Barnosky et al. 2011; Ceballos et al. 2015; Pievani 2014), collectively suggest that the diversity and distribution of nudibranchs could be impacted (Nimbs and Smith 2018).

The available information on Peruvian nudibranchs remains limited primarily due to a lack of research effort (Uribe and Pacheco 2012). Explorations specifically targeting nudibranchs have been extremely scarce, and most sightings and reports are sporadic (Nakamura 2006; Schrödl and Hooker 2014; Uribe et al. 2013). Given that the species richness of nudibranchs is likely underestimated in Peru (Hooker pers. comm.), this taxonomic order warrants further attention. Our aim was to update and revise the list of nudibranchs in the Peruvian sea, based on a comprehensive review of the scientific literature.

Materials and methods

A comprehensive review was conducted to compile all available literature pertaining to the order Nudibranchia in Peru. The literature search encompassed diverse sources of information, including peer-reviewed journal articles, books, book chapters, "grey literature" (such as scientific reports and theses), and the Sea Slug Forum (http://www.seaslugforum.net/). Key terms such as 'Opisthobranchia,' 'Heterobranchia,' 'Nudibranch,' 'Nudibranchia,' 'sea slug,' 'phylogeny,' 'checklist,' 'Peru,' 'Humboldt,' and 'taxonomy' were employed. Pertinent data, such as type material, geographic distribution, sampling/reporting sites, bathymetric distribution, and biogeographical provinces, were meticulously included. The most up-to-date scientific names were validated through the World Register of Marine Species (WoRMS, https://www.marinespecies.org/), and reports (occurrences) were cross-referenced using the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/) and the iNaturalist database (https://www.inaturalist.org/). Any modifications, revalidations, or refutations pertaining to taxonomy are concisely presented as "Remarks", accompanied by justifications as needed. Endemic species of Peru are also duly indicated. The distribution map was made using QGIS 3.22.8 software (QGIS Development Team 2022), while VENNY 2.1 online software (Oliveros 2016) was employed to visualize the number of species shared with some neighboring countries. Potentially occurring species were clearly distinguished from the confirmed ones.

The acronyms corresponding to the collections where the type material for certain species is deposited have been included, as follows:

CASIZ	California Academy of Sciences Invertebrate Zoology, San Francisco
CZA	Colección de Zoología Acuática, Universidad Peruana Cayetano Heredia, Lima
NHMUK	Natural History Museum, London
MHNURP	Museo Historia Natural Vera Alleman Haeghebaert, Universidad Ricardo Palma, Santiago de Surco
RMNH	Naturalis Biodiversity Center, Leiden
SMNH	Swedish Museum of Natural History, Stockholm
USNM	Smithsonian National Museum of Natural History, Washington DC
ZMB	The Berlin Zoological Museum, Berlin
ZSM	The Bavarian State Collection of Zoology, Munich

Results

Overview

A total of 31 species, encompassing two suborders, ten superfamilies, 20 families, and 28 genera (Table 1), has been confirmed within Peruvian waters. The suborder Cladobranchia comprises 16 species, spanning five superfamilies and 13 families. The suborder Doridina consists of 15 species, distributed among five superfamilies and seven families (Table 1). Notably, the families Chromodorididae and Discodorididae, both belonging to the suborder Doridina, exhibit remarkable species richness with five and four species, respectively (Fig. 1). Additionally, potentially occurring species (*n* = 9) are distributed across five superfamilies, eight families, and nine genera (Table 2). A chronological overview of articles documenting nudibranch species in the Peruvian sea can be found in Table 3, revealing the progressive increase in reported species and the latest updates in scientific nomenclature. Regarding the distribution of species along the Peruvian coast, according to the coastal marine biogeographical classification proposed by Spalding et al. (2007), 23 species inhabit within the Warm Temperate Southeastern Pacific province, 18 species are found within the Tropical Eastern Pacific province, and ten species are common to both provinces (Table 4, Fig. 2).

Peru's inventory of nudibranch species is comparatively modest in comparison to other South American countries, such as Chile, Colombia, and Brazil (Fig. 3A). Concerning species shared with these countries, of the 31 species that have been conclusively documented in Peruvian waters, 19 species are found

Table 1. Nudibranch species confirmed for Peruvian waters according to the bibliographic compilation of this study.

Suborders (*n* = 2)	**Superfamilies (*n* = 10)**	**Families (*n* = 20)**	**Species (*n* = 31)**
Cladobranchia	Aeolidioidea	Aeolidiidae	*Spurilla braziliana* MacFarland, 1909
		Facelinidae	*Phidiana lottini* (Lesson, 1831)
			Bajaeolis bertschi Gosliner & Behrens, 1986
		Glaucidae	*Glaucus atlanticus* Forster, 1777
	Arminoidea	Arminidae	*Armina californica* (J.G. Cooper, 1863)
	Dendronotoidea	Dendronotidae	*Dendronotus* cf. *venustus* MacFarland, 1966
		Dotidae	*Doto uva* Er. Marcus, 1955
		Hancockiidae	*Hancockia schoeferti* Schrödl, 1999
		Phylliroidae	*Cephalopyge trematoides* (Chun, 1889)
			Phylliroe bucephala Lamarck, 1816
		Cuthonidae	*Cuthona* sp.
		Fionidae	*Fiona pinnata* (Eschscholtz, 1831)
		Flabellinidae	*Kynaria cynara* (Ev. Marcus & Er. Marcus, 1967)
			Coryphellina cerverai (M. A. Fischer, van der Velde & Roubos, 2007)
	Proctonotoidea	Janolidae	*Janolus rebeccae* Schrödl, 1996
	Tritonioidea	Tritoniidae	*Tritonia* sp.
Doridina	Chromodoridoidea	Chromodorididae	*Tyrinna delicata* (Abraham, 1877)
			Tyrinna evelinae (Er. Marcus, 1958)
			Felimare agassizii (Bergh, 1894)
			Felimare sechurana Hoover, Padula, Schrödl, Hooker & Valdés, 2017
			Felimida baumanni (Bertsch, 1970)
	Doridoidea	Discodorididae	*Baptodoris peruviana* (d'Orbigny, 1837)
			Diaulula variolata (d'Orbigny, 1837)
			Diaulula punctuolata (d'Orbigny, 1837)
			Rostanga pulchra MacFarland, 1905
		Dorididae	*Doris fontainii* d'Orbigny, 1837
	Onchidoridoidea	Corambidae	*Corambe lucea* Er. Marcus, 1959
			Corambe mancorensis Martynov et al., 2011
		Goniodorididae	*Okenia luna* Millen, Schrödl, Vargas & Indacochea, 1994
	Phyllidioidea	Dendrodorididae	*Doriopsilla janaina* Er. Marcus & Ev. Marcus, 1967
	Polyceroidea	Polyceridae	*Polycera priva* Er. Marcus, 1959

in Chilean waters, four species in Colombian waters, and four species in Brazilian waters (Fig. 3B). The recorded collection/reporting sites of nudibranch species found in Peruvian waters, limited to South America, within the framework of the coastal-marine biogeographical classification proposed by Spalding et al. (2007), are illustrated in Fig. 4.

Table 2. Nudibranch species that could potentially occur in Peruvian waters based on the bibliographic compilation of this study.

Suborder (*n* = 2)	Superfamilies (*n* = 5)	Families (*n* = 8)	Species (*n* = 9)
Cladobranchia	Aeolidioidea	Aeolidiidae	*Aeolidia campbellii* (Cunningham, 1871)
		Glaucidae	*Glaucus* sp.
		Phylliroidae	*Phylliroe lichtensteinii* Eschscholtz, 1825
	Fionoidea	Coryphellidae	*Itaxia falklandica* (Eliot, 1907)
	Flabellinoidea	Flabellinidae	*Coryphellina marcusorum* (Gosliner & Kuzirian, 1990)
Doridina	Chromodoridoidea	Cadlinidae	*Cadlina sparsa* (Odhner, 1922)
		Discodorididae	*Gargamella immaculata* Bergh, 1894
	Polyceroidea	Polyceridae	*Polycera* cf. *alabe* Collier & Farmer, 1964
			Thecacera darwini Pruvot-Fol, 1950

Table 3. Chronologically ordered publications listing nudibranch species in the Peruvian sea. Legend: First reports for Peruvian waters: [a]d'Orbigny (1835–1846), [b]Dall (1909), [c]Millen et al. (1994), [d]Nakamura (2006), [e]Schrödl (2003), [f]Schrödl (1999), [g]Schrödl (2000), [h]Martynov et al. (2011), [i]Uribe et al. (2013), [j]Uribe and Pacheco (2012), [k]Schrödl and Hooker (2014), [l]Hoover et al. (2017), [m]Quesquen (2017), sp = The total count of nudibranch species mentioned in each checklist. Single asterisk (*) shows potentially occurring species and double asterisk (**) shows recent confirmation of previously predicted species in Peruvian waters. § shows that the scientific name has undergone changes.

d'Orbigny (1835–1846) (sp = 5)	Dall (1909) (sp = 6)	Álamo and Valdivieso (1997) (sp = 7)	Paredes et al. (1999) / Ramírez et al. (2003) (sp = 18)	(Nakamura 2006) (sp = 23)	Uribe et al. (2013) (sp = 25)	Schrödl and Hooker (2014) (sp = 14)	This study (sp = 31) * nine potentially ocurring species
Doriopsis peruviana[a]	Doris peruviana	Dendrodoris peruviana	Doris peruviana	Baptodoris peruviana	Baptodoris peruviana	Baptodoris? peruviana	Baptodoris peruviana§
Diphyllidia cuvieri	Pleurophyllidia cuvieri			Armina cuvieri	Armina californica		Armina californica§
Phidiana natans[a]	P. natans/ Fiona pinnata	Phidiana natans	P. natans / Fiona pinnata	Fiona pinnata	Fiona pinnata		Fiona pinnata§
Phidiana inca[a]	Phidiana inca	Phidiana inca	Phidiana lottini	Phidiana lottini	Phidiana lottini	Phidiana lottini	Phidiana lottini§
Glaucus distichoicus	Glaucus distichoicus		Glaucus atlanticus		Glaucus atlanticus		Glaucus atlanticus§
	Doris punctuolata[b]	Doris punctuolata	Anisodoris punctuolata	Diaulula punctuolata			Diaulula punctuolata§
		Okenia luna[c]	Okenia luna	Okenia luna	Okenia luna	Okenia luna	Okenia luna
		Cadlina? sparsa*	Cadlina sparsa*	Cadlina sparsa*			Cadlina sparsa*
		Rostanga pulchra*	Rostanga pulchra*	Rostanga pulchra*		Rostanga pulchra**	Rostanga pulchra
			Aeolidia serotina*	Aeolidia serotina*			Aeolidia campbellii*§
			Hypselodoris cf. agassizii	Hypselodoris agassizii	Felimare agassizii		Felimare agassizii§
			Flabellina cf. falklandica*	Flabellina falklandica			Itaxia falklandica*§
			Dendronotus frondosus	Dendronotus frondosus	Dendronotus cf. venustus		Dendronotus cf. venustus§

d'Orbigny (1835–1846) (sp = 5)	Dall (1909) (sp = 6)	Álamo and Valdivieso (1997) (sp = 7)	Paredes et al. (1999) / Ramírez et al. (2003) (sp = 18)	(Nakamura 2006) (sp = 23)	Uribe et al. (2013) (sp = 25)	Schrödl and Hooker (2014) (sp = 14)	This study (sp = 31) * nine potentially ocurring species
			Doto cf. *uva*	*Doto uva*	*Doto uva*	*Doto uva*	*Doto uva*
			Polycera cf. *alabe*	*Polycera alabe*	*Polycera alabe*		*Polycera* cf. *alabe*
			Tyrinna evelinae	*Tyrinna evelinae*	*Tyrinna evelinae*		*Tyrinna evelinae*
			Bajaeolus bertschi	*Bajaeolis bertschi*	*Bajaeolis bertschi*		*Bajaeolis bertschi*
			*Phylliroe lichtensteini**				*Phylliroe lichtensteinii**
				Flabellina cynara[d]	*Flabellina cynara*		*Kynaria cynara*§
				Glossodoris baumanni[d]	*Glossodoris baumanni*		*Felimida baumanni*§
				Cuthona sp.[d]	*Cuthona* sp.		*Cuthona* sp.
				Doriopsilla janaina[d]	*Doriopsilla janaina*		*Doriopsilla janaina*
				Flabellina sp. 2[e]	*Flabellina cerverai*	*Flabellina* cf. *cerverai*	*Coryphellina cerverai*§
				*Gargamella immaculata**[f]			*Gargamella immaculata**
				Doris fontainei[g]	*Doris fontainei*	*Doris fontainei*	*Doris fontainii*§
					Corambe mancorensis[h]		*Corambe mancorensis*
					Diaulula variolata[i]	*Diaulula variolata*	*Diaulula variolata*
					Tyrinna nobilis[i]		*Tyrinna delicata*§
					Tritonia sp[i]		*Tritonia* sp.
					Spurilla cf. *neapolitana*[j]	*Spurilla braziliana*	*Spurilla braziliana*
				*Thecacera darwini**	*Thecacera darwini**	*Thecacera darwini**	*Thecacera darwini**
						Polycera priva[k]	*Polycera priva*
						Corambe lucea[k]	*Corambe lucea*
						Janolus rebeccae[k]	*Janolus rebeccae*
						Hancockia schoeferti[k]	*Hancockia schoeferti*
							Felimare sechurana[l]
							Cephalopyge trematoides[m]
							Glaucus sp.*[m]
							Phylliroe bucephala[m]
							*Coryphellina marcusorum**

Table 4. Presence of nudibranch species inhabiting Peruvian waters along several marine coastal biogeographic provinces according the reporting sites. Legend: the asterisk (*) indicates potentially occurring species in Peruvian waters.

Families	Species	Magellanic	Pacific					Atlantic						Mediterranean Sea	Circumtropical	Cosmopolitan
			Warm Temperate Southeastern Pacific	Tropical Eastern Pacific	Warm Temperate Northeast Pacific	Cold Temperate Northeast Pacific	Galapagos	North Brazil Shelf	Tropical Northwestern Atlantic	Tropical Southwestern Atlantic	West African Transition	Warm Temperate Southwestern Atlantic	Lusitanian			
Aeolidiidae	*Spurilla braziliana*		X	X	X				X	X						
	Aeolidia campbellii (*)	X	X													
Facelinidae	*Phidiana lottini*	X	X													
	Bajaeolis bertschi			X	X											
Glaucidae	*Glaucus atlanticus*		X	X	X		X		X	X				X	X	X
	Glaucus sp. (*)		X													
Arminidae	*Armina californica*			X	X	X										
Dendronotidae	*Dendronotus* cf. *venustus*		X		X	X										
Dotidae	*Doto uva*	X	X									X				
Hancockiidae	*Hancockia schoeferti*	X	X													
Phylliroidae	*Cephalopyge trematoides*		X	X	X						X		X		X	
	Phylliroe bucephala		X	X					X				X	X	X	
	Phylliroe lichtensteinii (*)		X							X						X
Coryphellidae	*Itaxia falklandica* (*)	X	X									X				
Cuthonidae	*Cuthona* sp.	X		X												
Fionidae	*Fiona pinnata*		X													X
Flabellinidae	*Kynaria cynara*		X	X	X											
	Coryphellina cerverai		X	X												
	Coryphellina marcusorum (*)			X					X							
Janolidae	*Janolus rebeccae*		X	X												
Tritoniidae	*Tritonia* sp.		X	X												
Cadlinidae	*Cadlina sparsa* (*)	X	X		X											
Chromodorididae	*Tyrinna delicata*	X	X													
	Tyrinna evelinae			X	X											
	Felimare agassizii		X	X	X		X									
	Felimare sechurana			X												
	Felimida baumanni			X	X											

Families	Species	Magellanic	Pacific					Atlantic						Mediterranean Sea	Circumtropical	Cosmopolitan
			Warm Temperate Southeastern Pacific	Tropical Eastern Pacific	Warm Temperate Northeast Pacific	Cold Temperate Northeast Pacific	Galapagos	North Brazil Shelf	Tropical Northwestern Atlantic	Tropical Southwestern Atlantic	West African Transition	Warm Temperate Southwestern Atlantic	Lusitanian			
Discodorididae	*Baptodoris peruviana*		X													
	Diaulula variolata	X	X													
	Diaulula punctuolata		X													
	Rostanga pulchra	X	X		X	X										
	Gargamella immaculata (*)	X	X													
Dorididae	*Doris fontainii*	X	X													
Corambidae	*Corambe lucea*	X	X	X												
	Corambe mancorensis			X												
Goniodorididae	*Okenia luna*		X													
Dendrodorididae	*Doriopsilla janaina*			X	X		X									
Polyceridae	*Polycera priva*	X	X													
	Polycera cf. *alabe* (*)			X	X											
	Thecacera darwini (*)	X	X													

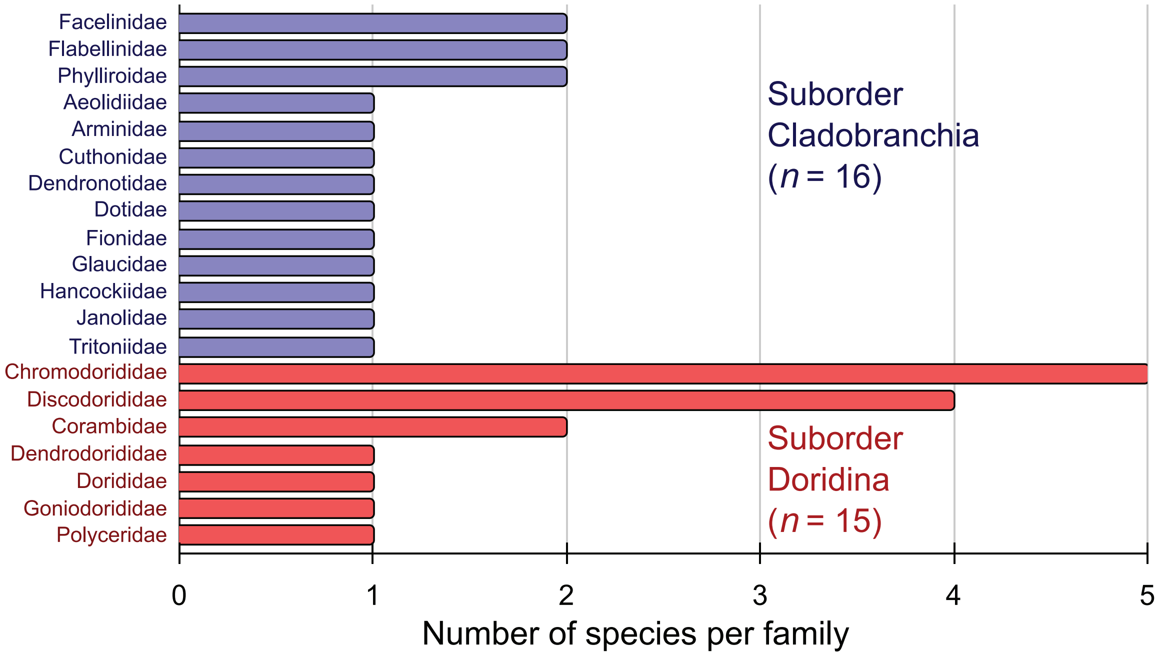

Figure 1. Number of species by family and suborder of Nudibranchia in Peruvian waters.

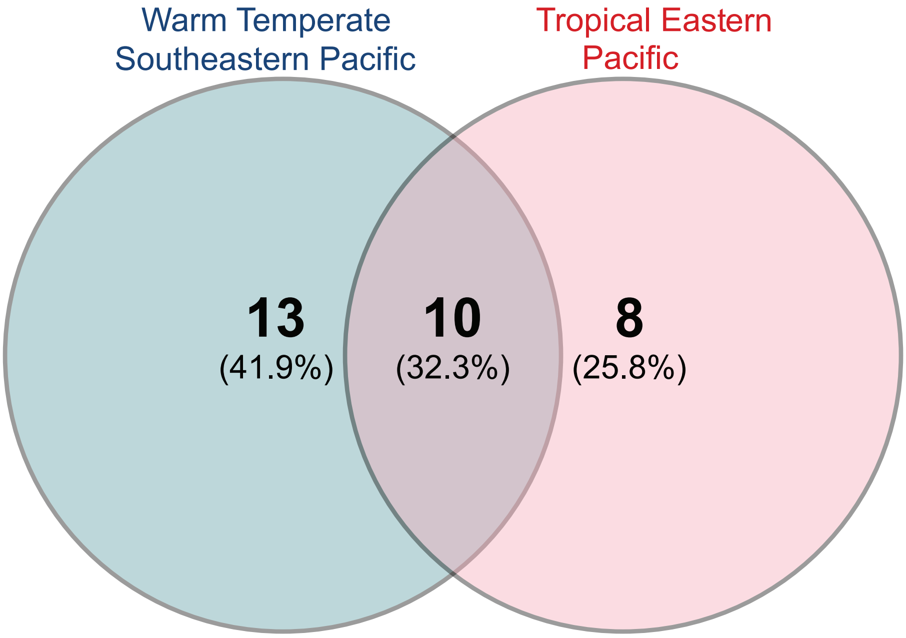

Figure 2. Venn diagram showing the number of species shared between the two coastal marine biogeographic provinces present in Peru.

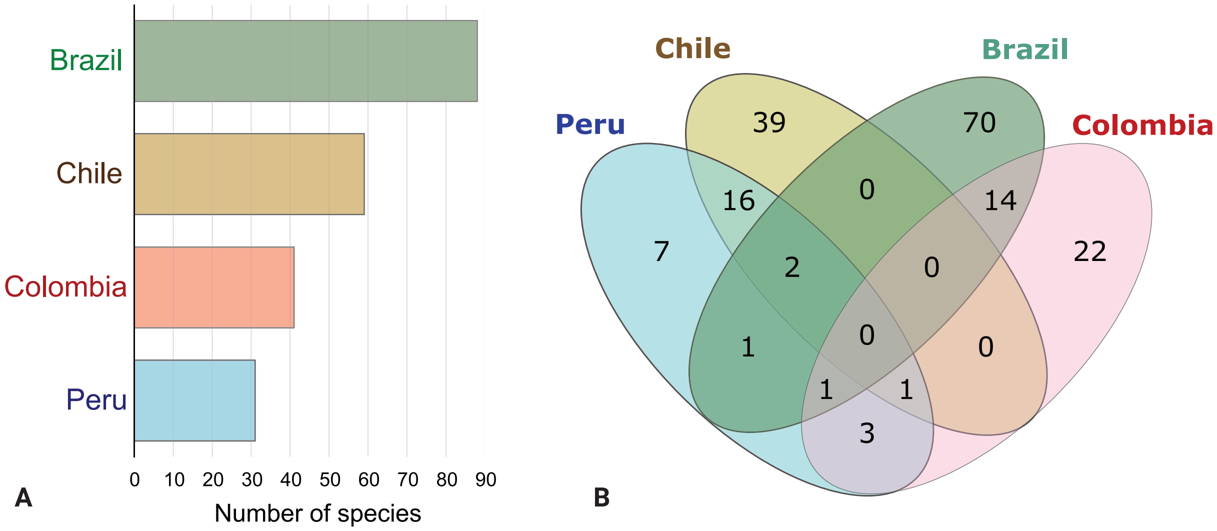

Figure 3. A Nudibranch species richness by country **B** Venn diagram illustrating the shared species count among countries. Only the confirmed species from Peru were considered. The counts of nudibranch species for neighboring countries were derived from a comprehensive literature review (data not shown).

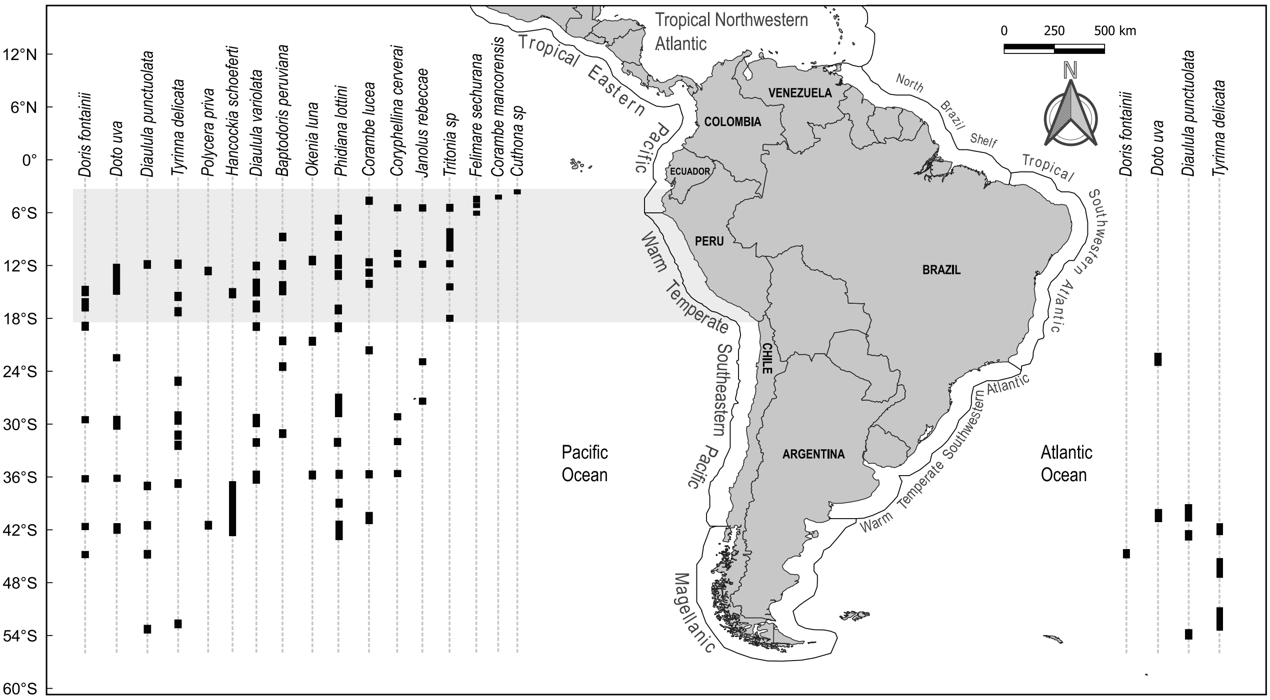

Figure 4. Reporting sites for nudibranch species found in Peruvian waters whose geographic ranges are limited to South America. The gray band highlights the locations where these species have been reported along the Peruvian coastline. The coastal marine biogeographic classification introduced by Spalding et al. (2007) is presented to provide context.

Confirmed species in Peruvian waters

Suborder Cladobranchia

Superfamily Aeolidioidea Gray, 1827
Family Aeolidiidae Gray, 1827

Spurilla braziliana **MacFarland, 1909**
Peruvian specimen photographs: Uribe and Pacheco (2012), Uribe et al. (2013)
Common name: Brazilian Aeolid

Habitat. Benthic.

Depth. 0–10 m (Gosliner 1979; Uribe and Pacheco 2012).

Type material. Holotype CASIZ 019731–Alagoas, Brazil (Carmona et al. 2014).

Distribution. This species exhibits a distribution range spanning the western Atlantic, extending from Florida to Brazil (Behrens and Hermosillo 2005; Marcus 1959), as well as the Pacific Ocean.

Sampling/reporting sites. In Peru, it was reported in Ferrol Bay (Chimbote, 09°06'S) (Uribe and Pacheco 2012) and Pucusana (Lima, 12°25'S) (Uribe et al. 2013) under the name *Spurilla neapolitana*. In the western Pacific, this species has been reported in Japan (Hamatani 2000), China (Lin 1992), and Australia (Willan 2006). Within the Eastern Pacific, it was reported in Mexico, Costa Rica, and Colombia (Carmona et al. 2013). Additionally, a specimen was reported from Hawaii (Gosliner 1979).

Remarks. Carmona et al. (2013), based on mitochondrial and nuclear sequences, revealed that *Spurilla neapolitana* MacFarland, 1909 comprises a complex of five cryptic species. In the light of this discovery, the name *Spurilla braziliana* has been assigned to populations found in the western Atlantic and Pacific regions. Carmona et al. (2013) further speculated that the occurrence of this species in the Pacific Ocean might be attributed to human-mediated introductions.

Family Facelinidae Bergh, 1889

Phidiana lottini (Lesson, 1831)

Peruvian specimen photographs: Fig. 5, iNaturalist
Common name: Flameback Sea Slug

Habitat. Benthic.

Depth. 0–15 m (Schrödl and Hooker 2014).

Type material. Not available.

Distribution. From Puerto Malabrigo (La Libertad, Peru, 07°42'S) (Flores 2014) to Melinka (Guaitecas islands, Chilean fjord region, 43°52'S) (Schrödl 2003).

Sampling/reporting sites. In Peru, it was initially reported in Callao as *Phidiana inca* by d'Orbigny, (1835–1846) and Dall (1909). It was also reported in Isla Santa (Ancash, 09°01'S), Ancon (Lima, 11°47'S), San Bartolo (12°22'S), Pucusana (Lima, 12°25'S) and San Juan de Marcona (Ica, 15°21'S) (Uribe et al. 2013). Schrödl and Hooker (2014) also collected individuals in Pucusana (12°25'S), Paracas (13°48'S) and La Punta (Callao). The report by Flores (2014) in Puerto Malabrigo (La Libertad, 07°42’S) revealed the northernmost locality of this species distribution. Valdivia-Chavez et al. (2020) presented a recent report of this species in Arequipa (15–17°S). Other reports (occurrences) for

Figure 5. *Phidiana lottini*, courtesy of Yuri Hooker.

this species in the Peruvian coast are Playa Tartacay (El Paraíso, Huaura, Lima, 11°13'44.9"S) (Zanabria 2020e), Isla Chuncho (Pucusana, Lima, 12°28'S) (Guzman 2018a; Cuba 2019) and Puerto General San Martín (Paracas Bay, Pisco, Ica, 13°48'37.3"S) (Zanabria 2020d). In Chile, it was reported in Playa Brava (Caldera, Atacama, 27°03'S), Calderilla (Atacama, 27°05'S) (Araya and Valdés 2016), Punta Blanca (Arica, 18°29'S), Comau Fjord (42°15'S), and Melinka (Guaitecas islands, Chilean fjord region, 43°52'S) (Schrödl 2003). Other reports (occurrences) for this species in the Chilean coast include Chascos Bay (Copiapó, Atacama, 27°40'S), Reserva Nacional Pingüino de Humboldt (Huasco, Atacama, 29°01'S), Coquimbo (29°58'S), Valparaíso (33°21'S), Concepción (36°45'S), Valdivia (39°57'S), and Chiloé (41°53'S) (iNaturalist 2023a).

Remarks. Initially designated as *Phidiana inca* (d'Orbigny, 1837) until research by Schrödl (1997) who considered it conspecific with *Eolidia lottini* Lesson, 1831, proposing *Phidiana lottini* as a valid name.

Bajaeolis bertschi **Gosliner & Behrens, 1986**

Peruvian specimen photographs: Uribe et al. (2013), Nakamura (2007)

Habitat. Benthic.

Depth. 3–8 m (Nakamura 2006).

Type material. Holotype CASIZ 059589—Punta la Gringa, Baja California (Gosliner and Behrens 1986).

Distribution. Eastern Pacific, from Baja California (Mexico, 28°N) to the northern coast of Peru (04°S) (Nakamura 2006).

Sampling/reporting sites. In Peru, it was reported in Playa Las Pocitas (Mancora, Piura, 04°06'S) (Nakamura 2006) and Playa El Rubio (Tumbes) (Uribe et al. 2013, based on a personal communication with Sandra Millen). It was also reported in Panama (Camacho-García et al. 2005) and Mexico (Baja California) (Gosliner and Behrens 1986).

Family Glaucidae Gray, 1827

Glaucus atlanticus **Forster, 1777**

Peruvian specimen photographs: Uribe et al. (2013), iNaturalist
Common name: Sea Swallow

Habitat. Pelagic.

Depth. Neustonic (Churchill et al. 2014b).

Type material. Not available.

Distribution. Cosmopolitan and circumtropical (Churchill et al. 2014b; Thompson and McFarlane 1967).

Sampling/reporting sites. Off the northern coast of Chile (Schrödl 2003). On the coast of El Salvador (13°N) (Segovia and López 2015). In Peru, it was mentioned by Paredes et al. (1999) and Ramírez et al. (2003) based on the records of d'Orbigny (1854) in Callao (10°15'S). Recently reported in Isla Santa, Ancash (09°01'S) by Uribe et al. (2013).

Remarks. Included in Paredes et al. (1999), probably based on a personal communication with Sandra Millen. The records of *Glaucus distichoicus* d'Orbigny, 1837 (d'Orbigny 1854; Dall 1909; Paredes et al. 1999; Ramírez et al. 2003) do not have enough evidence to formalize the species within the genus *Glaucus* and could refer to *G. atlanticus*.

Superfamily Arminoidea Iredale & O'Donoghue, 1923 (1841)
Family Arminidae Iredale & O'Donoghue, 1923 (1841)

***Armina californica* (J.G. Cooper, 1863)**
Peruvian specimen photographs: not available
Common name: Striped Nudibranch

Habitat. Benthic.

Depth. 11–268 m (Báez et al. 2011).

Type material. Not available.

Distribution. Eastern Pacific, from the Gulf of Alaska (Báez et al. 2011) to Piura (northern coast of Peru) (Dall 1909; Baez et al. 2011).

Sampling/reporting sites. In Peru, it was reported in Paita (Piura) (Dall 1909). Baez reported a Peruvian specimen (USNM 805043 South Pacific Ocean, Peru) but lacks geographic reference. It was also reported in Alaska (Central Aleutian Islands), Canada (Scott Islands), United States (California), Mexico (Isla Tortuga, Baja California, Socorro Island), and Panama (Islas Ladrones) (see Báez et al. 2011).

Remarks. In Peru, it was initially reported in Paita (Piura) (Dall 1909) under the name *Pleurophyllidia* (*Diphyllidia*) *cuvierii* d'Orbigny, 1837 and later considered as *Armina cuvieri* (d'Orbigny, 1837). However, *D*. *cuvieri* currently corresponds to *Armina tigrina* Rafinesque, 1814, a species from the Mediterranean Sea (Thompson et al. 1990). Nakamura (2006), referring to Dall (1909), listed *A. californica* as *A. cuvieri*. Báez et al. (2011) examined specimens of *A. cuvieri* and discovered an identification error, as it was actually *A. californica*. This correction is adopted in Uribe et al. (2013).

Superfamily Dendronotoidea Allman, 1845
Family Dendronotidae Allman, 1845

***Dendronotus* cf. *venustus* MacFarland, 1966**
Peruvian specimen photograph: Uribe et al. (2013)
Common name: Branched Dendronotid

Habitat. Benthic.

Depth. 5–20 m (Korshunova et al. 2020).

Type material. Not available.

Distribution. From Alaska (Stout et al. 2010) to Coliumo Bay (Chile, 36°32'S) (Schrödl 2003).

Sampling/reporting sites. in Peru, it was reported in Pucusana (12°25'S) as *Dendronotus* cf. *venustus* (Uribe et al. 2013). It was also reported in Alaska (Stout et al. 2010), United States (Morro Bay and Crescent City, California) (MacFarland 1966), and Chile (Coliumo Bay, 36°32'S) (Schrödl 2003).

Remarks. Paredes et al. (1999) listed this species as *Dendronotus frondosus* (Ascanius, 1774), a North Atlantic species (Ekimova et al. 2015), based on personal communication with Sandra Millen. *Dendronotus frondosus* was confirmed to have morphological and molecular differences with *Dendronotus venustus* (Stout et al. 2010; Ekimova et al. 2015) that is exclusive to the Pacific.

Family Dotidae Gray, 1853

Doto uva **Er. Marcus, 1955**

Peruvian specimen photographs: Uribe et al. (2013), Schrödl and Hooker (2014)
Common name: Grape-cluster Nudibranch

Habitat. Benthic.

Depth. 0–15 m (Schrödl and Hooker 2014).

Type material. Not available.

Distribution. Amphi-South American. On the Pacific side of South America, it extends from Callao (Peru, 12°S) to Comau Fjord (Chile, 42°S) (Schrödl et al. 2005). On the Atlantic side it ranges from San Matías gulf (Argentina, 41°26'S) (Cetra and Roche 2023) to Sao Paulo (Brazil, 21°S) (Marcus 1959).

Sampling/reporting sites. In Peru, it was reported in Callao (12°S), San Juan de Marcona (15°21'S), Islas Ballestas (13°44'S) (Schrödl and Hooker 2014) and Independencia Bay (14°14'S) (Uribe et al. 2013). In Chile, it was collected in Tocopilla (22°05'S), La Herradura (Coquimbo, 29°59'S), Tongoy (Coquimbo, 30°15'S) (Fischer and Cervera 2005b), Coliumo Bay (36°32'S) (Schrödl 2003), Canal de Calbuco (41°45'S) (Marcus 1959), and in Comau Fjord (42°22'S) (Schrödl et al. 2005). In Argentina, it was collected in San Matías Gulf (Patagonia, 41°30'S) (Cetra and Roche 2023). In Brazil, it was reported in Sao Paulo (21°21'S) (Marcus 1959).

Remarks. Molecular studies are needed to clarify the genetic identities of the populations on both sides of South America (Schrödl 2003; Uribe et al. 2013).

Family Hancockiidae MacFarland, 1923

Hancockia schoeferti **Schrödl, 1999**

Peruvian specimen photograph: Schrödl and Hooker (2014)

Habitat. Benthic.

Depth. 0–3 m (Schrödl and Hooker 2014).

Type material. Holotype ZSM Moll 19983471—Coliumo Bay (36°32'S), Chile (Schrödl 1999a).

Distribution. San Juan de Marcona, Peru (15°21'S) (Schrödl and Hooker 2014) and southern Chile (37–43°S) (Schrödl 2009).

Sampling/reporting sites. In Peru, it was reported for the first time in San Juan de Marcona (Ica, 15°21'S) (Schrödl and Hooker 2014). In Chile, it was reported in Coliumo Bay (36°32'S) and Queule (39°23'S) (Schrödl 1999a).

Family Phylliroidae Menke, 1830

Cephalopyge trematoides **(Chun, 1889)**

Peruvian specimen photograph: Quesquen (2017)

Habitat. Pelagic.

Depth. 40 m (Fernández-Alamo 1997).

Type material. Not available.

Distribution. Circumtropical (van der Spoel et al. 1997).

Sampling/reporting sites. In Peru, it was reported in Piura (Quesquen 2017). It was also reported in the Canary Islands and Cape Verde (Hernández et al. 2001, 2017), New South Wales (Steinberg 1956), Gulf of California (Fernández-Alamo 1997), and central and northern Chilean coast (Tokioka 1963).

Remarks. Originally described as *Phylliroe trematoides* Chun, 1889. The samples described in Quesquen (2017) were reported in grey literature (Quesquen 2008) and had been previously reported by Quesquen and Guzmán (1999).

Phylliroe bucephala **Lamarck, 1816**

Peruvian specimen photograph: Quesquen (2017)

Habitat. Pelagic.

Depth. 40–60 m (Fernández-Alamo 1997).

Type material. Not available.

Distribution. Circumtropical (van der Spoel et al. 1997).

Sampling/reporting sites. In Peru, it was reported in Tumbes and Piura (Quesquen 2017). It was also reported in the Canary Islands (Hernández and Jiménez 1996), off the coasts of Florida and Bermuda (Abbott 1974), in northeastern Atlantic waters near the African coast (van der Spoel 1970), and in the western Atlantic Ocean (Spencer et al. 2009). In the Mediterranean Sea there are reports from France and Syria (Durgham et al. 2016; Durgham and Ikhtiyar 2020; Pruvot-Fol 1954). Recorded south of the Pacific Ocean in Australia and New Zealand (Powell 1979; Spencer and Willan 1995). In the Indo-Pacific it has been reported from Vietnam (Sachidhanandam et al. 2000).

Family Cuthonidae Odhner, 1934

Cuthona **sp.**

Peruvian specimen photograph: Nakamura (2006)

Habitat. Benthic.

Depth. 5–7 m (Nakamura 2006).

Distribution. Northern coast of Peru.

Sampling/reporting sites. Cancas (Tumbes, 03°56'S) (Nakamura 2006).

Remarks. Description provides a length of 5 mm, body completely white, including rhinophores and oral tentacles with a translucent base. In addition, the specimen had dark, reddish-brown cerata without the white tip, which would differentiate it from other species of the genus (Nakamura 2006).

Family Fionidae Gray, 1857

Fiona pinnata **(Eschscholtz, 1831)**

Peruvian specimen photographs: not available
Common name: Feather Aeolid

Habitat. Pelagic.

Depth. Neustonic (Willan 1979).

Type material. Not available.

Distribution. Cosmopolitan (Gosliner 1987; Schmekel and Portmann 1982).

Sampling/reporting sites. In Peru, it was reported in Lima (d'Orbigny 1835–1846; Álamo and Valdivieso 1997; Dall 1909). It was also reported in Chile (Mehuín, Valdivia, Juan Fernández Islands and Talcahuano) (Fischer and Cervera 2005b; Bergh 1898).

Remarks. Originally named *Eolidia pinnata* Eschscholtz 1831 (type specimen from Alaska), until its current designation as *F. pinnata*; it is a species with a wide latitudinal range (Trickey 2013).

Family Flabellinidae Bergh, 1889

Kynaria cynara (Ev. Marcus & Er. Marcus, 1967)

Peruvian specimen photographs: Fig. 6, Uribe et al. (2013), Nakamura (2006)
Common name: Swimming Cynara

Habitat. Benthic.

Depth. 5–8 m (Nakamura 2006).

Type material. Holotype USNM 678417—La Choya Bay (31°20'30"N, 113°38'06"W), Puerto Peñasco, Sonora, Mexico.

Distribution. Eastern Pacific, from Gulf of California (Mexico, 28°N) (Millen and Hermosillo 2007) to Isla Tortuga (Peru, 09°S) (Uribe et al. 2013).

Sampling/reporting sites. In Peru, it was reported in Punta Sal (03°56'S), Cancas (03°56'S), Mancora (04°6'S), Chimbote (09°4'S), and Ancash (09°S) (Nakamura 2006; Uribe et al. 2013). It was recently reported in Isla La Viuda (09°20'57"S) and Isla Tortuga (09°21'48"S) in Ancash (Uribe et al. 2019). It was also reported in Mexico (Gulf of California) (Millen and Hermosillo 2007) and in

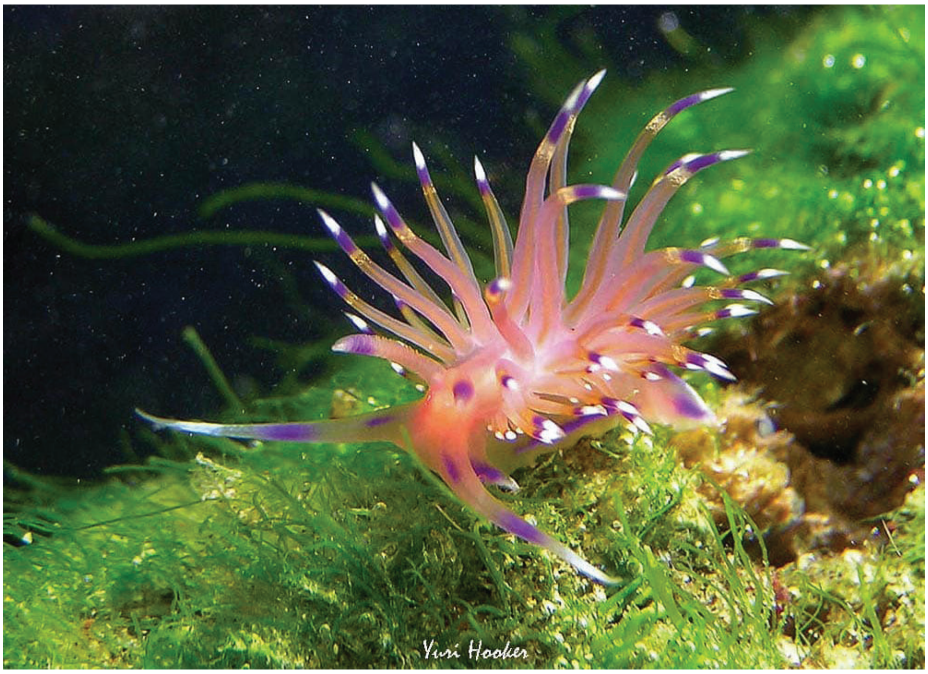

Figure 6. *Kynaria cynara*, courtesy of Yuri Hooker.

other several points (occurrences) such as Mexico (Guerrero), Costa Rica (Tamarindo Bay, Parque Nacional Santa Rosa), Ecuador (Salinas) (iNaturalist 2023b).

Remarks. The species was originally described as *Coryphella cynara* Ev. Marcus & Er. Marcus, 1967 and reported along the Peruvian coast as *Flabellina cynara* (Nakamura 2006; Uribe et al. 2013, 2019). These designations are currently invalid and have been replaced by *Kynaria cynara* (Korshunova et al. 2017).

Coryphellina cerverai **(M. A. Fischer, van der Velde & Roubos, 2007)**

Peruvian specimen photograph: Schrödl and Hooker (2014)

Habitat. Benthic.

Depth. 0–10 m (Schrödl and Hooker 2014).

Type material. Holotype RMNH Moll. 98130—La Herradura, Coquimbo (29°58'S, 071°22'W), Chile.

Distribution. From Sechura Bay (Peru, 05°49'S) to Coliumo Bay (Chile, 36°32'S).

Sampling/reporting sites. In Peru, it was reported in Sechura Bay (05°49'S) (Schrödl and Hooker 2014), Ancon (Lima, 11°47'S) and Pucusana (Lima, Peru, 12°25'S) (Schrödl 1996a). In Chile, it was reported in La Herradura (Coquimbo, 29°59'S) (Fischer et al. 2007), Peñón de Vida Marina (Montemar, Viña del Mar, 32°57'26.8"S) (Molina 2021), Coliumo Bay (36°32'S) (Schrödl 1996a).

Remarks. The species was first reported as *Flabellina* sp. 2 (Schrödl 1996a) and then as *Flabellina cerverai* van der Velde & Roubos, 2007 (Fischer et al. 2007; Schrödl and Hooker 2014).

Superfamily Proctonotoidea Gray, 1853
Family Janolidae Pruvot-Fol, 1933

Janolus rebeccae **Schrödl, 1996**

Peruvian specimen photographs: Fig. 7, Schrödl and Hooker (2014)

Habitat. Benthic.

Figure 7. *Janolus rebeccae*, courtesy of Yuri Hooker.

Depth. 2–12 m (Schrödl 1996a, b; Schrödl and Hooker 2014).

Type material. Holotype ZSM 19960557—English Bay (27°07'S, 070°53'W), Chile.

Distribution. From Sechura Bay (Peru, 05°49'S) (Schrödl and Hooker 2014) to English Bay (Chile, 27°07'51"S) (Schrödl 1996b, 2003).

Sampling/reporting sites. In Peru, it was reported in Sechura Bay (05°49'S) and Paracas (13°43'S) (Schrödl and Hooker 2014). In Chile, it was reported in Juan Lopez (23°30'S) and English Bay (27°07'S) (Schrödl 1996a, 1996b).

Superfamily Tritonioidea Lamarck, 1809
Family Tritoniidae Lamarck, 1809

***Tritonia* sp.**

Peruvian specimen photographs: Fig. 8, Uribe et al. (2013), Uribe et al. (2019), iNaturalist

Habitat. Benthic.

Depth. 5–15 m (Uribe et al. 2013).

Distribution. From Foca Island (Piura, Peru, 05°12'S) to Punta Picata (Tacna, Peru) (Uribe et al. 2013).

Sampling/reporting sites. In Peru, it was reported in Foca Island (Piura, 05°12'S), Santa Island (09°01'S), Ferrol Bay (Chimbote, 09°06'S), Punta El Huaro (Casma, Ancash, 09°37’S), La Gramita (Casma, Ancash, 09°43'S), Punta Patillos (Huarmey, 09°53'S), Punta Colorado (Huarmey, Ancash, 10°29'S), Pucusana (Lima, 12°25'S), Isla Asia (Lima, 12°47'S), Isla La Vieja (Independencia Bay, Pisco, Ica, 14°16'S) and Punta Picata (Tacna, 17°52'S) (Uribe et al. 2013).

Remarks. It bears resemblance to *Tritonia odhneri* (common in Chile) in terms of its external morphology, while displaying similarities to *Tritonia festiva* (found in Alaska, Baja California, and Japan; Uribe et al. 2013). Anatomical and molecular analyses are necessary to describe this species (Uribe et al. 2013).

Figure 8. *Tritonia* sp., courtesy of Jaime Calvo-Pérez.

Suborder Doridina

Superfamily Chromodoridoidea Bergh, 1891
Family Chromodorididae Bergh, 1891

Tyrinna delicata (Abraham, 1877)

Peruvian specimen photographs: Fig. 9, Uribe et al. (2013)

Habitat. Benthic.

Depth. 0–22 m (Uribe et al. 2013).

Type material. Holotype NHMUK 1995038—Chiloé Island, Chile.

Distribution. Amphi-South American. From Pucusana (Lima, Peru, 12°25'S) (Fabián Avilés pers. comm.) to Strait of Magellan (Chile, 53°S) and Peninsula Valdés (Argentina, 42°S) (Schrödl and Millen 2001; Uribe et al. 2013; Araya and Valdés 2016).

Sampling/reporting sites. In Peru, two specimens were collected (MHNURP, specimens currently lost) in Playa Las Ninfas (Pucusana, Lima, 12°28'49"S) on 23 October 2019, at 1.5–2.0 m depth, 55–60 mm length (Fig. 9) (Fabián Avilés pers. comm.). In Peru, this species was also reported in San Juan de Marcona (Ica, 15°21'S) and Isla Blanca (Arequipa, 17°00'S) (Uribe et al. 2013). In Chile, it was reported in Caleta Obispito (Caldera, Atacama, 26°45'51"S) (Araya and Valdés 2016) and in several points (occurrences) such as Reserva Nacional Pingüino de Humboldt (29°15'S), Playa El Francés (Coquimbo, 30°05'S), Pichicuy (32°20'S), Caleta Cocholgüe-Tomé (36°35'S), Caleta Chome (Península de Hualpén, 36°48'S), Caleta Chaihuín (39°56'S), Faro San Isidro (Strait of Magellan, 53°28'S) (iNaturalist 2023a). It was also reported in San Juan Fernández Islands (33°38'S) (Araya and Valdés 2016). In Argentina it was observed (occurrences) in Baliza Davison (Tierra del Fuego, 54°56'S), Grand Jason (Ja-

Figure 9. *Tyrinna delicata*, courtesy of Fabián Avilés.

son Islands, 51°04'S), La Tranquera (46°02'S), Golfo San Jorge (46°00'S), Punta del Marqués (Rada Tilly, 45°57'S), Parque Interjurisdiccional Marino Costero Patagonia Austral (PIMCPA, 45°02'S), Camarones Bay (44°46'S), Golfo Nuevo (42°47'S), and Golfo San José (42°24'S) (iNaturalist 2023c).

Remarks. This species was originally identified as *Tyrinna nobilis* Bergh, 1898, a name that is currently not accepted.

Tyrinna evelinae (Er. Marcus, 1958)

Peruvian specimen photographs: not available

Habitat. Benthic.

Depth. 0–5 m (Welch 2010).

Type material. Not available.

Distribution. Amphi-American and West Africa.

Sampling/reporting sites. In Peru, it was reported in El Rubio (Tumbes, 03°52'S) (Schrödl and Millen 2001). It was also reported in Mexico (Gulf of California, 28°N), Jamaica (Schrödl and Millen 2001). In the Atlantic, it has been sampled in the Gulf of Mexico (de la Cruz-Francisco et al. 2017), Panama (Goodheart et al. 2016), Brazil (Marcus 1958), Ghana, and Cape Verde (Camacho-García et al. 2005).

Remarks. It was initially listed by Paredes et al. (1999). According to Uribe et al. (2013) molecular studies are necessary to confirm the consistency of reports of *T. evelinae* in both the Atlantic and Pacific populations that are morphologically difficult to distinguish (Valdés et al. 2006).

Felimare agassizii (Bergh, 1894)

Peruvian specimen photographs: Uribe et al. (2013), Nakamura (2007)
Common name: Agassiz's Chromodorid

Habitat. Benthic.

Depth. 7–8 m (Nakamura 2006).

Type material. Not available.

Distribution. From the Gulf of California to the coast of northern Peru.

Sampling/reporting sites. In Peru, it was reported in Cancas (Tumbes, 03°56'S) (Nakamura 2006) and Lobos de Tierra Island (Lambayeque, 06°25'S) (Uribe et al. 2013). It was also reported in Mexico (Gulf of California, 28°N), Ecuador (Galapagos Islands, 0°S), Colombia (Malpelo Island, 04°N) (Behrens and Hermosillo 2005).

Remarks. Originally described as *Chromodoris agassizii* Bergh, 1894 and reported in Peruvian waters as *Hypselodoris agassizii* by Nakamura (2006).

Felimare sechurana Hoover, Padula, Schrödl, Hooker & Valdés, 2017

Peruvian specimen photographs: Fig. 10, iNaturalist, Hoover et al. (2017)

Habitat. Benthic.

Depth. 6–15 m (Hoover et al. 2017; Bravo et al. 2020)

Figure 10. *Felimare sechurana*, courtesy of Yuri Hooker.

Type material. Holotype CZA 402—Foca Island (05°12'13.8"S, 81°12'38.0"W), Piura, Peru.

Distribution. Northern coast of Peru's transition zone.

Sampling/reporting sites. In Peru, it was reported in Punta Veleros (Los Organos, Piura, 04°10'28.7"S) (Zavala, 2022), Quebrada Verde (Piura, 04°13'34.8"S), Foca Island (Piura, 05°12'13.8"S) (Hoover et al. 2017), and Lobos de Afuera Islands (Lambayeque, 06°56'S) (Bravo et al. 2020).

Remarks. This species is endemic to the northern coast of Peru. It was initially reported as *Felimare ghiselini* (Bertsch 1978) by Thompson (2006) but later corrected by Hoover et al. (2017) and described as a new species.

Felimida baumanni (Bertsch, 1970)

Peruvian specimen photograph: Fig. 11
Common name: Baumann's Chromodorid

Habitat. Benthic.

Depth. 5–8 m (Nakamura 2006).

Type material. Not available.

Distribution. Eastern Pacific, from Gulf of California (28°N) to Cancas (Tumbes, Peru) (Nakamura 2006).

Sampling/reporting sites. In Peru, it was reported in Cancas (Tumbes, 03°56'S) (Nakamura 2006). It was also reported in Mexico (Gulf of California, 28°N), Panama (08°N), Ecuador (Galapagos Islands, 00°S) and Colombia (Malpelo Islands, 04°N) (Behrens and Hermosillo 2005).

Remarks. The species was originally reported as *Chromodoris baumanni* Bertsch, 1970 in the Eastern Pacific (Rudman 1983). Years later, Gosliner et al. (2004) discussed the anatomical characteristics of this species to be like those attributed to the genus *Glossodoris*, suggesting a reclassification. Finally, the phylogenetic study by Johnson and Gosliner (2012) defined its new classification as part of the genus *Felimida*.

Figure 11. *Felimida baumanni*, courtesy of Fabián Encinas.

Superfamily Doridoidea Rafinesque, 1815
Family Discodorididae Bergh, 1891

Baptodoris peruviana **(d'Orbigny, 1837)**
Peruvian specimen photographs: Uribe et al. (2013), Schrödl and Hooker (2014)

Habitat. Benthic.

Depth. 4–15 m (Schrödl and Hooker 2014).

Type material. Holotype ZMB 50748—Isla de Pajargo (Pájaros), Chile (as *Platydoris punctatella* Bergh, 1898), poorly preserved. Neotype SSUC 6977 (*Doris peruviana*), Iquique (Chile, 20°12'S), February 1965, undissected specimen (Fischer and Cervera 2005a).

Distribution. From San Lorenzo Island (Callao, Peru, 12°S) (d'Orbigny 1835–1846) to Los Molles (Valparaíso, 32°15'S) (Fischer and Cervera 2005).

Sampling/reporting sites. In Peru it was reported for first time as *Doriopsis peruviana* in San Lorenzo Island (12°05') by d'Orbigny (1835–1846). It was also reported in Callao (12°S, as *Doris peruviana*, Dall 1909), Pucusana (Lima, 12°28'S, as *Platydoris punctatella*, Schrödl 1996a), Tortugas Bay (Ancash, 09°21'S), Independencia Bay (Ica, 14°14'S), and San Juan de Marcona (Ica, 15°21'S) (Uribe et al. 2013) In Chile, it was collected in Iquique (20°12'S), Mejillones (23°20'S), La Portada (Antofagasta, 23°39'S), and Los Molles (Valparaíso, 32°15'S) (Fischer and Cervera 2005a).

Remarks. Initially reported as *Doris peruviana* d'Orbigny 1836, transferred to *Platydoris* Bergh, 1877 by Schrödl (2003), who also included Bergh's (1898) description of *P. punctatella* as a junior synonym. Dorgan et al. (2002) ruled out that these reports were about a species belonging to *Platydoris*, based on a photograph of a live specimen (Schrödl 1996a). Based on the description of the radular teeth, this species was transferred from *Platydoris* to the genus *Baptodoris* (Fischer and Cervera 2005a). Regarding its northernmost distribution, Fischer and Cervera (2005a) considered the reports from Tagus Cove (Albermarle, Galapagos Islands) (Pilsbry and Vanatta 1902) as doubtful.

Diaulula variolata **(d'Orbigny, 1837)**
Peruvian specimen photographs: Fig. 12, Schrödl and Hooker (2014), iNaturalist

Habitat. Benthic.

Depth. 2–15 m (Schrödl and Hooker 2014).

Type material. Not available.

Distribution. From Pucusana (Lima, Peru, 12°28'S) (Guzman 2018b) to Punta Hualpén (Concepción, Chile, 36°44'S) (Marcus 1959).

Sampling/reporting sites. In Peru, it was reported in Pucusana (Lima, 12°28'S) (Guzman 2018b), El Chaco (Ica, 13°49'S), Caleta Atenas (Ica, 13°49'S), Independencia Bay (Ica, 14°14'S), San Juan de Marcona (Ica, 15°21'S) (Uribe et al. 2013), Dos Playas (Arequipa, 17°00'S) and Playa Calera (Arequipa, 17°15'S) (Tejada-Pérez et al. 2018; Valdivia-Chavez et al. 2020), Caleta La Huata (Camaná, Arequipa, 16°50'S) (Zanabria 2020a), Terminal Portuario Matarani (Islay, Arequipa, 16°59'S) (Zanabria 2020b), and Playa Jaboncillo (Ilo, Moquegua, 17°59'S) (Zanabria 2020c).

In Chile, it was reported in Arica (18°26'S) (Schrödl 2003), Coquimbo Bay (29°57'S) (Bergh 1898; Valdés and Muniaín 2002), English Bay-Guanaqueros-Los Hornos (30°10'S), El Tabo (Valparaíso, 33°27'S) (Fischer and Cervera 2005b), Coliumo Bay (36°32'S) (Schrödl 1996a, 1997), San Vicente Bay (36°44'S), and Punta Hualpén (Concepción, 36°44'S) (Marcus 1959). Within its Chilean distribution, several other recent observations (occurrences) have been reported (iNaturalist 2023d).

Remarks. This species had not been recorded outside Chile (Fischer and Cervera 2005b) until the first reports from Peru (Uribe et al. 2013). The specimen collected in Bernardo O'Higgins National Park (Chilean fjord region, 51°S) (Aldea et al. 2011) needs confirmation (Uribe et al. 2013).

Figure 12. *Diaulula variolata*, courtesy of Jaime Calvo-Pérez.

***Diaulula punctuolata* (d'Orbigny, 1837)**
Peruvian specimen photographs: not available
Common name: Dotted Bristle Sea Slug

Habitat. Benthic.

Depth. 0–7 m.

Type material. ZSM Moll 20040984—Ipún Island (44°33'S, 74°48'W), Aysén, Chile.

Distribution. Amphi-South American. It is frequently found on the Magellanic coasts of Chile and Argentina.

Sampling/reporting sites. In Peru, it was collected in Callao (12°S) (Dall 1909; Schrödl 2003). In Chile, it was collected in Lota (37°05'S), Lacuy Peninsula (Greater Island of Chiloé, 41°49'S) (Valdés and Muniaín 2002), Ipún Island (Chonos Archipelago, 44°33'S) (Schrödl and Grau 2006) and Strait of Magellan (53°35'S) (Roche et al. 2023). In Argentina, it was collected in San Matías Gulf (41°30'S) (Roche et al. 2023; Cetra 2019), Gulf Nuevo (42°42'S) (Valdés and Muniaín 2002), Peninsula Valdés (42°30'S), Punta Pardelas (42°36'S), Puerto Madryn (42°46'S) (Roche et al. 2023; Cetra 2019), Comodoro Rivadavia (45°51'S) (Valdés and Muniaín 2002), and Tierra del Fuego (54°21'S) (Roche et al. 2023).

Remarks. This species was listed as *Anisodoris punctuolata* (d'Orbigny, 1836) and *Doris punctuolata* d'Orbigny, 1837 in previous Peruvian articles listing nudibranch species. Both names are currently not accepted.

***Rostanga pulchra* MacFarland, 1905**
Peruvian specimen photographs: Schrödl and Hooker (2014)
Common name: Red Sponge Dorid

Habitat. Benthic.

Depth. 6–12 m (Schrödl and Hooker 2014).

Type material. Holotype USNM 181292—Monterey Bay, California, United States.

Distribution. This species presents a bipolar distribution in the Eastern Pacific and an amphi-South American distribution (Schrödl 2003; Schrödl and Grau 2006).

Sampling/reporting sites. In Peru it was considered a predicted species (Uribe et al. 2013) until the first specimen was finally reported in Punta San Juan (Marcona, Ica, 15°22'S) (Schrödl and Hooker 2014). It has been also reported in Alaska (Point Craven) (Lee and Foster 1985), Mexico Los Angeles Bay) (Lance 1966), Chile (Coliumo Bay, Playa Brava and Chonos archipelago) (Marcus 1959; Schrödl 2003; Schrödl and Grau 2006), and Argentina (Camarones Bay) (Marcus and Marcus 1969).

Remarks. Its distribution in Peruvian Waters was not certain; however, it was listed by Álamo and Valdivieso (1997) and Paredes et al. (1999). *Rostanga pulchra* is the only species of the genus in Peruvian and Chilean waters. Schrödl and Hooker (2014) mentioned that populations in the northern and southern hemispheres are likely to be distinct species based on preliminary unpublished molecular data.

Family Dorididae Rafinesque, 1815

Doris fontainii d'Orbigny, 1837

Peruvian specimen photographs: Fig. 13, iNaturalist
Common name: Large Warted Doris

Habitat. Benthic.

Depth. 8–17 m (Schrödl and Hooker 2014).

Type material. Holotype ZSM 19983417. Coliumo Bay, Chile (36°32'S, 72°57'W).

Distribution. Amphi-South American.

Sampling/reporting sites. In Peru it was reported in Independencia Bay (14°14'S) (Schrödl 1996a Schrödl and Grau 2006; Rudman 2002a, 2002b; Uribe et al. 2013), San Fernando National Reserve (14°58'S) (Ampuero 2010), and recently in Arequipa (15–17°S) (Valdivia-Chavez et al. 2020). Other reports (occurrences) for this species in the Peruvian coast are Pucusana, Ilo, Marcona, Pisco, Islay and Camaná, (iNaturalist 2023e). In Chile it was reported in Arica (18°28'S), Coquimbo (29°57'S), Dichato (36°32'S), Puerto Montt (41°27'S) (Valdés and Muniaín 2002; Schrödl 2003), and the Chonos Archipelago (45°08'S) (Schrödl and Grau 2006). In Argentina, it was collected from 37°50'S to Rada Tilly (45°55'S) (Valdés and Muniaín 2002).

Remarks. Erroneously named as *Doris fontainei* in previous articles.

Superfamily Onchidoridoidea Gray, 1827
Family Corambidae Bergh, 1871

Corambe lucea Er. Marcus, 1959

Peruvian specimen photograph: Schrödl and Hooker (2014)

Habitat. Benthic.

Figure 13. *Doris fontainii*, courtesy of Jaime Calvo-Pérez.

Type material. Neotype ZSM 1912—Caleta Buena (Chile, 22°25'S, 70°15'W).

Depth. 0–27 m (Schrödl and Hooker 2014)

Distribution. From Bayóvar (Sechura Bay, Peru, 05°49'S) (Schrödl and Hooker 2014) to Gulf of Corcovado (Chile, 42°46'50"S) (Schrödl 1996a).

Sampling/reporting sites. In Peru, it was reported in Bayóvar (Sechura Bay, 05°49'S), Callao (pier of IMARPE, 12°03'59"S), Ballestas Islands (Paracas, 13°43'54"S) and San Juan de Marcona (Ica, 15°21'S) (Schrödl and Hooker 2014). In Chile, it was reported in Caleta Buena (22°25'S), Coliumo Bay (36°32'S) (Schrödl 1997), Faro Punta Corona (Chiloé, 41°50'S) (Marcus, 1959), Ancud Bay (41°52'S) (Schrödl 1996a), Comau Fjord (42°22'S) (Schrödl et al. 2005) and Gulf of Corcovado (42°46'S) (Schrödl 1996a).

Remarks. It was first described as *Neocorambe lucea* (Schrödl 1996a). A morphological analysis confirmed its status as a valid species (Schrödl and Wägele 2001).

Corambe mancorensis Martynov, Brenzinger, Hooker & Schrödl, 2011

Peruvian specimen photograph: Martynov et al. (2011)

Habitat. Benthic.

Depth. 0–3 m (Martynov et al. 2011).

Type material. ZSM 20080543—Mancora (04°06'36"S, 81°04'02"W), Piura, Peru.

Distribution. Species only reported off the coast of Mancora (Piura, Peru) (Martynov et al. 2011).

Remarks. Endemic species of northern coast of Peru.

Family Goniodorididae H. Adams & A. Adams, 1854

Okenia luna Millen, Schrödl, Vargas & Indacochea, 1994

Peruvian specimen photographs: Fig. 14, Schrödl and Hooker (2014)

Figure 14. *Okenia luna*, courtesy of Yuri Hooker.

Habitat. Benthic.

Depth. 4–20 m (Schrödl 1996a).

Type material. Holotype CASIZ 089293—Coliumo Bay (36°32'S, 73°57'W), north of Concepción, Chile.

Distribution. From Ancon Bay (Lima, Peru) to Coliumo Bay (Chile).

Sampling/reporting sites. In Peru, it was collected in Ancon Bay (Lima, 11°47'S) (Millen et al. 1994). In Chile, it was collected in Iquique (22°13'S) (Fischer 2006) and Coliumo Bay (36°32'S) (Schrödl 2003).

Remarks. First record of the genus *Okenia* reported in the Southeast Pacific (Millen et al. 1994). It is distributed in Peruvian and Chilean waters.

Superfamily Phyllidioidea Rafinesque, 1814
Family Dendrodorididae O'Donoghue, 1924 (1864)

Doriopsilla janaina **Er. Marcus & Ev. Marcus, 1967**
Peruvian specimen photograph: Nakamura (2007)

Habitat. Benthic.

Depth. 0–3 m (Nakamura 2006).

Type material. Holotype USNM 576269—Panama Canal (09°05'N, 79°41'W), Panama.

Distribution. From the Gulf of California (28°N) to Cancas (Peru, 03°56'S).

Sampling/reporting sites. In Peru, it was reported in Cancas (Tumbes, 03°56'S) (Nakamura, 2006). It was also reported in Mexico (Punta Lobos, Sonora), Panama (08°N), and Ecuador (Galapagos Islands, 00°S) (Gosliner 1991).

Superfamily Polyceroidea Alder & Hancock, 1845
Family Polyceridae Alder & Hancock, 1845

Polycera priva **Er. Marcus, 1959**
Peruvian specimen photographs: Schrödl and Hooker (2014)

Habitat. Benthic.

Depth. 10 m (Schrödl 1996a).

Type material. Holotype ZSM Moll. 20041005—Punta Llonco, Comau Fjord (42°22'10"S, 072°27'18"W), Chile.

Distribution. From Paracas (Ica, Peru, 13°43'S) (Schrödl and Hooker 2014) to Chilean Patagonia (54°S) (Schrödl 2009; Schrödl et al. 2005).

Sampling/reporting sites. In Peru, it was reported in Ballestas Islands (Lima, 13°43'S) (Schrödl and Hooker 2014). In Chile, it was reported in Gulf of Ancud (42°06'S) (Marcus 1959) and Comau Fjord (42°22'S) (Schrödl et al. 2005).

Remarks. Peruvian presence of this species was surprising for the discoverers (Schrödl and Hooker 2014) since it was considered a Magellanic species, being very abundant in the fjords of southern Chile (41–44°S) (Schrödl 1996a, 1999b, 2003, 2009).

Potentially occurring species (unconfirmed)

Thecacera darwini **Pruvot-Fol, 1950**

Common name: Dalmatian Sea Slug

Habitat. Benthic.

Type locality. Orange Bay (55°31'S), Nassau Bay, Chile (Valdés and Héros 1998).

Distribution. From Juan López, northern coast of Chile, to Strait of Magellan (Schrödl 2003).

Remarks. This species was listed by Nakamura (2006) based on Schrödl (1999b), who listed this species as present in the Peruvian province, referring to Chilean waters. Fischer and Cervera (2005b) argued against its occurrence off the Chilean coast, although they acknowledge a high likelihood of its presence in Peruvian waters. Uribe et al. (2013) and Fischer (2006) referred to Zagal and Hermosilla's (2001) presumed finding of this species in Peru.

Glaucus **sp.**

Habitat. Pelagic.

Type material. Not available.

Distribution. Ica (Peru).

Sampling/reporting sites. In Peru, Quesquen (2017) reported specimens twice off the coast of Ica in 1995 and 1998. Currently, the only valid species of the genus *Glaucus* is *G. atlanticus*, reported in Peru by Uribe et al. (2013) from Isla Santa, Ancash.

Remarks. Quesquen (2017) offered a description of the specimens collected in Ica. He described a slender body that was ventrally flattened, with a small head and two cephalic tentacles. The dorsum was navy blue and green, and the ventral area was white. Additionally, the specimens had three or four branches on both sides of the body, and their body length could reach up to 43 mm. According to Valdés and Campillo's (2004) description, *G. atlanticus* is characterized by its slim and elongated body, along with a small head and sleek oral tentacles and rhinophores. The coloration of its dorsum can vary from deep blue to brown hues. It possesses up to three groups of cerata, and its ventral region exhibits a silver shade.

A confirmation of the taxonomic status of these specimens is necessary, using morphological and molecular analyses.

Aeolidia campbellii **(Cunningham, 1871)**

Habitat. Benthic.

Type material. ZSM 20020700 (Chile), designated as the neotype due to absence of the holotype (Kienberger et al. 2016).

Distribution. Falkland Islands (50°S), Argentine and Chilean Patagonia (41°S) to Valparaiso (32°S) (Schrödl 2003). In Peru, this species was listed by Paredes et al. (1999) as *Aeolidia serotina* Bergh, 1873 and replicated by Ramírez et al. (2003).

Remarks. It was reported off the coast of Chile by Schrödl (2003) as *Aeolidia papillosa* (Linnaeus, 1761), later reassigned to the species *A. campbellii* according to molecular studies by Kienberger et al. (2016).

Gargamella immaculata Bergh, 1894

Common name: Unicolored Sea Lemon

Habitat. Benthic.

Type material. SMNH 1015—Tierra del Fuego, Chile.

Distribution. A common species on the southern coast of Chile and Argentina.

Sampling/reporting sites. Cabo Metalqui, Chiloé (Fischer and Cervera 2005b; Odhner 1926), in Última Esperanza, Tierra del Fuego (Marcus 1959), in Cabo San Antonio; Cabo Delgado; Gulf of Ancud, between Isla Quenu and Calbuco; Seno Otway, Queule and Coliumo Bay (Schrödl 1996a). In Argentina, in the north (Bergh 1894; Odhner 1926), also in Argentine Patagonia and on the Burdwood Bank (Odhner 1926; Schrödl 2003).

Remarks. According to Schrödl (2003), the Peruvian records of this species by Zagal and Hermosilla (2001) are doubtful. However, in that compilation and in the most up-to-date publication (Zagal and Hermosilla 2007), *Gargamella immaculata* is not mentioned from Peruvian waters, but as an inhabitant of the Peruvian zoogeographic province from Juan López (Atacama) to the south. The species was mistakenly included in Kentrodorididae by Schrödl (1996a), until Valdés (2002) transferred it back to the Discodorididae.

Cadlina sparsa (Odhner, 1922)

Common name: Dark-spot Cadlina

Habitat. Benthic.

Type locality. Juan Fernández Islands and Desventuradas Islands, Chile (Odhner 1922).

Distribution. It presents disjunct populations with a bipolar distribution in the eastern Pacific and an amphi-South American pattern.

Sampling/reporting sites. In the Pacific, the northernmost location is Baja California (Behrens 1991; Jaeckle 1983) and the southernmost location is the Comau Fjord in southern Chile. In the Atlantic, it was recorded in Camarones Bay in the central region of Argentina (Schrödl 2000, 2003). In Chile, it has also been sampled in the Juan Fernández Islands (Odhner 1922), Chiloé Islands (Marcus 1959), and Coliumo Bay (Schrödl 1996a, 2003).

Remarks. *Cadlina sparsa* was initially proposed as probable species in Peruvian water by Álamo and Valdivieso (1997). Subsequently, its presence was consistently mentioned in the lists compiled by Paredes et al. (1999), Ramírez et al. (2003), and Nakamura (2006). However, no actual specimens have been collected from intermediate Pacific locations, including Peru. Despite the absence of direct observations, a hypothetical distribution for Peru has been predicted through extrapolation, assuming a continuous geographic range (Uribe et al. 2013).

Studies have demonstrated that *C. sparsa* does not fall within the family Chromodorididae, as initially suggested (Johnson 2011), but rather belongs to the family Cadlinidae. However, there have been no other revisions or updates regarding its scientific name. The taxonomy of the genera involved has been thoroughly elucidated in previous literature (Schrödl 2000).

***Polycera* cf. *alabe* Collier & Farmer, 1964**

Habitat. Benthic.

Type material. CASIZ 18190—Cedros Island, Baja California (28°12'13"N, 115°15'28"W), Mexico.

Distribution. From Baja California (Behrens 2004; Behrens and Hermosillo 2005; Camacho-García et al. 2005), Puerto Vallarta (20°40'N) in Mexico to Costa Rica (Behrens 2004); and northern Chile with a single isolated record (Schrödl 2003).

Remarks. In Peru, Paredes et al. (1999) listed this species as *Polycera* cf. *alabe*, likely based on information obtained through personal communication with Sandra Millen. Subsequently, Uribe et al. (2013) included this species in their listing, also citing personal communication with Sandra Millen, who observed this species at El Rubio (Tumbes).

***Phylliroe lichtensteinii* Eschscholtz, 1825**

Habitat. Pelagic.

Type material. Not available.

Distribution. Cosmopolitan (Padula 2015).

Sampling/reporting sites. Espiritu Santo, southeastern Brazil (Ralph 1959).

Remarks. For Peru, the species was listed in Ramírez et al. (2003). There are no reports of collections of this species in Peruvian waters.

***Itaxia falklandica* (Eliot, 1907)**

Habitat. Benthic.

Depth. 1–15 m (Schrödl 1996a).

Type material. Not available.

Distribution. Abundant in the Magellanic Province, in the Southeast Pacific (Aldea et al. 2011; Velasco-Charpentier et al. 2021) and with records in the South Atlantic (Eliot 1907; Odhner 1926, 1944), Pacific Ocean (Marcus 1959; Meyers-Muñoz et al. 2009; Schrödl 1996a, 2003), and Indian Ocean (Odhner 1944).

Remarks. It was included in the list of mollusks of Peru by Paredes et al. (1999) as *Flabellina falklandica* (Eliot 1907); this information was repeated by Ramírez et al. (2003).

According to Uribe et al. (2013), its presence in Peru requires confirmation since the inclusion by Paredes et al. (1999) was based on unfounded records.

Coryphellina marcusorum **(Gosliner & Kuzirian, 1990)**
Common name: Marcus's Aeolid

Habitat. Benthic.

Depth. 3–22 m (Welch 2010).

Type material. CASIZ 066151—San Diego Reef (25°12'N, 110°42'W), Gulf of California, Mexico.

Distribution. From Brazil to Gulf of California (Mexico) (Gosliner and Kuzirian 1990; Fischer et al. 2007).

Sampling/reporting sites. In Peru, this species was recently photographed in Los Organos (Piura, 04°10'S) on 11 March 2022 (Torrejón 2023).

Remarks. Originally named *Flabellina marcusorum* Gosliner & Kuzirian, 1990. Its presence in Peru needs be confirmed by future surveys.

Discussion

Overview

This article presents an updated compilation of nudibranchs found in Peru, derived from an extensive literature review. The revised and updated scientific names are presented, while species not verified or erroneously listed in previous articles, referred to here as 'potentially occurring', are separated from those confirmed.

Despite the presence of two distinct coastal marine biogeographic provinces and a transitional zone between them (Schrödl and Hooker 2014; Ibanez-Erquiaga et al. 2018; Barahona et al. 2019), the species richness of nudibranchs remains relatively low ($n = 31$) compared to other marine regions, such as the Gulf of Mexico, the Caribbean, the South Atlantic, the Indian Ocean, and the Mediterranean Sea (Behrens 2004; Chavanich et al. 2013). In neighboring South American countries, such as Colombia ($n > 40$) (Ardila et al. 2007; Londoño-Cruz 202 1), Chile ($n > 50$) (Schrödl 1996a, 1999b, 2003; Fischer and Cervera 2005b; Schrödl et al. 2005; Schrödl and Grau 2006; Aldea et al. 2011), and Brazil ($n > 80$) (Marcus and Marcus 1969; Padula et al. 2011; Pereira et al. 2012; Padula 2015), a greater diversity of nudibranch species is found (Fig. 3A, B).

It is worth noting that there are areas along the Peruvian coast that remain unexplored. In the Tropical Eastern Pacific, only a limited number of locations have been sampled, including Pocitas, Punta Sal, Mancora, and Cancas. Within the transition zone, Sechura Bay and Foca Island are the common reporting sites, while within the Warm Temperate Southeastern Pacific, Santa, Casma, Huarmey (Ancash), Ancon, Callao, San Lorenzo Island, Pucusana (Lima), Pisco, Independencia Bay, San Juan de Marcona (Ica), Matarani, and Isla Blanca (Arequipa) are frequently mentioned. Factors such as limited exploration efforts, challenging diving conditions, a scarcity of nudibranch taxonomists, and a general lack of interest in this group in Peru should be highlighted. In addition, it is highly likely that several species remain unreported and undescribed, particularly in deeper waters. Therefore, the confirmed number of nudibranch species in Peruvian waters ($n = 31$) is presumed to represent only a fraction of the actual diversity present.

Potential influence of the Humboldt Current and El Niño

Several confirmed species exhibit a biogeographical affinity for the Warm Temperate Southeastern Pacific (*n* = 23) (Fig. 2). The Humboldt Current plays a significant role as an oceanographic factor, facilitating the influx of various species from the Chilean sea into Peruvian waters, with 19 of them occurring in both. For instance, *Phidiana lottini*, found in Callao and Puerto Malabrigo, may extend into tropical waters due to the influence of the Humboldt Current (Uribe et al. 2013). The occurrence of *Polycera priva* in Peru came as a surprise to researchers, considering that this species is typically Magellanic and endemic to the Patagonian fjords (Schrödl 1996a, 1999b, 2003, 2009). Therefore, its presence in Peru is attributed to the influence of the Humboldt Current (Schrödl 1996a, 1999b, 2003, 2009; Schrödl and Hooker 2014). Species such as *Corambe lucea*, *Coryphellina cerverai*, *Janolus rebeccae*, and *Tritonia* sp., collected in the Warm Temperate Southeastern Pacific province, have also been reported in Sechura Bay and Foca Island, situated in the biogeographical transition zone. This suggests their adaptation to slightly warmer waters (Fig. 4).

El Niño events can induce shifts in the distribution ranges of sea slugs (Goddard et al. 2018) and even result in species turnover (Valqui et al. 2021). During strong El Niño events, it is noteworthy that mollusk species endemic to tropical areas have been observed in Peruvian waters due to the displacement of warm-water masses (Velez and Zeballos 1985; Paredes et al. 1998; Ramírez et al. 2003). Species such as *Armina californica*, *Felimida baumanni*, *Felimare agassizii*, *Tyrinna evelinae*, and *Bajaeolis bertschi*, which are abundant in the Tropical Eastern Pacific province, Warm Temperate Northeast Pacific, or Cold Temperate Northeast Pacific, have only been recorded in Tumbes or Piura (the northernmost coastal areas of Peru) (Table 4). This suggests that their presence may be temporary, resulting from the displacement of warm-water masses, or they may have permanently adapted to similar conditions following multiple El Niño events (Ashton et al. 2008).

The distribution patterns of cosmopolitan and circumglobal species can be attributed to various biological factors, including their remarkable dispersal capabilities. Take, for instance, *Glaucus atlanticus*, which possesses intriguing adaptations for dispersal such as larval gas bubbles and specialized anatomy enabling it to exploit water surface tension (Thompson and McFarlane 1967; Valdés and Campillo 2004; Churchill et al. 2014a). Additionally, abiotic factors like ocean currents (Miller 1993) and indirect human influences such as buoy rafting (Astudillo et al. 2009), ballast water, or shipping activities can facilitate the dispersal of these species, expanding their geographic ranges (Borg et al. 2009).

Potentially occurring species

This group of species poses a challenge as they have been consistently listed and referenced in several previous articles (indicated by asterisks in Table 3) despite lack of substantiated records. One notable example is *Cadlina sparsa*. Initially mentioned as a probable species by Alamo and Valdivieso (1997), it persisted in the subsequent publications of Paredes et al. (1999), Ramírez et al.

(2003), and Nakamura (2006) without acknowledging its speculative status. In the work of Uribe et al. (2013), the authors discussed the predicted occurrence of *Cadlina sparsa* in Peruvian waters based on extrapolation, assuming a continuous distribution, due to its presence in Baja California and Chile.

Paredes et al. (1999), based on personal communication with Sandra Millen, included *Aeolidia campbellii* (referred to as *Aeolidia serotina*) and *Itaxia falklandica* (referred to as *Flabellina falklandica*), which persisted in the subsequent lists of Nakamura (2006) and Ramírez et al. (2003), respectively. However, no additional published reports have surfaced to substantiate their presence. Ramírez et al. (2003) also included the species *Phylliroe lichtensteinii* without providing any justification for its inclusion. On the other hand, Nakamura (2006) mistakenly listed *Gargamella immaculata* and *Thecacera darwini* as present in Peruvian waters, referencing Schrödl (1999b). Nevertheless, Schrödl (1999b) only listed these species in a table as occurring in the 'zoogeographic Peruvian province', indicating their presence in the Chilean waters corresponding to this biogeographical province, but not in Peruvian waters.

A solitary specimen of *Coryphellina marcusorum* was recently documented through photography on the northern coast of Peru (Los Organos, Piura) (Torrejón 2023). Given the species' tropical distribution and a previous record near countries such as Ecuador (GBIF 2022), its occurrence in Peru is plausible. However, these two records do not provide evidence of an established population in this region, suggesting the possibility that it may have arrived independently through shipping or been displaced by ocean currents. Further collections and taxonomic verifications are necessary to confirm its presence definitively.

Potentially occurring species should not be included in the official list of Peruvian nudibranch species. However, considering their disjointed or patchy geographic distributions or unique observations, they may be reported in Peruvian waters in forthcoming papers. *Rostanga pulchra*, for instance, was a long-standing predicted species for Peruvian waters until its initial sighting in San Juan de Marcona (Ica) by Schrödl and Hooker (2014). As a cosmopolitan species, the presence of *Phylliroe lichtensteinii* in Peru is plausible. Fischer and Cervera (2005b) have deemed the presence of *Thecacera darwini* in Peruvian waters highly probable. The term "potentially occurring" signifies a provisional status, indicating that these species have not yet been officially confirmed.

Identification uncertainties

The genus *Polycera* displays remarkable color variability, seemingly correlated with its geographic range (Behrens and Hermosillo 2005). A genetic investigation identified clades with overlapping distributions in the Northeastern Pacific, strongly suggesting the existence of a species complex (Santander and Valdés 2013). One year later, a morphology-based study by Pola et al. (2014) revealed that specimens previously collected by Camacho-García et al. (2005) in Costa Rica, Panama, and Mexico actually belong to a new species, *Polycera anae*. Considering that *P. alabe* was solely "observed" by Millen in Tumbes, it is possible that it represents another *Polycera* species, such as *P. anae*, given its inclusion in a species complex (Santander and Valdés 2013).

The report of *Glaucus* sp., documented by Quesquen (2017), is based on a single poorly preserved specimen sampled in 1995, warranting verification through molecular methods. Currently, within the genus *Glaucus*, only the species *G. atlanticus* has been confirmed, as many previous records turned out to be synonyms (e.g., *G. distichoicus*) or were later reassigned to the genus *Glaucilla*. Similar circumstances apply to the reports of *Tritonia* sp. and *Cuthona* sp., as their species-level recognition is still pending.

Concluding remarks

This research contributes to the dissemination and diffusion of this understudied group of organisms in Peru. It is imperative to intensify monitoring efforts to verify the presence of doubtful species, evaluate anthropogenic impacts, and El Niño-driven displacements. Furthermore, considering the intricate nature of external morphological identification, frequent variability in coloration, and the probable existence of cryptic species, it is possible that a considerable number of species remain undiscovered. Consequently, there is an urgent need for comprehensive research involving detailed internal anatomy and the application of molecular tools, such as DNA barcoding and phylogenetic analyses. These methodologies will play a vital role in shedding light on the taxonomy and evolutionary relationships within this group.

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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 or Supplementary Information.

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Research Article

New subgenera and a new species of the genus *Raphignathus* Dugès (Prostigmata, Raphignathidae), with taxonomic notes on the genus *Neoraphignathus* Smiley & Moser

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Abstract

Four new subgenera in the genus *Raphignathus* Dugès are hereby proposed: *Raphignathus* (*Raphignathus*), **subgen. nov.**, *Raphignathus* (*Monoraphignathus*), **subgen. nov.**, *Raphignathus* (*Diraphignathus*), **subgen. nov.**, and *Raphignathus* (*Triraphignathus*), **subgen. nov.** These subgenera are diagnosed by the number of setae on the interscutal membrane of females. A new species, *R.* (*D.*) *neohecmatanaensis* **sp. nov.,** is described and illustrated based on females collected from *Ziziphus spina-christi* Mill. (Rhamnaceae). The taxonomic status of the monotypic genus *Neoraphignathus* Smiley & Moser and three species (*R. evidus*, *R. hsiufui*, and *R. johnstoni*) are discussed. A key to world species of the family Raphignathidae is given.

Key words: Acari, new combinations, predatory mite, Raphiganthoidea, Saudi Arabia

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Introduction

Members of the family Raphignathidae Kramer (Prostigmata, Raphignathoidea) are active predators feeding on small arthropods (Meyer and Ueckermann 1989). They are mostly found in humus soil under dense bushes, leaf litter, lichens, and mosses and on a wide range of plants (Fan and Zhang 2005). Raphignathids have cervical peritremes and contiguous coxae and are divided into two genera: *Raphignathus* Dugès (76 species) and *Neoraphignathus* Smiley & Moser (one species, *N. howei* Smiley & Moser) (Smiley and Moser 1968; Khanjani et al. 2013). These genera are mainly differentiated by the presence and absence of dorsal shields, respectively. *Raphignathus* species have a worldwide distribution, whereas *Neoraphignathus* is only known from Louisiana, USA (Beron 2020).

The genus *Raphignathus* (type species: *R. ruberrimus* Dugés) was diagnosed as having three, or sometimes four, dorsal shields (Atyeo et al. 1961). While discussing the chaetotaxy of the superfamily Raphignathoidea, Atyeo et al. (1963) provided a general description of the genus *Raphignathus*. They considered the number of setae on prodorsal shields and on interscutal membrane as important taxonomic characteristics. In addition, the number of setae on the interscutal membrane was also used to differentiate among the species in recently published diagnostic keys (Nasrollahi et al. 2018; Pishehvar and Khanjani

2021). The validity of some *Raphignathus* species has been questionable due to ontogenetic development, and more females need to be collected and observed (Dönel and Doğan 2011). *Raphignathus broomicus* Podder was considered a species inquirenda due to uncertain and doubtful characters (Doğan and Erman 2019). Until now, most of published work on the family Raphignathidae has been regional and includes identification keys for China, India, Iran, and Turkey (Fan and Yin 2000; Doğan and Erman 2019; Pishehvar and Khanjani 2021).

In the present study, four new subgenera of *Raphignathus* are erected based on the prominent and consistent morphological character (number of setae on interscutal membrane). A new species, *R.* (*D.*) *neohecmatanaensis* sp. nov. is described and illustrated based on females. Some taxonomic notes on the monotypic genus, *Neoraphignathus* Smiley & Moser, and the identity of three species (*R. evidus*, *R. hsiufui*, and *R. johnstoni*) are concisely discussed. A diagnostic key to the world species is also provided.

Material and method

All published taxonomic literature on the family Raphignathidae was critically reviewed to confirm the validity of the species, subgeneric divisions and to prepare a diagnostic key of world species. The new raphignathoid species was collected by shaking foliage of *Ziziphus spina-christi* Mill. (Rhamnaceae) over a white sheet of paper; mite specimens were preserved in small vials containing 70% ethanol. The specimens were permanently mounted on glass slides in Hoyer's medium and identified under a phase-contrast microscope (BX51, Olympus, Tokyo, Japan). All measurements of the holotype specimen are given in micrometers (μm), followed by those of paratypes in the parenthesis. The terminology and abbreviations used in the description of the new species follow those of Kethley (1990) and Grandjean (1939, 1944, 1946). The holotypes and paratypes were deposited at the King Saud Museum of Arthropods (**KSMA**, Acarology section), Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia.

Results

Four new subgenera of the genus *Raphignathus* are proposed: *Raphignathus* (*Raphignathus*), subgen. nov., *Raphignathus* (*Monoraphignathus*), subgen. nov., *Raphignathus* (*Diraphignathus*), subgen. nov., and *Raphignathus* (*Triraphignathus*), subgen. nov., on the basis of the number of setae on the interscutal membrane, a prominent and consistent morphological character. A new species, *R.* (*D.*). *neohecmatanaensis* sp. nov., is described and illustrated based on adult females. Furthermore, taxonomic notes on the status of the monotypic genus, *Neoraphignathus* Smiley & Moser, and the identity of three species (*R. evidus*, *R. hsiufui* and *R. johnstoni*) are discussed. A diagnostic key to the world species is also presented.

Family Raphignathidae Kramer, 1877

Raphignathidae Kramer, 1877: 215

Type genus. *Raphignathus* Dugès, 1834: 53

Diagnosis (based on Krantz and Walter (2009) and Fan and Zhang (2005)). Peritremes linear, not imbedded in dorsal surface of stylophore; paired peritremes running laterally from base of stylophore to make short loops in collar membrane between gnathosoma and podosoma; coxae II and III contiguous; stigmata opening at base of chelicerae.

Taxonomic divisions of the genus *Raphignathus*

The presence or absence of prodorsal shields are diagnostic for the differentiation of the two existing raphignathid genera. Atyeo (1963) discussed in detail the chaetotaxy of the superfamily Raphignathoidea while describing seven *Raphignathus* species. This author also provided comprehensive diagnosis of *Raphignathus* and stated that the number of setae on the shields and the interscutal membrane is a consistent and important diagnostic character. The number of setae on the interscutal membrane is considered to be a strong character and has been used in identification keys to distinguish species (Nasrollahi et al. 2018; Pishehvar and Khanjani 2021). Through our extensive study of the literature of all 76 *Raphignathus* species, we find that the number of setae on the interscutal membrane can be used to erect subgenera.

In the current study, we categorize species of the genus *Raphignathus* into four new subgenera based on the number of setae on the interscutal membrane. These four subgenera are *Raphignathus* (*Raphignathus*), subgen. nov. without setae (11 spp.), *Raphignathus* (*Monoraphignathus*), subgen. nov. with one seta (14 spp.), *Raphignathus* (*Diraphignathus*), subgen. nov. with two setae (33 spp.), and *Raphignathus* (*Triraphignathus*), subgen. nov. with three setae (10 spp.).

***Raphignathus* (*Raphignathus*), subgen. nov.**

https://zoobank.org/4A81EA35-DC89-4971-BD24-0459A27A8621

Type species. *Raphignathus ruberrimus* Dugès, 1834: 53.

Diagnosis. Interscutal membrane without setae.

Etymology. The subgeneric epithet refers to the nominotypical subgenus.

***Raphignathus* (*Monoraphignathus*), subgen. nov.**

https://zoobank.org/D332A8E8-0390-4D39-95D4-A8C6E7E0B8D5

Type species. *Raphignathus bathursti* Meyer & Ryke, 1960: 229.

Diagnosis. Interscutal membrane with one pair of setae.

Etymology. The subgeneric epithet refers to the one pair of setae on interscutal membrane.

***Raphignathus* (*Diraphignathus*), subgen. nov.**

https://zoobank.org/0D7C8B12-10E8-4019-A2FA-591934FD9B17

Type species. *Raphignathus gracilis* (Rack, 1962): 281.

Diagnosis. Interscutal membrane with two pairs of setae.

Etymology. The sub-generic epithet refers to the two pairs of setae on interscutal membrane.

The species included in this new subgenus are widely distributed over the world.

Raphignathus **(*Triraphignathus*), subgen. nov.**

https://zoobank.org/80C632B9-6521-47BB-A6E2-3EA44EE761DD

Type species. *Raphignathus domesticus* Shiba, 1969: 157.

Diagnosis. Interscutal membrane with three pairs of setae.

Etymology. The subgeneric epithet refers to the interscutal membrane with three pairs of setae.

Notes on the taxonomic status of the genus *Neoraphignathus*

To date, the family Raphignathidae has included two genera, *Raphignathus* and *Neoraphignathus*, which have been differentiated based on the presence or absence of shields on the dorsum. The monotypic genus, *Neoraphignathus* (type species: *N. howei* Smiley & Moser) was erected in 1968, based on a single female holotype specimen without detailed description and illustration. Based on observations and the collection of the immature specimens of the genus *Raphignathus*, prodorsal shields are weakly developed or absent in immatures. Atyeo et al. (1961) has reported that dorsal shields are sometime feebly developed. Since its first description, the type species, *N. howei*, has not been redescribed, nor have new *Neoraphignathus* species been described. We suggest that the type specimen of *N. howei* be re-examined and also that more specimens be collected from the type locality to confirm the absence of a dorsal shield to confirm the validity of *Neoraphignathus*.

Notes on the validity of *Raphignathus evidus, R. hsiufui*, and *R. johnstoni*

The taxonomic identity of *Raphignathus evidus* Fan, *R. hsiufui* Fan, and *R. johnstoni* Womersley are doubtful. These species were originally described based on single specimens, minor differential characteristics (i.e. number of dorsal setae on the lateral prodorsal shield; all three species have two pairs of setae on lateral shields), and small opisthosomal shields. In contrast, all other *Raphignathus* species have three pairs of setae on the lateral prodorsal shields along with the pores (*ia*). The immature stages of *Raphignathus* gradually develop the prodorsal shields, striation patterns, and leg setae (Fan and Yin 2000). For instance, the immature stages of *R. giselae*, *R. lenis*, and *R. caspicus* each have two setae on the lateral prodorsal shields (three setae in adult) and small lateral prodorsal shields with weakly developed striations. Moreover, we also observed the immatures from more than 10 populations of *Raphignathus* and found reduced size of weakly sclerotized lateral prodorsal shields and setae set on the edges of shields. Based on this evidence, *R. evidus*, *R. hsiufui*, and *R. johnstoni* should be revised and more specimens collected to confirm their validity.

New species

***Raphignathus* (*Diraphignathus*) *neohecmatanaensis* sp. nov.**
https://zoobank.org/12E13CEE-478F-4A3F-A47E-B3C7F34C73FA
Figs 1–8

Diagnosis. Female: endopodal shield absent between coxae I–IV; two small shields present posterolateral to median prodorsal shield; palp femora with two setae; femora 6–5–3–2; genua 5(+κ)-5(+κ)–4–4; tibiae 5(+φρ)–5(+φρ)–4(+φρ)–4 (+φρ); tarsi 21(1ω) –20(1ω)–15–14.

Description. Female (*n* = 4). Idiosoma oval, length of body (including gnathosoma) 533 (525–545); width of body 345 (338–353).

Dorsum (Fig. 1). Propodosoma with one medial and two lateral shields each containing three setae; medial sclerite with setae *vi*, *si* and *c1*; paired ovoid lateral shields each with an eye, one cupule (*ia*) and *sci*, *sce* and seta *c2* seta; opisthosomal setal pairs *d1*, *e1* and the pair of cupuli (*im*); posterior opisthosomal shield large, rectangular, bearing four pairs of setae (*f1*, *h1–3*) and one pair of the cupule (*ip*); all dorsal shields finely punctate; dorsal body setae setiform, smooth and acute; pseudanal setae *ps1* dorsally located. Lengths of dorsal setae: *vi* 25 (23–27), *ve* 28 (26–30), *sci* 27 (26–29), *sce* 28 (26–29), *c1* 23 (17–19), *c2* 25 (19–23), *d1* 21 (20–23), *e1* 22 (21–23), *f1* 21 (19–22), *h1* 24 (23–25), *h2* 25 (23–25), *h3* 22 (21–24), *ps1* 23 (21–25); distances between dorsal 195 setae: *vi–vi* 27 (29–31), *sci–sci* 123 (118–128), *vi– sci* 70 (66–72), *sce–c2* 68 (65–72), *c1–c1* 30 (28–32), *d1–d1* 99 (92–101), *c1–d1* 53 (49–55), *d1–e1* 22 (21–24), *f1–f1* 88 (83–90), *e1–f1* 65 (61–68), *h1–h1* 45 (42–48), *h1–h2* 43 (41–46), *h2–h2* 77 (72–80), *h3–h3* 101 (98–106).

Venter (Fig. 2). Venter entirely striated, without punctations; coxisternal shields absent (Fig. 2); ventral setae lengths: *1a* 42 (41–44), *1b* 38 (35–39), *1c* 34 (33–36), *2b* 36 (30–34), *2c* 32 (30–34), *3a* 24 (23–26), *3b* 18 (17–20), *3c* 36 (34–38), *4a* 22 (23–27), *4c* 38 (35–40); two pairs of aggenital setae (*ag1–2*) with one cupule (*ih*) on each side of the genital shield; anal opening and genital shields separate; genital shield prominent with a few punctations, bearing three pairs of genital setae (*g1–3*); anal opening terminal, with three pairs of setae (*ps1*–3), *ps1* dorsal *ps3* and *ps2* ventral; ventral setal lengths: *ag1* 29 (27–32); *ag2* 27 (25–28); *g1* 31 (28–33); *g2* 25 (23–28); *g3* 20 (19–22); *ps2* 22 (21–23); *ps3* 21 (20–22). Distances between ventral setae: *1a–1a* 55 (53–58), *3a–3a* 115 (97–109), *4a–4a* 70 (68–73), *ag2–ag2* 42 (41–43), *g1–g1* 38 (35–40), *g2–g2* 52 (48–55), *g3–g3* 75 (74–75), *2b–2c* 30 (25–29), *1a–3a* 50 (49–53), *3a–4a* 72 (68–75), *4a–ag1* 85 (82–88), *ag1–ag2* 65 (63–68), *ag2–g1* 63 (60–65), *g1–g2* 15 (14–17), *g2–g3* 22 (21–25), *ag–g1* 42 (40–45), *g3–ps3* 16 (15–18), *ps2–ps3* 16 (14–18).

Gnathosoma (Figs 3, 4). Ventral infracapitular with two pairs of very long setae (*m* and *n*), *m* 40 (39–42), *n* 52 (49–54) and two pairs of pilose adoral setae (*or1*–2), *or1* 23 (21–24), *or2* 20 (19–22) (Fig. 3); stylophore conical and striated; palp chaetotaxy (femur-tarsus) as follows: 3–2–4+1 claw 4+1ω+4 eupathidia (ζ) (Fig. 4).

Legs (Figs 5–8). Length of legs I–IV (without coxae): 340 (328–348); 270 (276–286); 325 (317–328); 375 (367–384), respectively. Chaetotaxy on legs

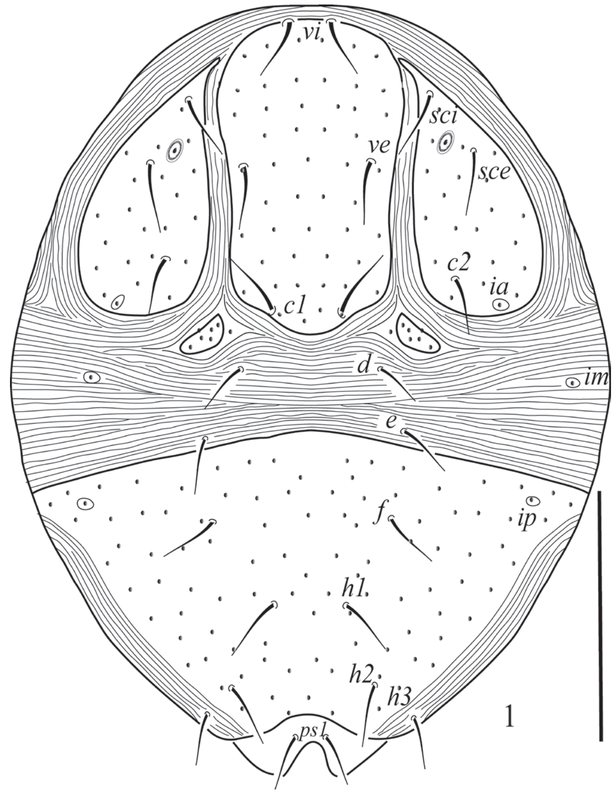


Figure 1. *Raphignathus* (*Diraphignathus*) *neohecmatanaensis* sp. nov. (female), dorsum. Scale bar: 100 µm.

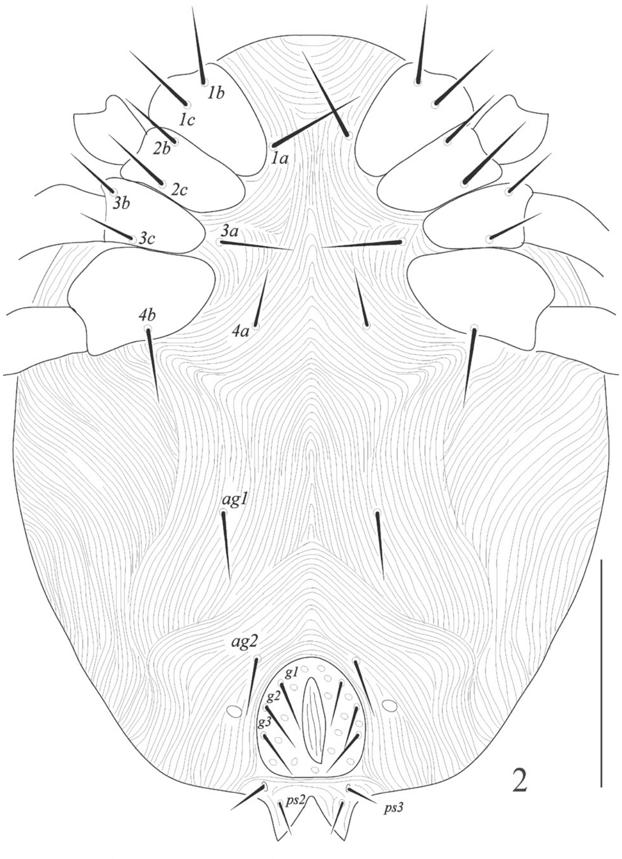


Figure 2. *Raphignathus* (*Diraphignathus*) *neohecmatanaensis* sp. nov. (female), venter. Scale bar: 100 µm.

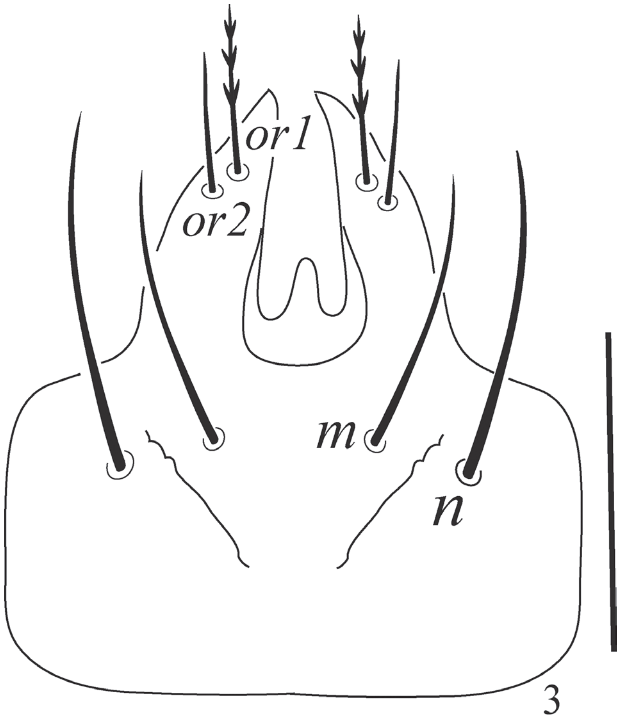


Figure 3. *Raphignathus* (*Diraphignathus*) *neohecmatanaensis* sp. nov. (female), gnathosoma. Scale bar: 50 µm.

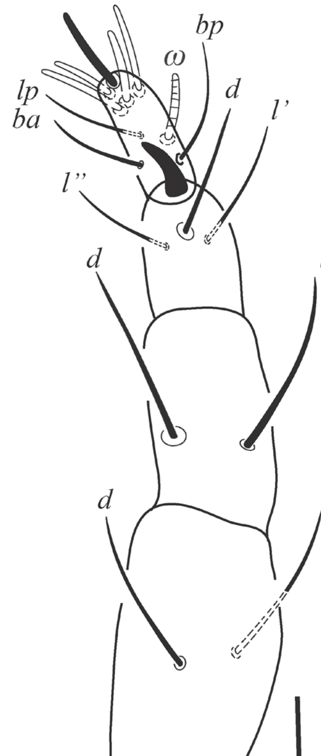
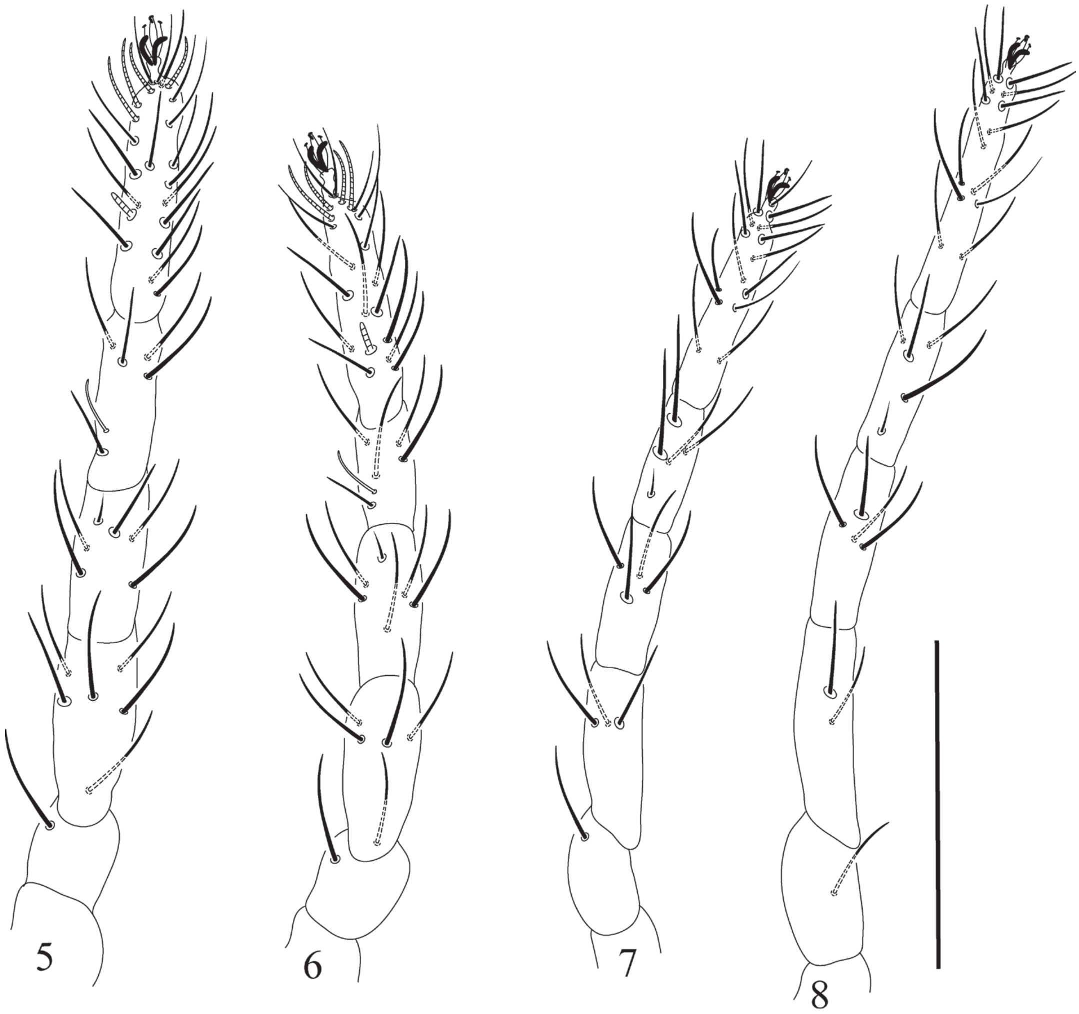


Figure 4. *Raphignathus* (*Diraphignathus*) *neohecmatanaensis* sp. nov. (female), palp. Scale bar: 20 µm.



Figures 5–8. *Raphignathus* (*Diraphignathus*) *neohecmatanaensis* sp. nov. (female), Legs I–IV. Scale bar: 20 μm.

I–IV (solenidia in parentheses and not included in setal counts): coxa 2–2–2–1; trochanter 1–1–2–1; femora 6–5–3–2; genua 5(+κ)-5(+κ)–4–4; tibiae 5(+φρ)–5(+φρ)–4(+φρ)–4(+φρ); tarsi 21(1ω) –20(1ω)–15–14.

Male and immature stages. Unknown.

Type materials. ***Holotype*** female and three paratype females, Faifa, Jazan, 24°30.412'N, 39°36.578'E, 8 Oct., 2020, collected from *Ziziphus spina-christi* Mill. (Rhamnaceae) by Eid M. Khan, Jawwad H. Mirza & Hafiz S. Mushtaq.

Etymology. The specific epithet is in reference to the similarity of the new species to *R.* (*D.*) *hecmatanaensis*; *neo* = new.

Remarks. *Raphignathus* (*D.*) *neohecmatanaensis* sp. nov., belongs to the subgenus *Diraphignathus* subgen. nov. The new species resembles to *R.* (*D.*) *hecmatanaensis* Khanjani & Ueckermann in having two pairs of setae (*d1* and *e1*) on the interscutal membrane, two setae on the palp femora, and two small plates present posterolateral to the median prodorsal shield. However, the new species differs from *R.* (*D.*) *hecmatanaensis* in the absence of an endopodal shield (vs present), femur IV with two setae (vs three), and leg tarsus I with one solenidion (vs two solenidia).

Key to genera, subgenera, and all known species of the family Raphignathidae

Five species are not included in the key. *Raphignathus longimanus* (Koch), *R. impressus* (Koch), *R. hispidus* (Dugès), and *R. deserticula* (Trägårdh) because their descriptions are incomplete, and *R. lanuginosus* Atyeo is excluded, as it was described on the male.

1 Dorsum with well-developed shields, one medial, one pair of lateral shields on prodorsum and one hysterosomal shield .... **Genus *Raphignathus* [2]**
– Dorsum without shields.... **Genus *Neoraphignathus*, *N. howei* Smiley & Moser**
2 Interscutal membrane without setae, hysterosomal shield with six pairs of setae.................................. ***Raphignathus* (*Raphignathus*) subgen. nov. [3]**
– Interscutal membrane with ≥1 setae **13**
3 Opisthosoma or opisthosomal shield reticulated **4**
– Opisthosomal shield smooth or otherwise, not reticulated........................ **6**
4 Prodorsal shields reticulated.... ***R.* (*R.*) *crustus* Fan & Zhang, New Zealand**
– Prodorsal shields smooth or punctate.. **5**
5 Tibia I with 5 +2φ, Tibia III with 5+1φ, dorsal body setae comparatively long (54–74) ....***R.* (*R.*) *kurdistaniensis* Nasrollahi, Khanjani & Mirfakhraei, Iran**
– Tibia I with 5 +1φ, Tibia III with 4+1φ, dorsal body setae comparatively short (24–36) .... ***R.* (*R.*) *darjeelingensis* Gupta, India**
6 Opisthosoma without distinct shield; setae *d1*, *e1*, and *f1* very minute, 1/3–1/2 length of setae *v1* .... ***R.* (*R.*) *guajavae* Gupta, India**
– Opisthosoma with distinct opisthosomal shield; setae *d1*, *e1*, and *f1* at least 2/3 length of setae *v1* **7**
7 Genu I and II each with a large leaf-like solenidion .... ***R.* (*R.*) *pycnonotus* (Gupta & Paul), India**
– Genu I and II each with a small, slender solenidion **8**
8 Setae *c1* short, reaching 1/3 length of interscutal membrane, far behind the anterior margin of opisthosomal shield; tarsus IV with large solenidion... ***R.* (*R.*) *inornata* (Gupta & Paul), India**
– Setae *c1* crossing interscutal membrane and anterior margin of opisthosomal shield; tarsus IV with small solenidion **9**
9 Tibia I with one solenidion **10**
– Tibia I with two solenidia **12**
10 Genu IV with three setae ....***R.* (*R.*) *hirtellus* Athias-Henriot, Algeria**
– Genu IV with four setae.. **11**
11 Ratios *d1–d1/c1–c1* = 3.00, *c2–c2/d1–d1* = 3, *d1–d1/e1–e1* = 0.58, *e1–e1/f1–f1* = 0.94–1.00................***R.* (*R.*) *neocardinalis* Atyeo, The Bahamas**
– Ratios *d1–d1/c1–c1* = 6.00, *c2–c2/d1–d1* = 1.22, *d1–d1/e1–e1* = 1.38, *e1–e1/f1–f1* = 0.65 ....***R.* (*R.*) *conspicuus* (Berlese), Colombia**
12 Dorsal setae comparatively long; setae *c1*, and *d1* crossing bases of *d1* and *e1*, respectively .... ***R.* (*R.*) *khorramabadensis* Bagheri, Jafari & Paktinat, Iran**
– Dorsal setae comparatively short; setae *c1*, and *d1* far behind bases of *d1* and *e1*, respectively .... ***R.* (*R.*) *cardinalis* (Ewing), USA**

13 Interscutal membrane with one pair of setae.. ....***Raphignathus* (*Monoraphignathus*) subgen. nov. [14]**

– Interscutal membrane with more than one pair of setae **27**

14 Palp femur with two setae; femur I with three setae ....**R. (*M.*) *arabicus* Gomaa & Hassan, Egypt**

– Palp femur with three setae; femur I with five or six setae.......................**15**

15 Femur IV with two or three setae **16**

– Femur IV with four setae..**21**

16 Femur IV with two setae **17**

– Femur IV with three setae..**19**

17 Genu II with five setae including micro setae... ....**R. (*M.*) *costatus* Chaudhri, Akbar & Rasool, Pakistan**

– Genu II with six setae including microsetae...**18**

18 Setae *e1* reaching to bases of *h1*; dorsal body setae with spinules along entire length......................................**R. (*M.*) *zhaoi* Hu, Jing & Liang, China**

– Setae *e1* reaching half distance to bases of *h1* (or distance *e1–h1*), dorsal body setae with spinules along entire length .... **R. (*M.*) *kuznetzovi* Dogan & Ayyildiz, Turkey**

19 Setae *c2* crossing bases of *d1*, Setae *c1* extending to bases of *e1*, setae *e1* extending to bases of *h1* ....**R. (*M.*) *ueckermanni* Koç & Kara, Turkey**

– Setae *c2* crossing setae *c1* far behind to the bases of *e1*, setae *e1* far behind to the bases of *h1* **20**

20 Dorsal body setae ensiform, setae *c1* far behind bases of *d1*, *d1–d1* distance almost five times more than *c1–c1* distance..................................... ....**R. (*M.*) *ensipilosus* Meyer & Ueckermann, South Africa**

– Dorsal body setae setiform, setae *c1* far behind bases of *d1*, *d1–d1* distance almost equal to *c1–c1* ....**R. (*M.*) *cometes* Atyeo, Bahama-Islands**

21 Small shields absent posterolateral to median prodorsal shield.............**22**

– Small shields present posterolateral to median prodorsal shield............**25**

22 Genital plates/covers with four pairs of setae ....**R. (*M.*) *koseiensis* Dönel & Doğan, Turkey**

– Genital plates/covers with three pairs of setae **23**

23 Femur I and II each with five setae... **R. (*M.*) *solimani* Hassan & Gomaa, Egypt**

– Femur I and II each with six setae...**24**

24 Dorsal setae comparatively long; most setae cross base of next consecutive setae..................................**R. (*M.*) *kelkitensis* Dönel & Doğan, Turkey**

– Dorsal setae comparatively short; most setae far behind base of next consecutive setae..................................**R. (*M.*) *fani* Doğan & Ayyildiz, Turkey**

25 Dorsal setae comparatively long; most setae reach or cross base of next consecutive setae ....**R. (*M.*) *bathursti* Meyer & Ryke, South Africa**

– Dorsal setae comparatively short; most setae far behind base of next consecutive setae..**26**

26 Trochanter III with three setae...... **R. (*M.*) *afyonensis* Akyol & Koç, Turkey**

– Trochanter III with two setae ....**R. (*M.*). *collegiatus* Atyeo, Baker, & Crossley, USA**

27 Interscutal membrane with two pairs of setae... .... **R. (*Diraphignathus*) subgen. nov. [28]**

– Interscutal membrane with three or four pairs of setae .... **R. (*Triraphignathus*) subgen. nov. [60]**

28 Medial prodorsal shield with two pairs of setae **29**

– Medial prodorsal shield with three pairs of setae **30**

29 Setae *c1* present, setae *vi* absents; presence of plates behind the anteromedian plate; femur IV with 2 setae .... **R.** (**D.**) ***evansi*** **Zaher & Gomaa, Egypt**

– Setae *c1* absent, setae *vi* present; dorsum without a pair of small plates behind anteromedian plate; femur IV with 3 setae .... **R.** (**D.**) ***ehari*** **Zaher & Gomaa, Egypt**

30 Genital plates/covers with four pairs of setae **31**

– Genital plates/covers with three pairs of setae **32**

31 Two small shields posterior to median prodorsal shield absent; endopodal shields absent; setae *f1* behind the anterior margin of opisthosomal shield..**R.** (**D.**) ***saboorii*** **Ghorbani & Bagheri, Iran**

– Two small shields posterior to median prodorsal shield present; endopodal shields present; setae *f1* on the anterior margin of opisthosomal shield... **R.** (**D.**) ***karabagiensis*** **Akyol & Koç, Turkey**

32 Palp femur with two setae **33**

– Palp femur with three setae...**44**

33 Two small shields posterior to median prodorsal shield present **34**

– Two small shields posterior to median prodorsal shield absent **38**

34 Endopodal shield present; femur IV with three setae **35**

– Endopodal shield absent; femur IV with two setae **36**

35 Tarsus I with two solenidia ....**R.** (**D.**) ***hecmatanaensis*** **Khanjani & Ueckermann, Iran**

– Tarsus I with one solenidion .... **R.** (**D.**) ***arcus*** **Akyol, Turkey**

36 Femur IV with two setae; tarsus I with one solenidion .... **R.** (**D.**) ***neohecmatanaensis*** **sp. nov. Alatawi & Kamran, Saudi Arabia**

– Femur IV with three setae; tarsus I with two solenidia **37**

37 Lateral prodorsal shield with one pair of pob; tibiae III 5(+1φp) tarsi 18(+1ω+1ω2) ....**R.** (**D.**) ***seraji*** **Pishehvar & Khanjani, Iran**

– Lateral prodorsal shield without pob; tibiae III 5 tarsi 19(+1ω+1ω2) .... **R.** (**D.**) ***rakhshandehi*** **Pishehvar & Khanjani, Iran**

38 Dorsal setae distally forked or tricarinate.. **R.** (**D.**) ***furcisetosus*** **Meyer & Ueckermann, South Africa**

– Dorsal setae simple, not distally forked or tricarinate **39**

39 Femur IV with two setae .... **R.** (**D.**) ***erzincanica*** **Doğan, Turkey**

– Femur IV with three setae **40**

40 Opisthosomal shield reduced; interscutal membrane more longer than opisthosomal shield..**41**

– Opisthosomal shield equally long or longer than interscutal membrane **43**

41 Dorsal setae stout, serrate and blunt-tipped .... **R.** (**D.**) ***membranus*** **Fan & Yin, China**

– Dorsal setae simple, distally pointed **42**

42 Interscutal membrane four times longer than much reduced opisthosomal shield; *f1* on anterior margin of opisthosomal shield .... **R.** (**D.**) ***vahiti*** **Doğan, Turkey**

– Interscutal membrane slightly longer than opisthosomal shield; *f1* behind anterior margin of opisthosomal shield.. **R.** (**D.**) ***giselae*** **Meyer & Ueckermann, Zimbabwe**

43 Median prodorsal shield anteriorly extending to peritremes and wider anteriorly near setae *sci* as compared to posterior half; setae *f1* on anterior margin of opisthosomal shield.................. ***R.*** **(*D.*) *gracilis* (Rack), Germany**

– Median prodorsal shield anteriorly far behind peritremes and almost equally wide anteriorly near setae *sci* and at posterior half; setae *f1* just behind anterior margin of opisthosomal shield........ ***R.*** **(*D.*) *bakeri* Zaher & Gomaa, Egypt**

44 Small shields posterior to median prodorsal shield absent **49**

– Two small shields posterior to median prodorsal shield present **45**

45 Coxae II with one seta .... ***R.*** **(*D.*) *atyeoi* Meyer & Ueckermann, South Africa**

– Coxae II with two setae **46**

46 Femur IV with two setae **47**

– Femur IV with three setae **48**

47 Coxae III and IV with endopodal shields; setae *f1* on posterior margin of interscutal membrane; distance *f1–f1* < *d1–d1*...... ***R.*** **(*D.*) *summersi* Robaux, USA**

– Coxae III and IV without endopodal shields; setae f1 far behind posterior margin of interscutal membrane; distance *f1–f1* > *d1–d1* .... ***R.*** **(*D.*) *aciculatus* Fan, China**

48 Tarsus I–IV 19+ ω, 15+1ω, 13, 12 .... ***R.*** **(*D.*) *africanus* Meyer & Ueckermann, South Africa**

– Tarsus I–IV 21+1 ω, 21+1ω, 15, 14.. .... ***R.*** **(*D.*) *hatamii* Khanjani & Pishehvar, Iran**

49 Coxa II with one seta .... ***R.*** **(*D.*) *rarus* Kuznetsov, USSR**

– Coxa II with two setae **50**

50 Coxae III and IV without endopodal shields **51**

– Coxae III and IV with endopodal shields **53**

51 *c1–f1*/*f1–f1* = 0.70; *c1–f1* < *f1–f1*; space between setae *f1–f1* twice as wide as between setae *d1–d1* ***R.*** **(*D.*) *atomatus* Fan & Zhang, New Zealand**

– *c1–f1*/*f1–f1* = 1.50–1.87; *c1–f1* 1.5–2.0 times more than *f1–f1*; distance *f1–f1* ≤ *d1–d1* **52**

52 Dorsal setae barbed; setae *c1*, *d1*, and *e1* reach or cross bases of next consecutive setae, distances *d1–f1* ≤ *f1–f1*; setae *f1* near anterior margin of opisthosomal shield .... ***R.*** **(*D.*) *satoi* Shiba, Malay Peninsula**

– Dorsal setae simple; setae *c1*, *d1*, and *e1* not reaching base of next consecutive seta; distances *d1–f1* 1.31 times more as *f1–f1*, *f1* behind anterior margin of opisthosomal shield .... ***R.*** **(*D.*) *kamiensis* Meyer & Ueckermann, South Africa**

53 Femur I and II with five and four setae, respectively .... ***R.*** **(*D.*) *hexeris* Chaudhri, Akbar & Rasool, Pakistan**

– Femur I and II with six and five setae, respectively **54**

54 Opisthosomal shield 2–4 times wider than interscutal membrane......... **55**

– Interscutal membrane as wide as or more wider than opisthosomal shield **56**

55 Setae *c1* far behind posterior margin of prodorsal shield; tibia III with five setae excluding solenidion; opisthosomal shield four times wider than interscutal membrane .... ***R.*** **(*D.*) *neogracilis* Robaux, USA**

– Setae *c1* on the posterior margin of prodorsal shield; tibia III with four setae excluding solenidion; opisthosomal shield twice as wide as interscutal membrane... ***R.*** **(*D.*) *scutatus* Kuznetsov, USSR**

56 Femur IV with two setae **57**
– Femur IV with three setae **59**
57 Dorsal shields without striations, tarsus I with 22 setae .... ***R.* (*D.*) *tumidus* Kuznetsov, USSR**
– Dorsal shields with fine, sparse puncta and faint striae; tarsi I with 21 setae **58**
58 Tarsi III–IV with 14 and 13 setae, respectively; femur II with five setae .... ***R.* (*D.*) *caspicus* Doustaresharaf and Kazemi, Colombia**
– Tarsi III–IV with 15 and 14 setae, respectively; femur II with six setae .... ***R.* (*D.*) *tamaricis* Poudineh, Ramroodi & Bagheri, Iran**
59 Setae *f1–f1* ≤ *c1–c1* and *d1–d1* .... ***R.* (*D.*) *giresuniensis* Doğan, Turkey**
– Setae *f1* twice as widely spaced as *c1–c1* .... ***R.* (*D.*) *orientalis* Fan & Li, China**
60 Medial prodorsal shield with two pairs of setae .... ***R.* (*T.*) *lenis* Barillo, Uzbekistan**
– Medial prodorsal shield with ≥3 pairs of setae **61**
61 Genital shield with four pairs of setae **62**
– Genital shield with three pairs of setae **63**
62 Two small shields present posterolateral to prodorsal shield; endopodal shields near coxae III and IV absent .... ***R.* (*T.*) *sceptrum* Chaudhri, Akbar & Rasool, Pakistan**
– Small shields absent posterolateral to prodorsal shield; endopodal shields near coxae III and IV present .... ***R.* (*T.*) *quadrigeminus* Dönel & Doğan, Turkey**
63 Palp femur with three setae .... ***R.* (*T.*) *aethiopicus* (Meyer & Ryke), South Africa**
– Palp femur with two setae **64**
64 Femur IV with two setae .... ***R.* (*T.*) *karrooi* Meyer & Ueckermann, South Africa**
– Femur IV with three setae **65**
65 Femur I with five setae, femur II with four setae .... ***R.* (*T.*) *domesticus* Shiba, Japan**
– Femur I with six setae, femur II with five setae **66**
66 Two small shields present posterolateral to prodorsal shield **67**
– Small shields absent posterolateral to prodorsal shield **68**
67 Tibiae III with four setae, solenidia absent .... ***R.* (*T.*) *hamooniensis* Poudineh, Ramroodi & Bagheri, Iran**
– Tibiae III with five setae with one solenidion .... ***R.* (*T.*) *larestanensis* Bagheri, Akrami & Majidi, Iran**
68 Genu II with four tactile setae; endopodal shields near coxae I–II present .... ***R.* (*T.*) *emirdagiensis* Akyol & Koç, Turkey**
– Genu II with five tactile setae; endopodal shields near coxae I–II absent .... ***R.* (*T.*) *ozkani* Doğan, Turkey**

Discussion

The taxonomic classification of predatory mites of the genus *Raphignathus* are revised, and for the first time, the genus is divided into four subgenera by considering the morphologically valid, persistent, and prominent characters (Atyeo 1963). The use of subgenera supports the identification of raphignathoid species and will help to avoid designation of new species based on variable characters. *Raphignathus evidus*, *R. hsiufui*, and *R. johnstoni* are considered doubtfully valid. They were described based on size of lateral prodorsal shields and number of setae, but these in these characteristics they resemble immature stages (Fan and Yin 2000). The monotypic genus *Neoraphignathus* was erected based on only the single type specimen with a restricted geographical region and its description is brief. We suspect it might have been described based on the immature stage of a *Raphignathus* species, and we suggest that the type species be revised and more specimens collected to confirm the validity of the species and genus.

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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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Research Article

Mitogenomes of the two historical species *Seira ferrarii* Parona, 1888 and *Seira pallidipes* Reuter, 1895 (Collembola, Entomobryidae, Seirinae) with their phylogenetic placement within Seirinae

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Abstract

The present paper reports the first occurrence of *Seira ferrarii* Parona, 1888 from Hungary. On this occasion, molecular analyses were performed on both *S. ferrarii* and another historical species of the genus, *S. pallidipes* Reuter, 1895, originally described from Hungary. Using low-coverage whole-genome sequencing, the complete mitogenomes were assembled and annotated using MitoZ. To test the phylogenetic placement of both species, we performed maximum likelihood and Bayesian analyses using a matrix containing 14 Seirinae species and two outgroups. Both resultant trees showed that the European populations of the sampled *Seira* spp. likely derive from ancestral branches of Seirinae, compared to the Asian and American populations. Our results put in question the monophyly of the genus *Seira*, as already observed in previous studies.

Key words: Entomobryoidea, Europe, gene order, Hungary, mitochondrial genomes, phylogeny, springtails

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Introduction

Although *Seira* Lubbock, 1870 is one of the most widespread genera of Entomobryidae worldwide, its high species richness is mainly found in the tropics (e.g., Mari Mutt 1986; Cipola et al. 2014, 2018b; Godeiro and Bellini 2014), with relatively few representatives occurring in Europe, most of which found in Mediterranean countries, under subtropical climates (e.g., Gama 1964; Tosi and Parisi 1990; Cipola et al. 2018a).

Seira ferrarii Parona, 1888 was originally described from Italy (Genova), and later also recorded in the Spanish mainland (Yosii 1959), Bulgaria, French mainland, Greek mainland, Malta, Republic of Moldova, Spain, Portugal, Romania, various islands (Canary Is., Corsica, Dodecanese Is., Sicily) (Bedos 2023), North Africa (Jacquemart and Jacques 1980; Barra 2004), and Israel (Gruia et al. 2000). From the capital of Hungary, Loksa and Loksa (1994) reported the presence of a "particular" *Seira* sp. juv., which however remained undetermined. On revisiting the sampling site, we managed to collect several specimens of this abovementioned *Seira* sp., which were identified as *S. ferrarii*, and thus represent the first occurrence of the species in Hungary.

Seira pallidipes Reuter, 1895, on the other hand, was originally described from Hungary, and was recently redescribed and synonymized with *S. pillichi* Stach, 1929 by Winkler and Dányi (2017). Outside Hungary, the species occurs only in Austria and Serbia (Bedos 2023).

Seira dollfusi Carl, 1899 is a widely distributed species in Europe (Bedos 2023). However, as its true identity is unclear (the species has not yet been redescribed in detail based on type material or specimens collected at the type locality), we refer to specimens collected in Hungary for our study as *S.* cf. *dollfusi*.

The main goal of our study was to investigate for the first time the phylogenetic position of three European species of Seirinae. Previous studies focused on the Neotropical region (Godeiro et al. 2020) and the most embracing was recently published, containing species from the Neotropics and Asia (Godeiro et al. 2023). The internal organization of the subfamily is uncertain, and extensive taxon sampling is currently underway to propose a global phylogenetic study with dispersion routes and possible new genera.

Materials and methods

Specimens of *S. ferrarii* were collected in a xerophilous dolomite-steppe meadow association, Tétényi Plateau, Budapest, Hungary (47°25'6"N, 18°56'58"E, 195 m a.s.l.) on 7.X.2022 (leg. D. Winkler & M. Korda), while specimens of *S. pallidipes* were collected in a secondary hay meadow, Sopron, Hungary (47°45'31"N, 16°36'58"E, 169 m a.s.l.) on 4.VI.2022 (leg. D. Winkler). *Seira* cf. *dollfusi* was sampled on a calcareous open rocky grassland, Zuppa-tető, Szárliget, Hungary (47°30'10"N, 18°30'52.41"E, 359 m) on 6.IX.2022 (leg. D. Winkler).

On each occasion, an entomological aspirator was used for collection. Specimens were stored in absolute ethanol until further analysis. A Zeiss Stemi 508 stereomicroscope was used to sort the material. Habitus of the two studied species was photographed with a Canon EOS 7D digital camera attached to the stereomicroscope using a C-mount adapter.

Specimens for morphological identification were cleared using Nesbitt's fluid and then mounted on permanent slides in Hoyer's medium, following the protocol described by Jordana et al. (1997). The slides were examined under a Leica DM2500 LED microscope with conventional bright light and phase contrast.

Part of the material preserved in absolute ethanol was sent to Shanghai Yaoen Biotechnology Co., Ltd, China, where all laboratory experiments, including DNA extraction, amplification, and library construction were made according to the procedures suggested by the kits manufacturers. For DNA extraction, the TIANamp MicroDNA extraction kit (Tiangen Co., Ltd, China) was used. Libraries were constructed using KAPA Hyper Prep Kit (Roche, Basel, Switzerland). Approximately 10 Gbp of paired-end reads from each species were sequenced by an Illumina NovaSeq 6000 platform. This amount of data was enough to have a good coverage to assemble complete mitogenomes.

Mitogenomes were assembled, annotated, and visualized using MitoZ v.2.4-alpha (Meng et al. 2019). A manual check was necessary to confirm the start and end points of protein-coding genes (PCGs). Mitochondrial genome sequences with annotations and raw sequence data were submitted to the NCBI nucleotides and SRA databases (https://www.ncbi.nlm.nih.gov/), with project number PRJNA971781. To complete our phylogenetic dataset, protein-coding genes (PCG's) of another 11 species of Seirinae and two Lepidocyrtinae (outgroups) were downloaded from NCBI (Table 1). The partial mitogenome of *S.* cf. *dollfusi* (9298 bp), also sequenced and assembled during the present study, was included in the phylogenetic analyses to test its closer relationship with *S. pallidipes*. The sequence is publicly available at: https://doi.org/10.6084/m9.figshare.23654097.

Previously to the alignment, nucleotide sequences of the 13 PCGs of the 16 species that comprise our phylogenetic dataset were translated into amino acids using TransDecoder v.5.5.0 (https://github.com/TransDecoder/TransDecoder). Previous phylogenetic studies of Collembola showed that due to the high heterogeneity of mitochondrial sequences, matrices created using amino acids produce better results than using nucleotides (Bellini et al. 2023; Godeiro et al. 2021). Also, according to a recent analysis, site-wise heterogeneity is typically a more significant source of bias in phylogenomic inference than protein-wise heterotachy (Wang et al. 2019; Yu et al. 2022). Independent files containing the PCGs were aligned by MAGUS (Smirnov and Warnow 2021) employing MAFFT (Katoh et al. 2019). An automated alignment trimming was performed by BMGE v.1.12 (Criscuolo and Gribaldo 2010). PhyKIT v.1.9.0 (Steenwyk et al. 2021) concatenated the alignments and generated the partition scheme. The final matrix had 3104 amino acid sites and 13 loci.

To test the phylogenetic placement of the European species of *Seira*, we performed two phylogenetic inferences. IQ-Tree v.2.0.7 (Minh et al. 2020) was used to make the maximum likelihood (ML) analyses with 1000 ultrafast bootstrap replicates (Hoang et al. 2018) and SH-aLRT support (Guindon et al. 2010). The best model for each partition was suggested by ModelFinder (Kalyaanamoorthy

Table 1. Taxonomical information of the species used in the phylogenetic analyses. The newly assembled mitogenomes are represented in bold. *Mitogenome partially recovered. NA: not applicable.

	Species	Subfamily	Country	GenBank number
1	*Lepidocyrtus fimetarius* Gisin, 1964	Lepidocyrtinae	China	NC_047189.1
2	*Lepidocyrtus sotoi* Bellini & Godeiro, 2015	Lepidocyrtinae	Brazil	MT928545.1
3	*Lepidocyrtinus dapeste* Santos & Bellini, 2018	Seirinae	Brazil	MF716609.1
4	*Lepidocyrtinus harena* (Godeiro & Bellini, 2014)	Seirinae	Brazil	MF716617.1
5	*Seira atrolutea* (Arlé, 1939)	Seirinae	Brazil	MF716602.1
6	*Seira boneti* (Denis, 1948)	Seirinae	China	OP181099.1
7	*Seira brasiliana* (Arlé, 1939)	Seirinae	Brazil	MF716619.1
8	*Seira dowlingi* (Wray, 1953)	Seirinae	Brazil	MF716615.1
9	***Seira* cf. *dollfusi****	Seirinae	Hungary	NA
10	***Seira ferrarii***	Seirinae	Hungary	OR206048.1
11	***Seira pallidipes***	Seirinae	Hungary	OR115504.1
12	*Seira ritae* Bellini & Zeppelini, 2011	Seirinae	Brazil	MF716616.1
13	*Seira sanloemensis* Godeiro & Cipola, 2020	Seirinae	Cambodia	MT997754.1
14	*Seira tinguira* Cipola & Bellini, 2014	Seirinae	Brazil	MF716620.1
15	*Tyrannoseira bicolorcornuta* (Bellini, Pais & Zeppelini, 2009)	Seirinae	Brazil	MF716599.1
16	*Tyrannoseira raptora* (Zeppelini & Bellini, 2006)	Seirinae	Brazil	MF716610.1

Table 2. Partitioning scheme and substitution models selected by ModelFinder used for maximum likelihood analyses.

Partition	Genes	Model
1	ATP6 / NAD1 / ND4L	mtART
2	ATP8 / ND2 / ND6	mtART+F
3	COX1/ COX2 / COX3 / CYTB	mtART
4	ND3 / ND4 / ND5	mtART

et al. 2017). The partitions and models used are listed in Table 2. The Bayesian inference (BI) was performed using PhyloBayes-MPI v.1.8 (Lartillot et al. 2013), default model CAT+GTR with four rate categories, discretized gamma distribution of rates across sites, sampling every 100 generations, with the first 1000 sampled trees discarded as “burn-in”. Two Markov Chain Monte Carlo (MCMC) chains were run until the likelihood had satisfactorily converged (maxdiff < 0.3). Phylogenetic trees were visualized in Figtree v.1.3.1 (Rambaut 2016).

Results

Mitochondrial genomes of *Seira pallidipes* and *S. ferrarii* have 14,856 bp and 14,916 bp in length, respectively (Figs 2, 3). All 13 PCGs, 2 rRNAs, and 22tRNAs were found. In *S. ferrarii*, the direction of transcription was anti-clockwise, and a rare gene translocation was observed in the region ranging from 8337 bp to 8550 bp. The order of tTNAs is normally tRNA-Asn (trnN) → tRNA-Ser 1 (trnS1) → tRNA-Glu (trnE), but in *S. ferrarii*, the gene order is tRNA-Ser 1 (trnS1) → tRNA-Glu (trnE) → tRNA-Asn (trnN), meaning that a translocation happened in the position of trnN (Figs 1, 3). For this reason, *S. ferrarii* does not present the Pancrustacean ancestral gene order, like most mitogenomes of Seirinae se-

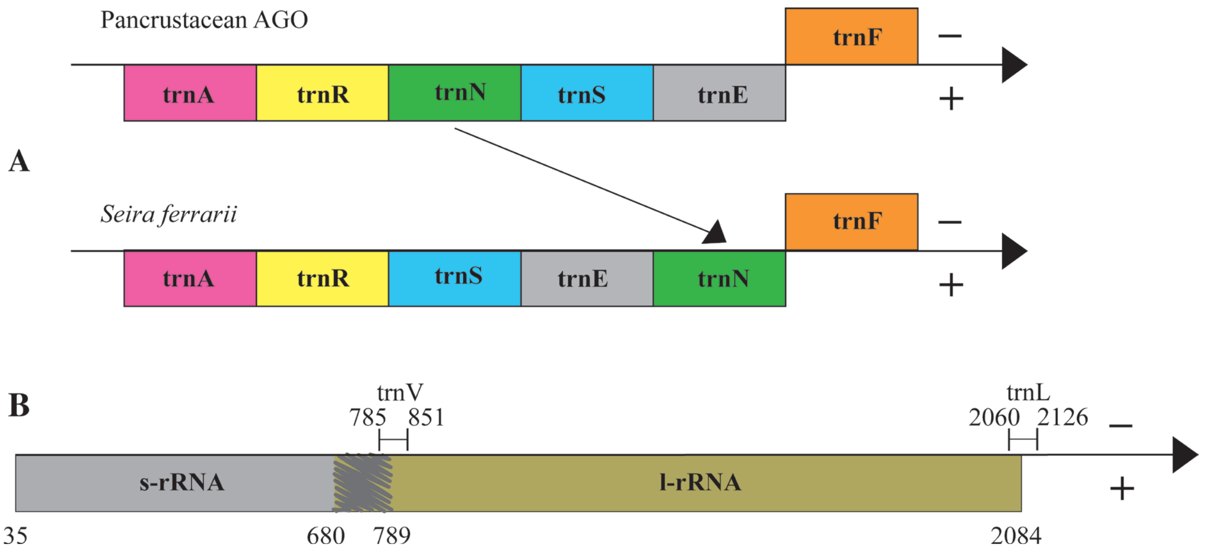

Figure 1. A gene order comparison between Pancrustacean and *Seira ferrarii* (clockwise direction) **B** region comprising rRNAs of *S. ferrarii* mitogenome (anti-clockwise direction). Scratch indicates the overlap between genes. Numbers represent the location of the genes in base pairs.

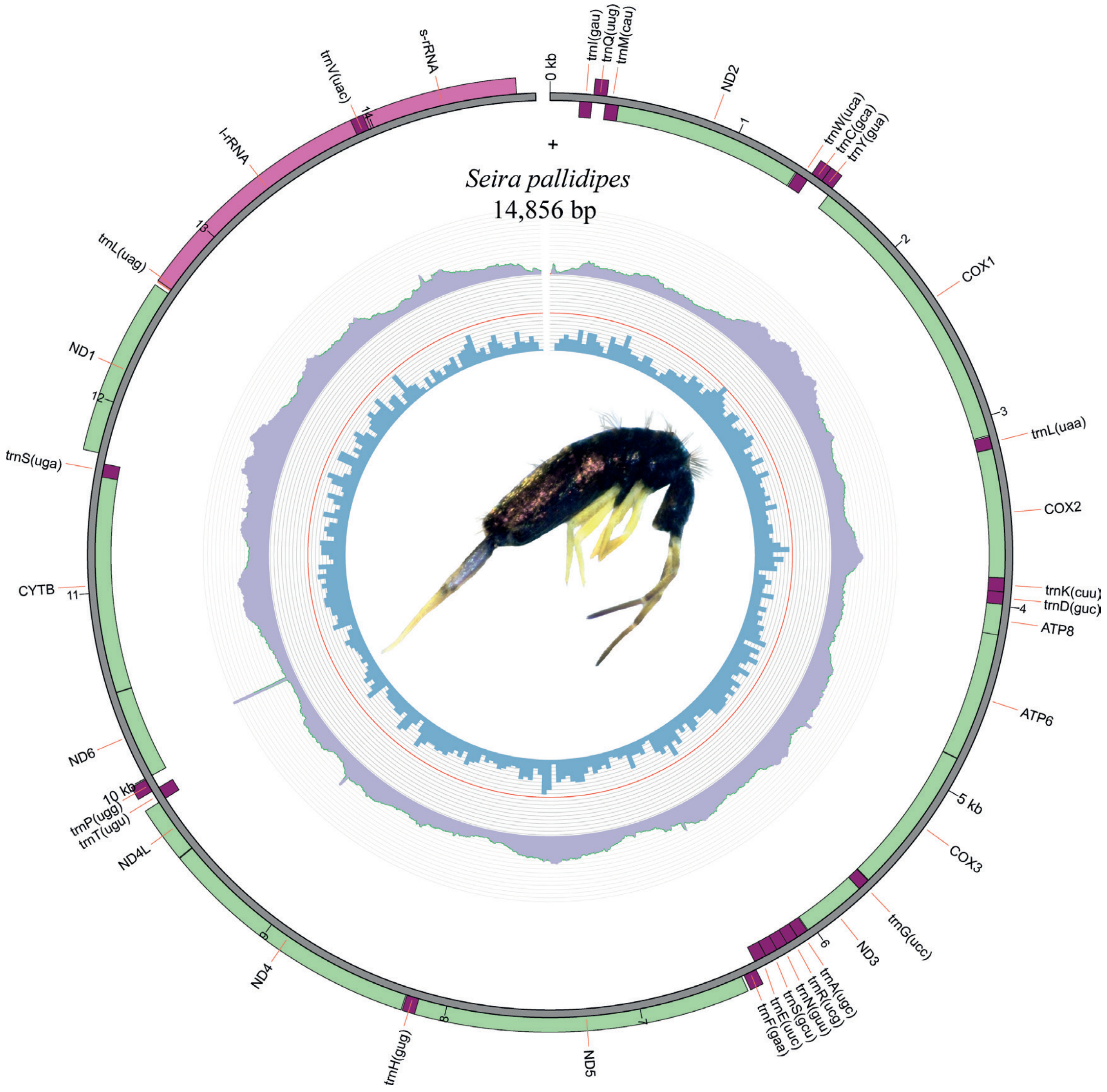

Figure 2. Circular representation of the mitogenome of *Seira pallidipes*. The innermost circle shows the GC content; the middle circle shows the reads coverage, and the outermost circle shows the gene features, rRNA, tRNA, and CDS. Photo in the center represents the original coloration of a specimen preserved in ethanol.

quenced until now, including *S. pallidipes*. Another interesting characteristic observed during the mitogenome analysis were long overlaps between the genes tRNA-Leu (trnL), 16S ribosomal RNA (l-rRNA), tRNA-Val (trnV), 12S ribosomal RNA (s-rRNA), with trnV totally inserted on the l-rRNA sequence (Figs 1–3). These overlaps were observed in all Seirinae mitogenomes sequenced to date but have never been reported before. A broader study needs to be carried out to compare the gene order of other Collembola taxa to verify whether these differences have significant evolutionary importance and phylogenetic signals.

The most common start-stop codons in *S. pallidipes* were ATT/ATG-TAA, and in *S. ferrarii* ATT/ATA-TAA (Tables 3, 4). This pattern is the most common in Collembola mitogenomes (Godeiro et al. 2022). AT-rich regions have lengths of 185bp and 421bp in *S. pallidipes* and *S. ferrarii*, respectively. The nucleotide composition of both mitogenomes presented higher AT content than other Seirinae mitogenomes, 78% in *S. pallidipes* and 80% in *S. ferrarii*.

Table 3. Gene order and features of the mitochondrial genome of *Seira pallidipes*.

***Seira pallidipes* – 14,856 bp**					
Start	**End**	**Length(bp)**	**Direction**	**Start/End code**	**Gene product [gene name]**
155	219	65	+		tRNA-Ile [trnI(gau)]
225	293	69	-		tRNA-Gln [trnQ(uug)]
290	359	70	+		tRNA-Met [trnM(cau)]
359	1354	996	+	ATG/TAA	NADH dehydrogenase subunit 2 [ND2]
1361	1429	69	+		tRNA-Trp [trnW(uca)]
1429	1492	64	-		tRNA-Cys [trnC(gca)]
1492	1558	67	-		tRNA-Tyr [trnY(gua)]
1563	3098	1536	+	ATT/TAA	cytochrome c oxidase subunit I [COX1]
3105	3171	67	+		tRNA-Leu [trnL(uaa)]
3171	3851	681	+	ATA/TAA	cytochrome c oxidase subunit II [COX2]
3853	3925	73	+		tRNA-Lys [trnK(cuu)]
3925	3991	67	+		tRNA-Asp [trnD(guc)]
3991	4158	168	+	ATT/TAA	ATP synthase F0 subunit 8 [ATP8]
4152	4832	681	+	ATG/TAG	ATP synthase F0 subunit 6 [ATP6]
4835	5623	789	+	ATG/TAA	cytochrome c oxidase subunit III [COX3]
5627	5690	64	+		tRNA-Gly [trnG(ucc)]
5690	6034	345	+	ATT/TAA	NADH dehydrogenase subunit 3 [ND3]
6039	6100	62	+		tRNA-Ala [trnA(ugc)]
6100	6164	65	+		tRNA-Arg [trnR(ucg)]
6163	6228	66	+		tRNA-Asn [trnN(guu)]
6225	6292	68	+		tRNA-Ser 1 [trnS1(gcu)]
6292	6358	67	+		tRNA-Glu [trnE(uuc)]
6370	6439	70	-		tRNA-Phe [trnF(gaa)]
6452	8146	1695	-	ATA/TAA	NADH dehydrogenase subunit 5 [ND5]
8148	8213	66	-		tRNA-His [trnH(gug)]
8218	9567	1350	-	ATG/TAA	NADH dehydrogenase subunit 4 [ND4]
9571	9843	273	-	ATT/TAA	NADH dehydrogenase subunit 4L [ND4L]
9855	9925	71	+		tRNA-Thr [trnT(ugu)]
9925	9995	71	-		tRNA-Pro [trnP(ugg)]
9997	10467	471	+	ATT/TAA	NADH dehydrogenase subunit 6 [ND6]
10470	11609	1140	+	ATG/TAA	cytochrome b [CYTB]
11608	11674	67	+		tRNA-Ser 2[trnS2(uga)]
11675	12616	942	-	ATT/TAA	NADH dehydrogenase subunit 1 [ND1]
12617	12684	68	-		tRNA-Leu [trnL(uag)]
12651	14093	1443	-		16S ribosomal RNA [l-rRNA]
13908	13975	68	-		tRNA-Val [trnV(uac)]
13988	14758	771	-		12S ribosomal RNA [s-rRNA]
14778	107	185			AT-rich region

Our phylogenetic results placed the European population of the sampled *Seira* spp. as ancestral to the Asian and American populations mostly with high SH-aLRT support and Bayesian posterior probability (Fig. 4). The geographic proximity on this scale can lead to genetic adaptation for specific conditions (e.g., different climate, habitat) and can result in a striking level of genetic differentiation within a genus, as observed in the case of *Seira*.

Table 4. Gene order and features of the mitochondrial genome of *Seira ferrarii*.

***Seira ferrarii* – 14,916 bp**					
Start	**End**	**Length (bp)**	**Direction**	**Start/End code**	**Gene name**
35	789	755	+		s-rRNA
785	851	67	+		trnV(uac)
680	2084	1405	+		l-rRNA
2060	2126	67	+		trnL(uag)
2126	3064	939	+	TTG/TAA	ND1
3055	3126	72	-		trnS2(uga)
3125	4261	1137	-	ATG/TAA	CYTB
4264	4758	495	-	ATA/TAA	ND6
4749	4815	67	+		trnP(ugg)
4815	4879	65	-		trnT(ugu)
4881	5159	279	+	ATT/TAA	ND4L
5159	6502	1344	+	ATA/TAA	ND4
6502	6568	67	+		trnH(gug)
6568	8259	1692	+	ATT/T--	ND5
8267	8333	67	+		trnF(gaa)
8337	8406	70	-		trnN(guu)
8409	8481	73	-		trnE(uuc)
8483	8550	68	-		trnS1(gcu)
8553	8618	66	-		trnR(ucg)
8618	8678	61	-		trnA(ugc)
8680	9024	345	-	ATT/TAA	ND3
9025	9086	62	-		trnG(ucc)
9092	9880	789	-	ATG/TAA	COX3
9880	10557	678	-	ATG/TAA	ATP6
10551	10712	162	-	ATA/TAA	ATP8
10713	10780	68	-		trnD(guc)
10780	10851	72	-		trnK(cuu)
10851	11534	684	-	ATA/TAA	COX2
11535	11600	66	-		trnL(uaa)
11595	13133	1539	-	ATT/TAA	COX1
13135	13199	65	+		trnY(gua)
13205	13268	64	+		trnC(gca)
13267	13333	67	-		trnW(uca)
13333	14298	966	-	ATA/TGA	ND2
14316	14384	69	-		trnM(cau)
14390	14458	69	+		trnQ(uug)
14464	14527	64	-		trnI(gau)
14530	35	421			AT-rich region

Discussion

Most of the European *Seira* species are from the Mediterranean (e.g., Gama 1964; Gruia et al. 2000; Cipola et al. 2018a). As several phylogeographic studies and reviews have pointed out, the Mediterranean region (in particular, but not exclusively, Iberia, Italia, and the Balkans) was also important refugia for thermophilic species during glacial periods (e.g., Provan and Bennett 2008;

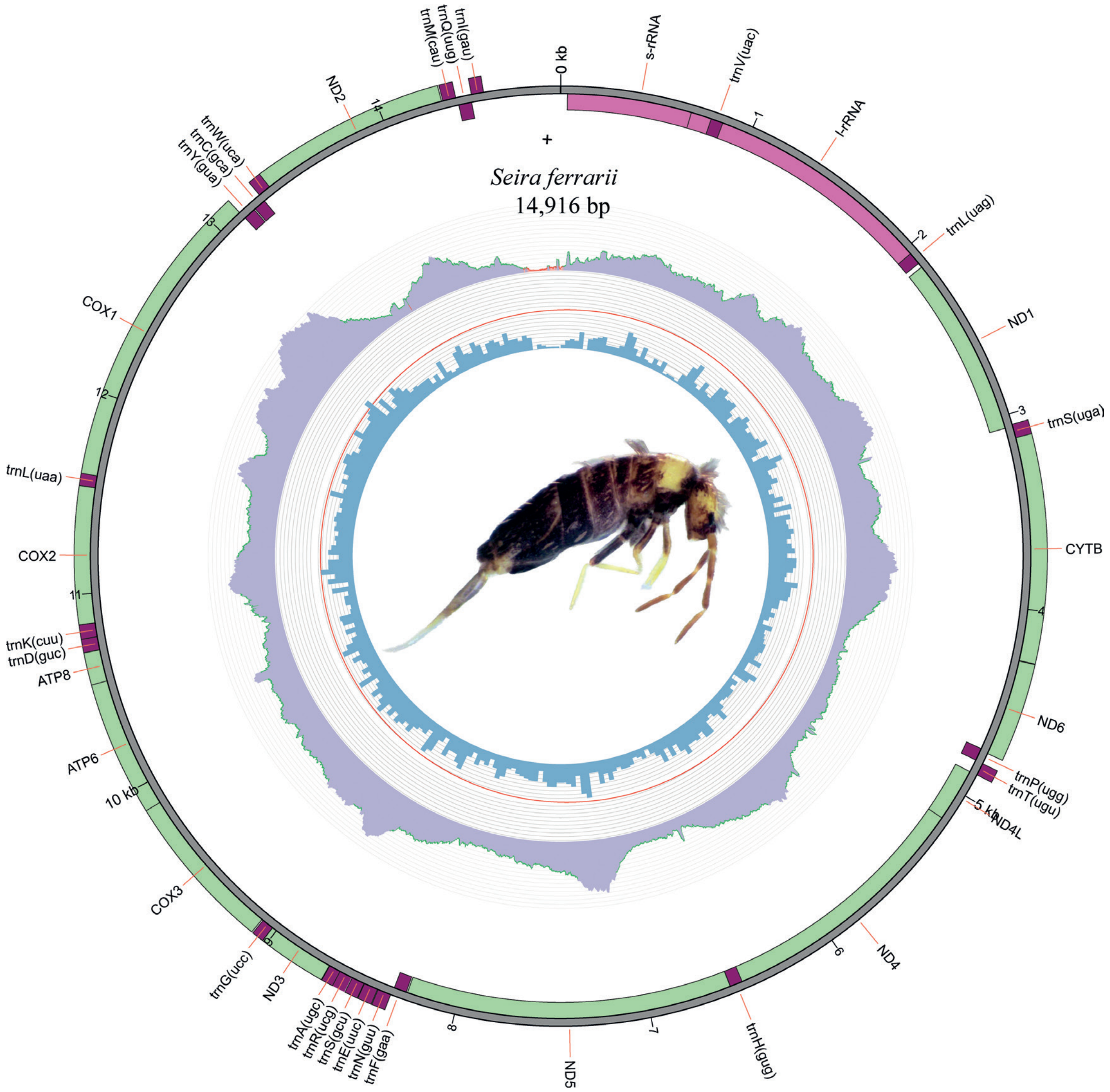

Figure 3. Circular representation of the mitogenome of *Seira ferrarii*. The innermost circle shows the GC content; the middle circle shows the reads coverage, and the outermost circle shows the gene features, rRNA, tRNA, and CDS. Photo in the center represents the original coloration of a specimen preserved in ethanol.

Cicconardi et al. 2010; Costa et al. 2013; Fiera et al. 2017). High mountains ranging to the north, like the Alps or the Pyrenees, acted as barriers to dispersal and, thus, to gene flow, resulting in long-term isolated evolution and formation of new lineages (Gómez and Lunt 2007; Hewitt 2011; Fiera et al. 2017).

Of the studied species, *S. ferrarii* has the broadest distribution area, ranging from the Iberian Penisula to the Near East in the Mediterranean and also occurring in some Central-European countries. *Seira pallidipes*, on the other hand, has a very narrow known distribution, and the data so far suggest that its range is restricted to the Carpathian Basin. Collembola species with limited distribution have either a limited dispersal ability, very narrow habitat requirements, or their distributions are limited by geographical barriers (Fiera et al. 2017). As a part of an entirely epigeic group, *S. pallidipes* is also characterized by a strong dispersal ability, and its habitat requirements are not restrictive either (the species apparently occurs in various types of grasslands). Nevertheless, due to the geographically transitional

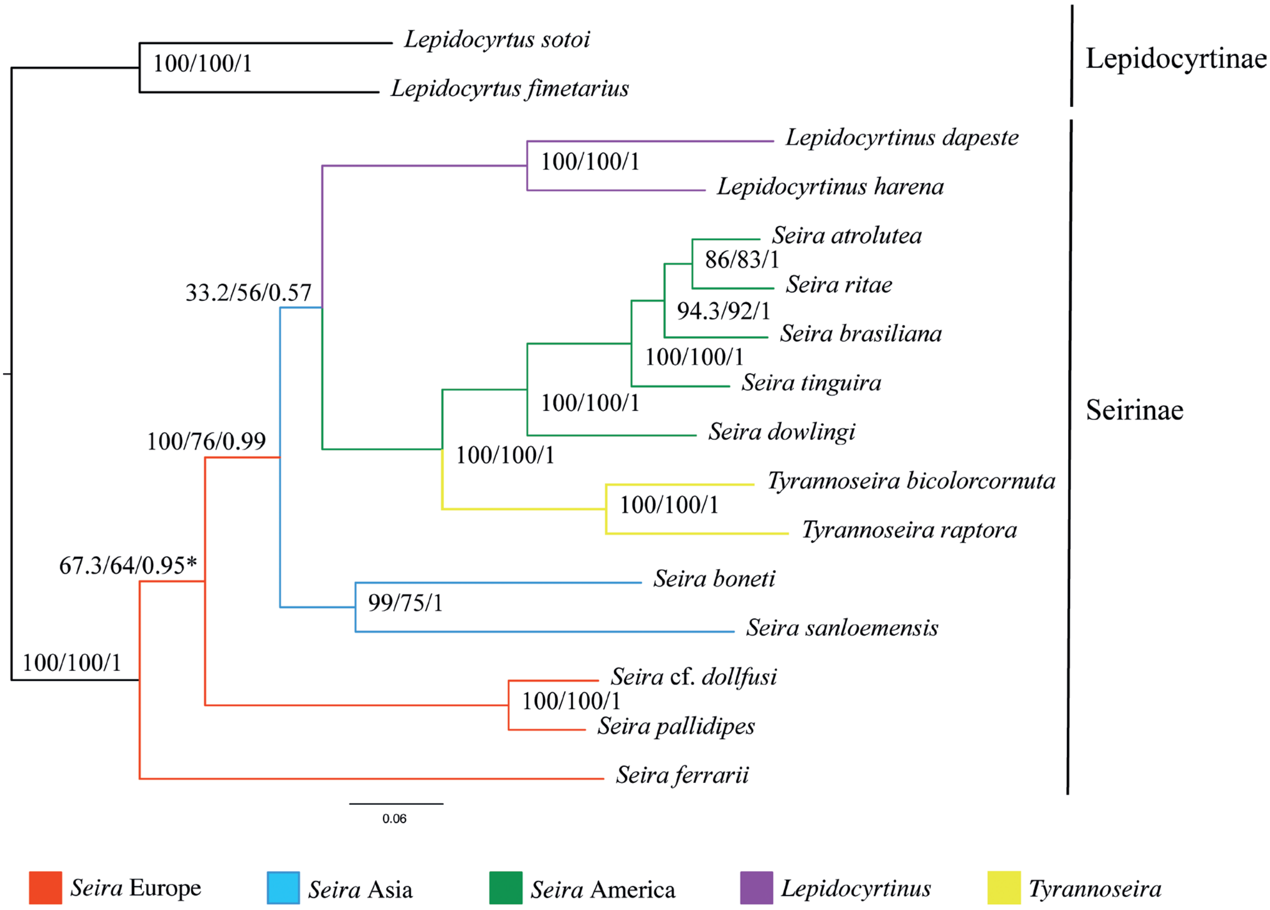

Figure 4. Phylogenetic placement of European *Seira*. Tree constructed based on maximum likelihood and Bayesian inferences (BI) from mitochondrial genomes. Numbers at the nodes represent the SH-aLRT support, bootstrap values (both for maximum likelihood), and the posterior probability (BI support), respectively. *In the BI the topology was: (*S.* cf. *dollfusi* + *S. pallidipes*) + *Seira ferrarii* + (other Seirinae).

position and the climatic conditions, the Carpathian Basin is rich in endemic and relict species (Varga 1995), and *S. pallidipes* may be one of its representatives.

Seira ferrarii represents a separate clade within Seirinae, while *S. pallidipes* with *S.* cf. *dollfusi* together form another clade (Fig. 4). The latter two species are more closely related morphologically by sharing a similar, mostly polichaetotic, dorsal macrochaetotaxy from Th II to Abd II, and are part of the "*squamoornata*-group" sensu Cipola et al. (2018a). On the other hand, *S. ferrarii* shows a clear reduction in dorsal macrochaetotaxy and thus was classified in the so-called "*domestica*-group" sensu Jacquemart (1974). This kind of division of species groups established based on the macrochaetotaxy distribution can be observed in other derived genera of Entomobryomorpha, most notably in *Lepidocyrtus* (Mateos et al. 2018; Winkler et al. 2020).

This study is part of a larger initiative to better understand the dispersion pathways of Seirinae species. Systematic research on this subfamily is impacted by the scarcity of specialists based in other continents, with the exception of South America. Despite the limited taxon sample, our phylogeny (Fig. 4) pointed out that the genus *Seira* is not monophyletic, possibly should be subdivided into more genera, and that the morphology of the subfamily needs to be more deeply investigated.

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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

NNG designed the research, performed and analysed the data. DW collected the samples. NNG, YB and DW wrote the manuscript.

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Data availability

All accession numbers and links for the data that support the findings of this study are available in the main text.

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Research Article

Four new terrestrial earthworm species from the northeast Thailand (Oligochaeta, Megascolecidae)

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Abstract

Earthworm specimens collected from Sakon Nakhon and Nakhon Phanom, northeast Thailand, were found to contain four new species in the family Megascolecidae, with one species in the genus *Metaphire* Sims & Easton, 1972, and the other three in the genus *Amynthas* Kinberg, 1867. These are herein named *Metaphire songkhramensis* Chanabun & Panha, **sp. nov.** in the sexthecal *houlleti* species group, and *Amynthas sakonnakhonensis* Chanabun & Panha, **sp. nov.**, *A. auriculus* Chanabun & Panha, **sp. nov.**, and *A. bantanensis* Chanabun & Panha, **sp. nov.** in the sexthecal *aelianus* species group. *Metaphire songkhramensis* Chanabun & Panha, **sp. nov.** occurs in dark clay soil of the oxbow lake of the river, *Amynthas sakonnakhonensis* Chanabun & Panha, **sp. nov.** occurs in wetland area, *A. auriculus* Chanabun & Panha, **sp. nov.** occurs in dark sandy loam habitats of mixed deciduous forest while the following species, *A. bantanensis* Chanabun & Panha, **sp. nov.** occurs in sandy loam habitats of paddy fields. Descriptions of the new species, including illustrations of the external and internal morphological characteristics, are provided.

Key words: *Amynthas*, *Metaphire*, new species, taxonomy, Thailand

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Introduction

Earthworms are common macro-soil invertebrates found in almost all parts of the world, in all types of habitats, such as terrestrial, aquatic, and semi-aquatic habitats. Earthworms play significant roles in the physical, chemical, and biological properties of soil (Edwards and Arancon 2022). As permanently soil-dwelling animals, their activities affect soil properties and create suitable habitats for other smaller soil flora and fauna. Moreover, earthworms can be used as bioindicators of the relative health of soil ecological systems (Fründ et al. 2011). There are approximately 6,000 species that have been recorded worldwide, and the estimated total global species diversity exceeds 8,000 species (Edwards and Bohlen 1996; Fragoso et al. 1999; Jeratthitikul et al. 2017).

Earthworms are known to decompose organic waste from houses and agricultural farms. They can improve the soil property in ecosystems by producing vermicompost and vermicompost tea that including micro-organisms without pathogenic bacteria (Wongsaroj et al. 2021), as shown for *Eisenia andrei* Bouché, 1972, *Eisenia fetida* (Savigny, 1826), *Dendrobaena veneta* (Rosa, 1886), *Perionyx excavatus* Perrier, 1872, and *Eudrilus eugeniae* (Kinberg, 1867) species. All these five species are widely cultivated due to their tolerance to a wide range of environmental conditions and have short life cycles, high reproductive rates, and good composting rates (Domínguez 2018; Heuzé et al. 2020).

In addition, humans have long used earthworms as a healthy diet or for medication and as a source of feed for other animals (Edwards and Bohlen 1996). Earthworm meal has plenty of nutrients and enzymes, which can help break down food and repair body tissue (Paoletti et al. 2003; Grdiša et al. 2009, 2013; Iannucci et al. 2009; Bamidele et al. 2016; Sun and Jiang 2017; Musyoka et al. 2018) as in several countries, including Japan, Korea, Taiwan, Myanmar, Laos, India, Singapore, South America, North America, Papua New Guinea, Australia, New Zealand and China (Price 1901; Benham 1904; Gates 1926; Carr 1951; Reynolds and Reynolds 1972; Paoletti and Dreon 2005; Grdiša et al. 2009; Cooper et al. 2012a, b; Sun and Jiang 2017).

Economically, most of the dried earthworms from Sakon Nakhon and Nakhon Phanom, both in northeast Thailand, are exported to countries like Hong Kong, China, and Taiwan, where a large number of earthworms are consumed per year as part of traditional diet and medicine. This results in a high economic return for the villages of the mentioned provinces. At the end of the rainy season and the onset of the cold season from August to December, the villagers will go out at night and early in the morning, at approximately 2.00–5.00 a.m., to paddy fields, meadows, lowland areas, freshwater islands, or to lake shores to collect with their bare hands when fresh earthworms crawling on the soil surface. In the past, after villagers collected earthworms from the field, they would wash them in water and then rewash them in water-soaked with Burmese rosewood bark (*Pterocarpus macrocarpus*) to discard the mucus. The earthworms were then dissected from the anterior to the posterior and completely sun-dried before selling them to the middlemen. Nowadays, the locals sell the fresh earthworms to a middleman who lives in Ban Tan, Nakhon Phanom. This province is a popular marketplace for dried earthworms. The middleman is now responsible for dissecting the earthworms with a machine and drying them in the sun. The price of dried earthworms ranges from 450–750 Thai baht/kg (14–23 USD/kg as of this writing), and that for fresh earthworms ranges from 20–45 Thai baht/kg (0.59–1.3 USD/kg as of this writing) (Fig. 1). Because of the aforementioned importance of earthworms in agriculture, food, traditional medicine, and their economic value, there is a vital need to know about earthworm species, their behaviors, habitats, and distribution ranges, in order to assure their future conservation and sustainable use. However, although Thailand sells dried earthworms, none of the species have been identified. In this paper, we report on the earthworm species that Thai people sell, and all four species described herein were from northeast Thailand and are newly discovered.

Figure 1. Collecting earthworms in the early morning of the winter season **A** villagers in Sakon Nakhon collect the migratory earthworms with their bare hands **B** large numbers of caught earthworms being sold to middlemen **C** *Metaphire songkhramensis* sp. nov. from Akat Amnuai, Sakon Nakhon, moving on the soil during the migration season, and **D** dried earthworms.

Material and method

Earthworm specimens from Kong Ngong from Sakon Nakhon were collected with the villagers at approximately 2.00–5.00 a.m. by hand when earthworms were crawling on the soil surface during the cold season (November). Specimens from Nong Tuet and Wut Tham Kham from Sakon Nakhon and Ban Tan from Nakhon Phanom were collected by digging and hand-sorting. All earthworm specimens are from northeastern Thailand (Fig. 2). The collected specimens were washed and killed in 30% (v/v) ethanol before being photographed and then transferred to 95% (v/v) ethanol for preservation and subsequent morphological studies. The anatomical and morphological observations were made with an ACCU-SCOPE 3075 stereo microscope. Illustrations included the body segments, distinct external characters, and internal organs. The type series are

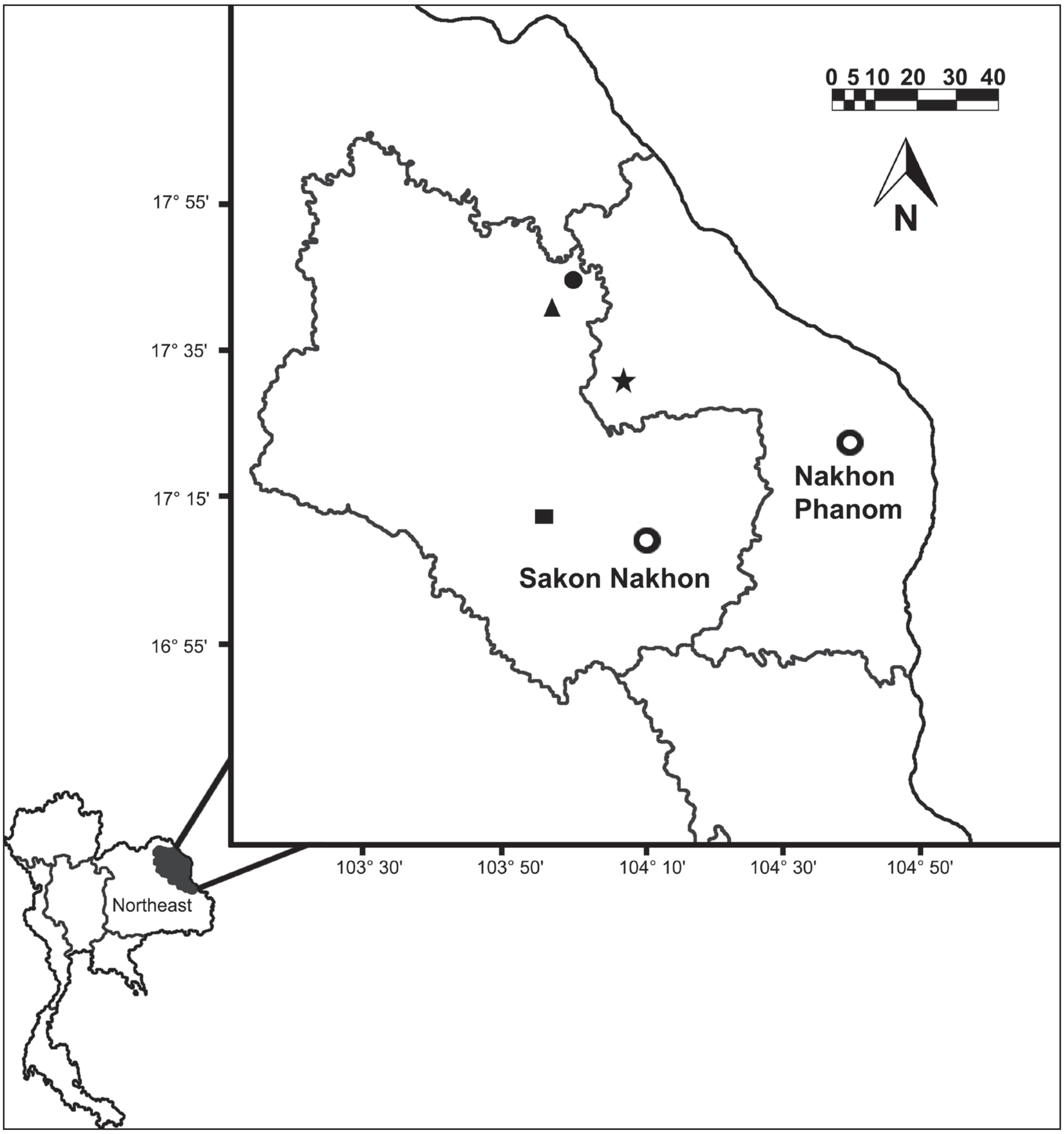

Figure 2. Map of the collecting localities of the new species described in this paper from northeast Thailand. Filled circle: *Metaphire songkhramensis* sp. nov. from Kong Ngong, Akat Amnuai, Sakon Nakhon, Thailand. Filled triangular: *Amynthas sakonnakhonensis* sp. nov. from Nong Tuet, Samakkee Pattana, Akat Amnuai, Sakon Nakhon, Thailand. Filled square: *Amynthas auriculus* sp. nov. from Wut Tham Kham, Phannanikom, Sakon Nakhon. Filled star: *Amynthas bantanensis* sp. nov. from Ban Tan, Nawah, Nakorn Phanom, Thailand.

deposited in Chulalongkorn University, Museum of Zoology, Bangkok, Thailand (**CUMZ**). Additional paratypes will be deposited in the Natural History Museum (**NHMUK**), London, and at the Biozentrum Grindel und Zoologisches Museum, University of Hamburg (**ZMH**). Specimens used in this study strictly followed the protocols approved by the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU) under approval number IACUC-KKU-32/65.

The following abbreviation used in the figures of the anatomy are as appeared in Bantaowong et al. (2011a, b): **sp**, spermathecal pores; **fp**, female pore; **gm**, genital markings; **mp**, male pores; **sc**, spermathecae; **sv**, seminal vesicles; **pg**, prostate gland; **ic**, intestinal caeca.

Taxonomy

Family Megascolecidae Rosa, 1891

Genus *Metaphire* Sims & Easton, 1972

Metaphire songkhramensis Chanabun & Panha, sp. nov.

https://zoobank.org/5E9E4FCF-FF48-4C38-9ED6-3A89627DC625
Figs 1C, 3D, 4, Table 1

Type material. ***Holotype*:** Adult specimen (CUMZ 3821), Kong Ngong, Akat Amnuai, Sakon Nakhon, northeast of Thailand, 17°45'19.4"N, 104°01'18.8"E, 148 m a.m.s.l., 8 November 2022, coll. R. Chanabun, A. Aoonkum. ***Paratypes*:** 45 adults (CUMZ 3822), 2 adults (NHMUK), 2 adults (ZMH), same collection data as for holotype.

Diagnosis. Large sized; length 229–427 mm, diameter 10–14 mm, 129–240 segments. Male pores paired in segment XVIII, each represented by a large invaginated area, conspicuous, deep holes that resemble eyes, genital markings absent. Spermathecal pores paired in intersegment 6/7–8/9. Spermathecae with large, round, and flat ampulla, with a long and thick duct, diverticulum thin, long, and zigzagged towards the distal end. No nephridia on the spermathecal duct. Holandric. Intestinal caeca simple. First dorsal pore in 12/13. Prostate gland large in XV–XXII, long, slender with U-shape duct, large paired of glandular masses on the copulatory sac, no genital marking glands.

Description of holotype. Length 333 mm, diameter 12 mm, cylindrical body with 167 segments. Preserved specimens are dark brownish on the dorsal part and pale gray on the ventral part. Setae regularly distributed around segmental equators, numbering 65 at segment VII, 85 at segment XX, and nine between male pores at segment XVIII. Setal formula AA:AB:ZZ:ZY=1:1:2:1 at segment XIII. Single female pore on the ventral side at segment XIV. Prostomium epilobic. First dorsal pore in 12/13. Clitellum annular in XIV–XVI with no dorsal pores or setae.

Male pores in copulatory pouches that have wrinkled and convex margins on ventrolateral sides of segment XVIII; pouches 5 mm apart, which is 0.13× the body circumference. These pores are large, conspicuous, deep holes that resemble eyes. There is no skin folding, and genital markings absent. Large spermathecal pores arranged in three pairs in ventral region 6/7–8/9, distance between each pair is ~ 0.26× the body circumference ventrally apart. There are no genital markings in this area.

The septa at 5/6–7/8 thick, absent in 8/9–9/10, slightly thick in 10/11–11/12, thin in 12/13–14/15, and very thin behind 15/16. Intestine begins at segment XV. Gizzard large within IX–X. Long and simple intestinal caeca in XXVII–XIX. Esophageal hearts four pairs in X–XIII. Holandric; testes and funnels in segments X and XI. Seminal vesicles are paired, one at XI–XII, the other at XIII–XIV; the hindmost pair is larger. Prostate glands large, located in segments XV–XXII, and divided into several lobules. Prostate duct long, slender with U-shape, and with large paired glandular masses on the copulatory sac at segments XVII–XVIII and XIX–XX.

The ovaries are located in segment XIII. Three pairs of spermathecae present on VII–IX. Ampulla large, round, and flat-shaped, with long and thick duct

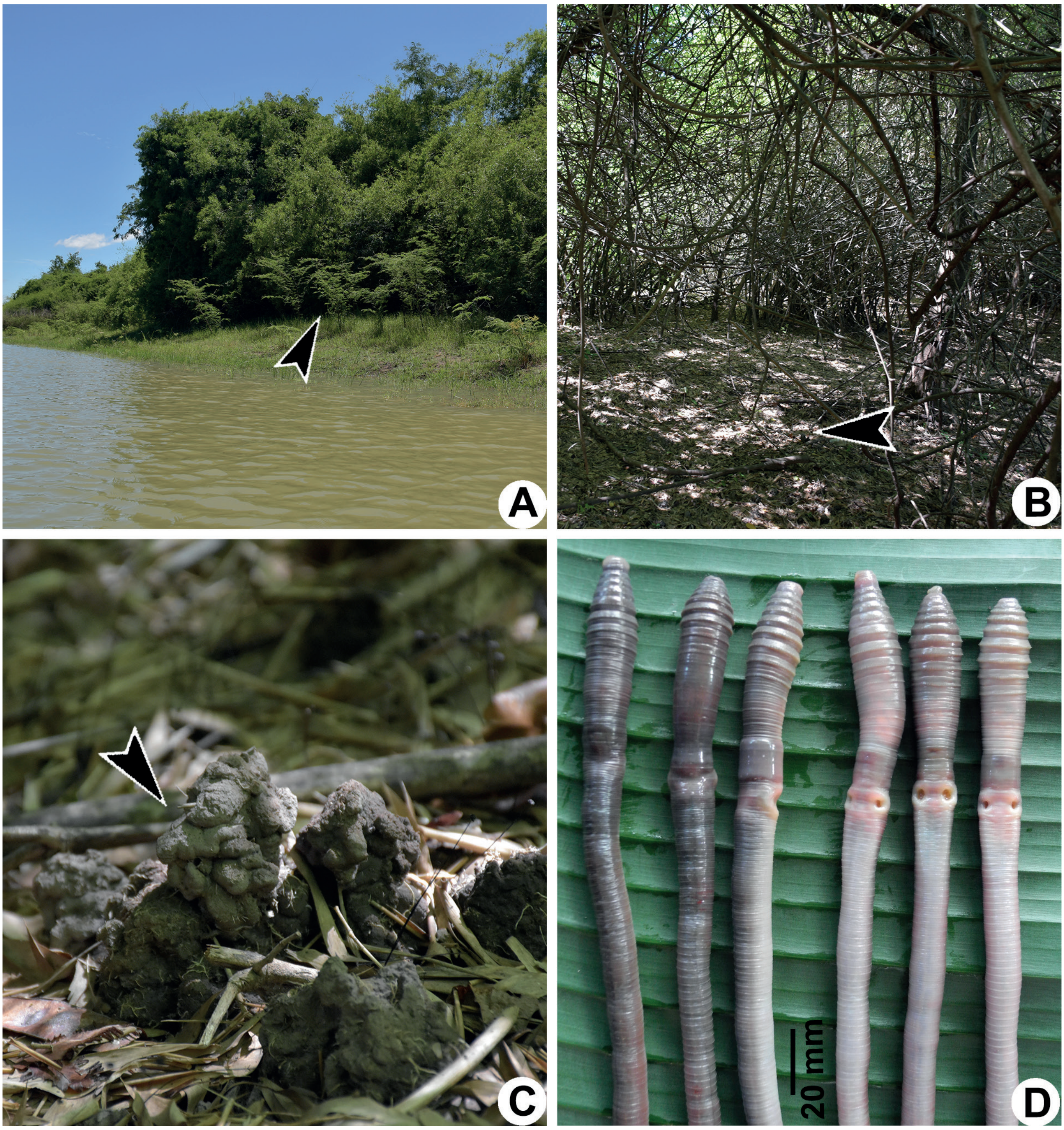

Figure 3. Photographs showing the **A** type locality of *Metaphire songkhramensis* sp. nov. from Kong Ngong, Akat Amnuai, Sakon Nakhon, Thailand **B** habitat of the new species covered with small bamboo and small shrubs **C** casts of the new species, and **D** coloration of newly collected paratype (CUMZ 3822) after the first preservation step in 30% (v/v) ethanol.

that can be clearly seen from the ampulla. Diverticulum is thin, long, and zigzagged towards the distal end.

Variation. Forty-nine paratypes ranged in size from 229–427 mm (318.75 ± 40.32 mm) body length with 129–240 (159.29 ± 19.77) segments.

Etymology. This new species was named after its type locality, Songkhram River.

Distribution. This species is known only from the type locality.

Remarks. *Metaphire songkhramensis* is a new species of sexthecal earthworm without postclitellar genital markings. It has three pairs of spermathecal

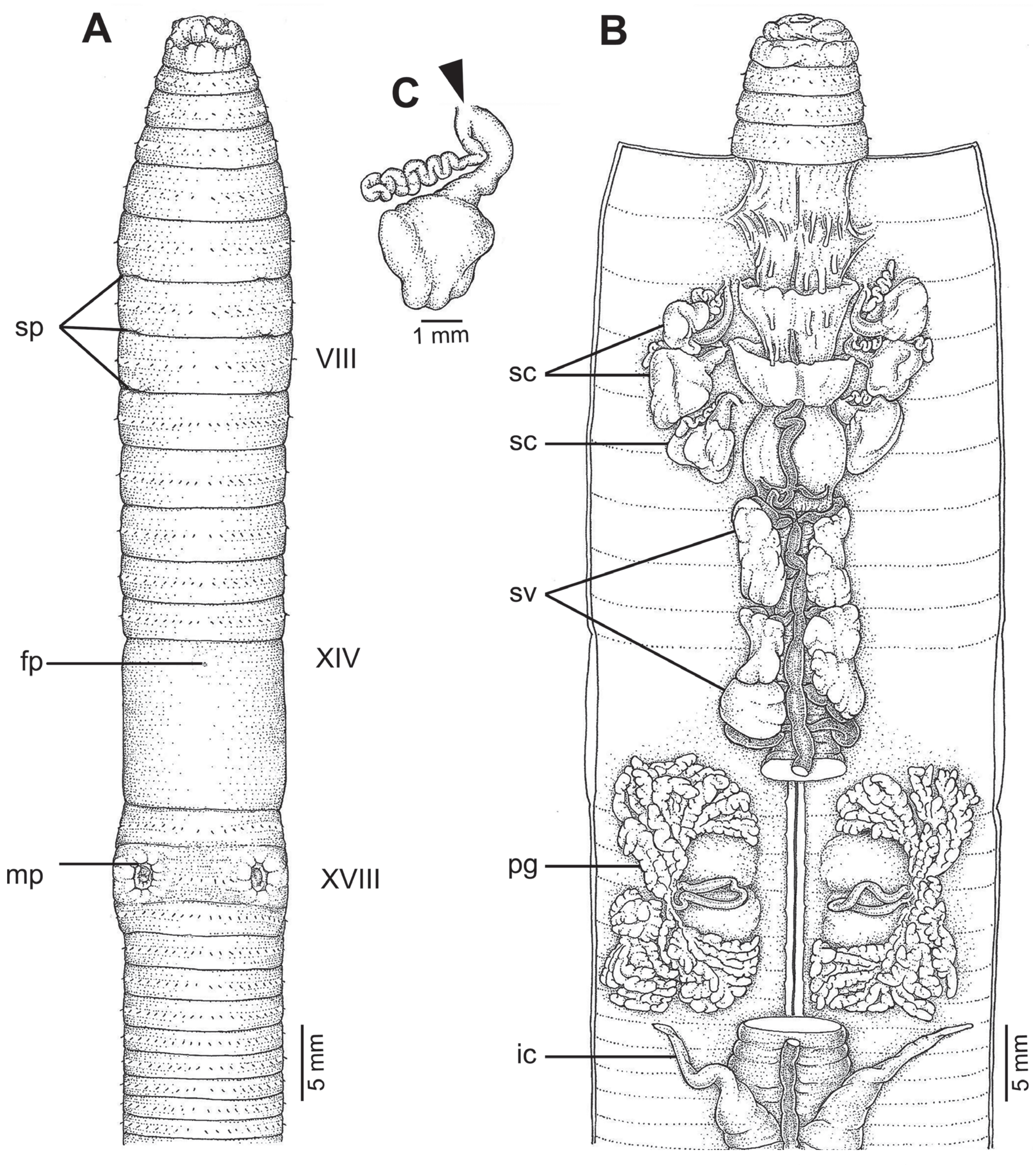

Figure 4. External and internal morphology of *Metaphire songkhramensis* sp. nov., holotype (CUMZ 3821) **A** external ventral view **B** internal dorsal view, and **C** spermatheca with location of spermathecal pore arrowed.

pores in intersegmental furrows 6/7–8/9. This species keys to the *houlleti* species group, which has more than 40 species (Sims and Easton 1972). Below, comparison of the new species with other species found in different regions in the *houlleti* species group: *M. umbraticola* (Gates, 1932) and *M. quadrigemina* (Gates, 1932) from Myanmar, *M. amplectens* (Michaelsen, 1934), *M. dawydovi* (Michaelsen, 1934), *M. acampanulata* Nguyen, 2022 from Vietnam (Nguyen et al. 2022), *M. bindjeyensis* (Michaelsen, 1899) from Sumatra, and *M. hijauensis* Ng & Panha, 2018 from Malysia (Ng et al. 2018) are stated.

Metaphire songkhramensis sp. nov. differs from *M. umbraticola* and *M. quadrigemina* from Myanmar by having smaller size than the new species. *Metaphire umbraticola* which has a body size that ranges from 115–122 mm, a diameter of 6–7 mm, with 125–135 segments, *M. quadrigemina* which has a size that ranges from 64–72 mm by 3–4 mm, with 115, while the new species ranges from 229–427 mm, a diameter of 10–14 mm, with 129–240 segments.

Metaphire songkhramensis sp. nov. differs from *M. amplectens*, *M. dawydovi*, and *M. acampanulata* from Vietnam through the body size and spermathecae. *Metaphire songkhramensis* sp. nov. differs from *M. amplectens* by *M. amplectens* has a smaller body size than the new species (body length 44–52 mm, diameter 2½–3½ mm with 90–112 segments). *Metaphire songkhramensis* sp. nov. differs from *M. dawydovi* in that *M. dawydovi* has a smaller size (body length 275 mm, diameter 7 mm, with 160 segments), and flask-shaped ampullae with small diverticulum, whereas the new species has a larger body size with large, round, and flat-shaped ampullae with thin, long, and zigzag diverticulum. *Metaphire songkhramensis* sp. nov. differs from *M. acampanulata* from Vietnam in body size and spermathecae; the latter has a smaller size than the new species (body length 77–198 mm, diameter 4.03–6.91 mm with 56–144 segments), and ampulla mushroom-shaped with grooves on the surface while the new species has a larger size and has large, round, and flat-shaped of ampulla.

Metaphire songkhramensis sp. nov. similar to *M. bindjeyensis* from Sumatra in body size length but *M. bindjeyensis* obclavate ampullae with zigzag diverticulum, while the new species has a large, round, and flat-shaped ampulla with a thin, long, and zigzagged diverticulum. *Metaphire songkhramensis* sp. nov. differs from *M. hijauensis* from Malysia by the latter species has a smaller size than the new species (body size range 66–87 mm, diameter of 2.8–3.1 mm, with 101–120 segments), and the first dorsal pores in 10/11 while the new species has first dorsal pores in 12/13.

In Thailand, five species have been reported, *M. houlleti* (Perrier, 1872), *M. virgo* (Beddard, 1900), *M. perichaeta* (Beddard, 1900), *M. khaochamao* Bantaowong & Panha, 2016, and *M. khaoluangensis* Bantaowong & Panha, 2016. *Metaphire songkhramensis* sp. nov. can be distinguished from this group of five as follows: *M. houlleti* and *M. virgo* have first dorsal pores in 11/12 while the new species has first dorsal pores in 12/13, and a large, round, and flat-shaped ampulla with a thin, long, and zigzagged diverticulum (spherical and small sac in *M. houlleti* and *M. virgo*, respectively). The new species does not have the contorted diverticulum stalk enveloped in connective tissue as found in *M. houlleti*, and also lacks the typhlosole present in *M. houlleti*. *Metaphire virgo* has a spermathecal diverticulum stalk with multiple folds, while the new species is absent. *Metaphire houlleti* and *M. virgo* have genital markings bearing stalked glands in association with spermathecae and copulatory sacs, while the new species lacks them. This new species differs from *M. perichaeta* in that *M. perichaeta* has a smaller size, inverted pear-shaped spermathecae with coiled diverticulum, and the last hearts in XII (Beddard 1900; Stephenson 1932). *Metaphire songkhramensis* sp. nov. differs from *M. khaochamao* and *M. khaoluangensis*, through the male opening, body size, and spermathecae. *Metaphire khaochamao* has smaller body size (body size range 100–148 mm, with 110–120 segments), the male field including the lateral slits associated with the male pores, the absence of setae between male pores, and an elliptic

Table 1. Morphological characteristics of the *Metaphire houlleti* species group in Thailand. The comma is used to separate body length and width. Data for *M. khaoluangensis* Bantaowong & Panha, 2016, *M. khaochamao* Bantaowong & Panha, 2016, and *M. perichaeta* (Beddard, 1900) are from Bantaowong et al. (2016), *M. houlleti* (Perrier, 1872) are from Gates (1972), and *M. virgo* (Beddard, 1900) are from Stephenson (1932).

Characters	*M. songkhramensis* sp. nov.	*M. khaoluangensis*	*M. khaochamao*	*M. perichaeta*	*M. virgo*	*M. houlleti*
Body length, width (mm)	229–427, 10–14	220, 10	166, 6	160, 5	152–157, 5	92–200, 4–7
Segment number	129–240	119	118	118	129	92–140
First dorsal pore	12/13	12/13	12/13	12/13	11/12	11/12
Spermathecae	large, round, flattened	elongate	elliptic	pear shape	small	large sac
Diverticulum	thin, long	slender	slender	zigzag	tubular	looped
Prostate gland	XV–XXII	XVI–XIX	XVII–XXIII	XVII–XIX	XVII–XVIII	XVI–XXI
Genital marking gland	absent	absent	absent	absent	absent	stalk
Copulatory sac	present	present	absent	present	present	present
Intestinal caeca	XXVII–XIX	XXVII–XXI	XXVII–XXIII	XXVII–XXIV	XXVII–XXV	XXVII–XXII
Type locality	Thailand	Thailand	Thailand	Thailand	Thailand	India

ampulla with a short duct. *Metaphire khaoluangensis* has smaller body size (body size range 130–265 mm, with 113–131 segments), a secondary male opening with puckered margin, an elongate ampulla, and a sac-like duct, whereas the new species has a larger size with setae between male pore, a large, round, and flat-shaped ampulla, and a thin, long, and zigzag diverticulum. A comparison of characters between *M. songkhramensis* sp. nov. and other related species is presented in Table 1.

Genus *Amynthas* Kinberg, 1867

Amynthas sakonnakhonensis Chanabun & Panha, sp. nov.

https://zoobank.org/361CB72B-D59A-47E3-90BC-B7945E33F3BD
Figs 5, 6, Tables 2, 5

Type material. ***Holotype*:** Adult specimen (CUMZ 3823), Nong Tuet, Samakkee Pattana, Akat Amnuai, Sakon Nakhon, northeast of Thailand, 17°40'54.1"N, 103°59'50.6"E, 149 m a.m.s.l., 6 October 2019, coll. R. Chanabun, A. Aoonkum. ***Paratypes*:** 25 adults (CUMZ 3824), 2 adults (NHMUK), 2 adults (ZMH), same collection data as for holotype.

Diagnosis. Medium-large size, length 134–238 mm, diameter 6–9 mm, 85–162 segments. Male pores paired in segment XVIII, each surrounded by four genital markings. Paired spermathecal pores in intersegments 6/7–8/9. Spermathecae large oval sacs of the ampulla, with stout and short duct, it is less than ampullar in length. Diverticulum long and zigzagged at the beginning and dilated towards the distal end, a baton-like chamber. Intestinal origin at XV. Intestinal caeca simple. Holandric. First dorsal pore in 12/13. Prostate glands large in segments XV–XXII, its ducts long and slender, surrounded by a large, long sessile glandular mass on the body wall.

Description of holotype. Length 217 mm, diameter 9 mm, body cylindrical with 156 segments. Preserved specimens are dark brownish on the dorsal

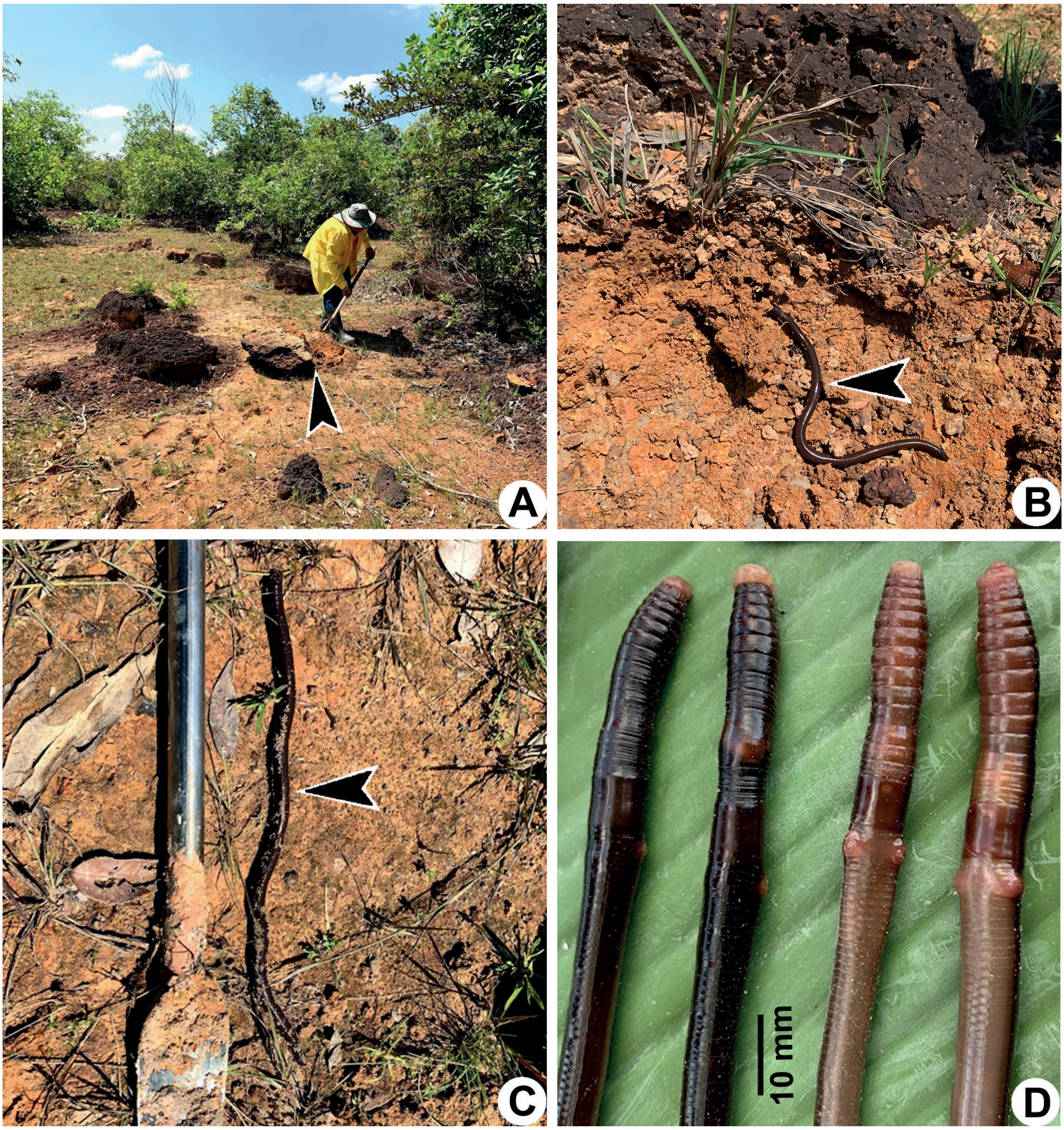

Figure 5. Photographs showing the **A** type locality of *Amynthas sakonnakhonensis* sp. nov. from Nong Tuet, Samakkee Pattana, Akat Amnuai, Sakon Nakhon, Thailand **B** living specimen under the stone near casts **C** coloration of a living paratype, and **D** coloration of a newly collected paratype (CUMZ 3824) after the first preservation step in 30% (v/v) ethanol.

part and pale gray on the ventral part. Setae regularly distributed around the segmental equators, numbering 49 at segment VII, 72 at segment XX, and 10 between male pores at segment XVIII. Setal formula is AA:AB:ZZ:ZY = 1:1:2:1 at XIII. Single female pore on the ventral side at segment XIV. Prostomium epilobic. First dorsal pore in 12/13. Clitellum annular in XIV–XVI with no dorsal pores or setae.

Male pores paired on the ventral side at XVIII, in a setal line ~ 5 mm and 0.18× the circumference apart ventrally. Male pores superficial, convex, and

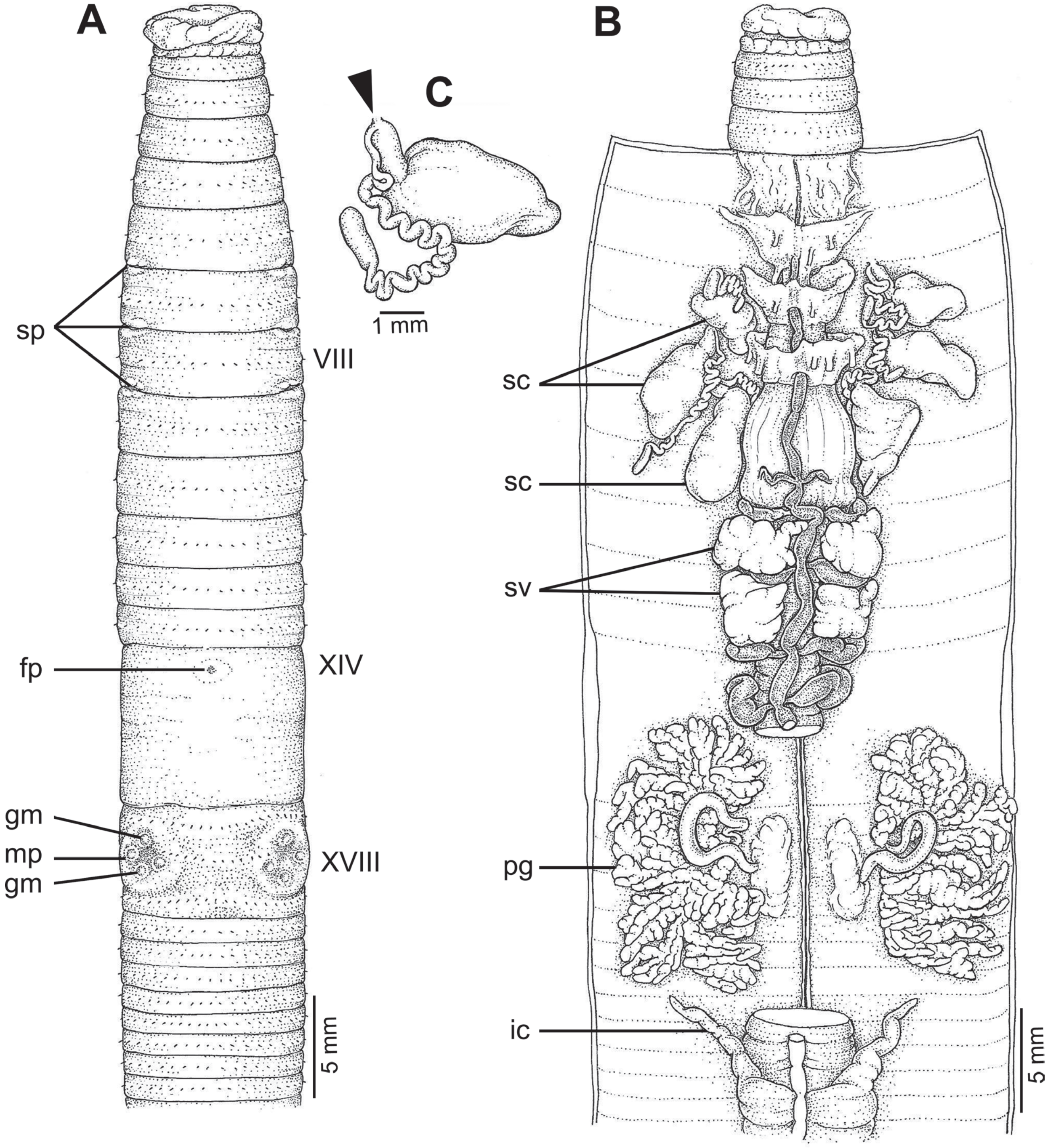

Figure 6. External and internal morphology of *Amynthas sakonnakhonensis* sp. nov., holotype (CUMZ 3823) **A** external ventral view **B** internal dorsal view, and **C** spermatheca with location of spermathecal pore arrowed.

surrounded by four genital markings each. Large spermathecal pores three pairs in 6/7–8/9. The distance between each pair ~ 0.27× the circumference ventrally apart, with no genital markings on this area.

The septa at 5/6–7/8 thick, absent in 8/9–9/10, slightly thick in 10/11–11/12, thin in 12/13–14/15, and very thin behind 15/16. Gizzard large in IX–X. Intestine begins in segment XV. Long and simple intestinal caeca in XXVII–XXII. Esophageal hearts four pairs in segment X–XIII. Holandric; testes and funnels in X and XI. Seminal vesicles are paired, one in XI, the other one in XII–XIII. The prostate glands are well developed, located in XV–XXII, and divided into several lobules. The prostate duct is long and slender, surrounded by a large, long sessile glandular mass on the body wall on segments XVII–XIX.

Ovaries located in segment XIII. Three pairs of spermathecae present on VII–IX. Spermathecae large oval sacs of the ampulla, with stout and short duct, it is less than ampullar in length. Diverticulum long and zigzagged at the beginning and dilated towards the distal end, a baton-like chamber.

Variation. Twenty-nine paratypes range in body length from 134–238 mm (197.33 ± 24.52 mm), with 85–162 (142.96 ± 21.25) segments (Table 2).

Etymology. The name *sakonnakhonensis* was derived from the province Sakon Nakhon.

Distribution. This species is known only from the type locality.

Remarks. *Amynthas sakonnakhonensis* sp. nov. is sexthecal with spermathecal pores in 6/7–8/9. This species keyed to the *sieboldi* species group (Sims and Easton 1972). After that, Easton (1981) moved *A. sieboldi* (Horst, 1883) to the genus *Metaphire*. Then James et al. (2005) investigated and proposed the *aelianus* species group after *A. aelianus* (Rosa, 1892), to replace the *sieboldi* species group name (James et al. 2005; Bantaowong et al. 2014).

The *aelianus* species group consists of more than 60 species (Sims and Easton 1972; Tsai et al. 1999, 2010; Shen et al. 2003; James et al. 2005; Blakemore 2011; Bantaowong et al. 2014). In the following, we compared the new species with regional species in the *aelianus* species group: *A. osmastoni* (Michaelsen, 1907) from Myanmar, *A. burchardi* Michaelsen, 1899 from Sumatra, *A. monsoonus* James, Shih & Chang, 2005 and *A. huangi* James, Shih & Chang, 2005 from Taiwan (James et al. 2005).

Amynthas sakonnakhonensis sp. nov. is similar to *A. osmastoni* from Myanmar and *A. burchardi* from Sumatra in the body size, but its easily distinguished by the new species having no median genital markings on segment VIII while *A. osmastoni* has them, the new species having no mid-ventral group of small circular papillae on segment XVIII while *A. burchardi* has them. *Amynthas sakonnakhonensis* sp. nov. can be distinguished from *A. monsoonus* and *A. huangi* from Taiwan in that the new species has a larger body size than these two species, *A. monsoonus* has genital markings in segments VII–IX. *Amynthas monsoonus* and *A. huangi* have no genital markings in the male pores region, whereas the new species contains these characters.

In Thailand, only four species within this species group have been reported from northeastern Thailand. These are *A. fucosus* (Gates, 1933), *A. siam* Blakemore, 2011, *A. arenulus* Bantaowong & Panha, 2014, *A. longicaeca* Bantaowong & Panha, 2014. *Amynthas sakonnakhonensis* sp. nov. is easily distinguished from *A. fucosus* from Burma and *A. siam* from Thailand because these species have body diameters of 6 mm and 3 mm, respectively, while the new species are wider at ~ 8–10 mm. *Amynthas fucosus* has two pairs of genital markings at 17/18 and 18/19, and *A. siam* has a single pair between the male pores, while the new species has four genital markings surrounded by male pores. This new species differs from *A. arenulus* in that the latter has a larger size and lacks genital markings in the male pore area. *Amynthas arenulus* has a large transverse elliptical disc surrounded by an elevated rim with an indistinct male aperture located at the outer edge of each poropore, while the new species has four genital markings surrounding the male pore area. The new species

Table 2. Holotype and paratype dimensions and other morphological characteristics of *Amynthas sakonnakhonensis* sp. nov.

Characters	Body length (mm)	Number of segments	Location of genital markings	Number of genital markings	First dorsal pore	Prostate glands	Intestinal caeca	Genital marking gland
Holotype CUZM 3823	217	156	XVIII	4	12/13	XV–XXII	XXVII–XXII	present
Paratype CUMZ 3824								
1	190	156	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
2	238	154	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
3	204	150	XVIII	4	12/13	XVI–XXII	XXVII–XXII	present
4	183	156	XVIII	4	12/13	XV–XXI	XXVII–XXII	present
5	172	154	XVIII	4	12/13	XV–XXII	XXVII–XXII	present
6	192	158	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
7	184	153	XVIII	4	12/13	XV–XXIII	XXVII–XXI	present
8	232	153	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
9	201	152	XVIII	4	12/13	XV–XXI	XXVII–XXI	present
10	217	157	XVIII	4	12/13	XV–XXIII	XXVII–XXI	present
11	144	110	XVIII	4	12/13	XV–XXIII	XXVII–XXI	present
12	134	85	XVIII	4	12/13	XV–XXII	XXVII–XXII	present
13	199	159	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
14	217	156	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
15	204	127	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
16	226	159	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
17	208	109	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
18	196	112	XVIII	4	12/13	XV–XXII	XXVII–XXII	present
19	212	156	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
20	191	158	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
21	166	112	XVIII	4	12/13	XV–XXII	XXVII–XXI	present
22	198	127	XVIII	4	12/13	XV–XXI	XXVII–XXI	present
23	176	121	XVIII	4	12/13	XVI–XXIII	XXVII–XXI	present
24	209	162	XVIII	4	12/13	XV–XXII	XXVII–XXII	present
25	193	150	XVIII	4	12/13	XV–XXI	XXVII–XXII	present
26	225	158	XVIII	4	12/13	XV–XXI	XXVII–XXI	present

has body dimensions similar to *A. longicaeca* but can be distinguished from *A. longicaeca* by the fact that *A. longicaeca* has small crescent-shaped genital markings in the male pore region and spermathecae consist of large oval ampullar with short duct, diverticulum has a small ovate knob and nephridia are present on diverticulum, while the new species has none of these characters.

Amynthas sakonnakhonensis sp. nov. is similar to *A. bantanensis* sp. nov. in body size but can be distinguished by the *A. sakonnakhonensis* sp. nov. having large oval sacs of the ampulla, with stout and short duct, with long and zigzag diverticulum, and has large, long sessile glandular mass on the body wall region of XVII–XIX, as opposed to *A. bantanensis* sp. nov., which has elongated sacs of the ampulla with slender and long stalks, and has no such glandular mass. *Amynthas sakonnakhonensis* sp. nov. differs from *A. auriculus* sp. nov. by the latter has a bit larger size, male pores located in between small three genital markings, each male pore region somewhat ear-shaped after fixation, and consists of a large, elongated ampullar with very short and zigzag diverticulum; however, *A. sakonnakhonensis* sp. nov. has four genital markings surrounding the male pore area, and has a large oval sacs of the ampulla, with long and zigzag diverticulum. *Amynthas sakonnakhonensis* sp. nov. different genital marking pattern from the other new *Amynthas* species here, and the other related species is presented in Table 5.

Amynthas auriculus **Chanabun & Panha, sp. nov.**

https://zoobank.org/2CA10F55-D79F-4D5C-972F-22FA890B6340

Figs 7, 8, Tables 3, 5

Type material. ***Holotype*:** Adult specimen (CUMZ 3825) Wut Tham Kham, Phannanikom, Sakon Nakhon, northeast of Thailand, 17°13'06.2"N, 103°54'00.7"E, 403 m a.m.s.l., 9 October 2019, coll. R. Chanabun, A. Aoonkum. ***Paratypes*:** 2 adults (CUMZ 3826), 2 adults (NHMUK), same collection data as for holotype.

Diagnosis. Medium-large size, length 184–267 mm, diameter 9–11 mm, 95–151 segments. Paired male pores at segment XVIII, surrounded by three genital papillae, each male pore region somewhat ear-shaped after fixation. Paired spermathecal pores in intersegments 6/7–8/9. Spermathecae are large, elongated sacs, with slender stalks of the ampulla, and diverticulum very short and zigzagged from its origin until the end. Holandric. Intestinal caeca simple. First dorsal pore in 12/13. Prostate glands large in XVI–XXIV, the prostate duct large, slender, smooth, and a bit zigzagged at the end, surrounded by a large sessile glandular mass on the body wall.

Description of the holotype. Length 267 mm, diameter 10 mm, body cylindrical. 139 segments. Preserved specimens are dark brownish on the dorsal part and pale gray on the ventral part. Setae are regularly distributed around segmental equators, numbering 27 at segment VII, 62 at segment XX, and 20 between male pores at segment XVIII. Setal formula AA:AB:ZZ:ZY = 1:1:2:1 at XIII. At segment XIV, a single female pore mid-ventrally. Prostomium epilobic. First dorsal pore in 12/13. Clitellum annular in segments XIV–XVI with no dorsal pores or setae.

Male pores paired, superficial, convex, located on ventral side of segment XVIII, in between small genital markings; each male pore region somewhat ear-shaped after fixation in ethanol. Male pores ~ 7 mm apart, distance 0.29× the body circumference. Single female pore at XIV. At segment XVIII, each male pore is lined by two anterior genital markings above the setal line, and one posterior genital marking below the setal line. Spermathecal pores three pairs in 6/7–8/9, distance between each pair is ~ 0.37× the circumference ventrally, there are no genital markings in this area.

The septa are thick in 5/6–7/8; absent in 8/9–9/10; slightly thick in 10/11–11/12; thin in 12/13–14/15; and very thin behind 15/16. Gizzard large at IX–X. Intestine begins at segment XV. Long and simple intestinal caeca in XXVII–XXI. Esophageal hearts four pairs in segment X–XIII. Holandric; testes and funnels in X and XI. Seminal vesicles paired in XI–XII. Prostate glands divided into several lobules, in XVI–XXIV, large and well-developed. Prostate duct large, slender,

Figure 7. Photographs showing the **A** type locality of *Amynthas auriculus* sp. nov. from Wut Tham Kham, Phannanikom, Sakon Nakhon, Thailand **B** casts of the new species **C** coloration of a living paratype, and **D** coloration of a newly collected paratype (CUMZ 3826) after the first preservation step in 30% (v/v) ethanol.

smooth, and a bit zigzagged, surrounded by a large sessile glandular mass on the body wall on segments XVIII–XXI.

Ovaries in segment XIII. Three pairs spermathecae in VII–IX. Spermathecae large, elongated sacs with slender stalks of the ampulla, diverticulum very short and zigzagged from its origin until the end.

Variation. Four paratypes range in size from 184–224 mm (218.20 ± 30.80 mm) body length with 95–151 (128.40 ± 22.65) segments (Table 3).

Table 3. Holotype and paratype dimensions and other morphological characteristics of *Amynthas auriculus* sp. nov.

Characters	Body length (mm)	Number of segments	Location of genital markings	Number of genital markings	First dorsal pore	Prostate glands	Intestinal caeca	Genital marking gland
Holotype CUZM 3825	267	139	XVIII	3	12/13	XVI–XXIV	XXVII–XXI	present
Paratype CUMZ 3826								
1	224	151	XVIII	3	12/13	XV–XXV	XXVII–XXI	present
2	207	116	XVIII	3	12/13	XVI–XXIV	XXVII–XXI	present
3	209	141	XVIII	3	12/13	XVI–XXIV	XXVII–XXI	present
4	184	95	XVIII	3	12/13	XVI–XXIII	XXVII–XXI	present

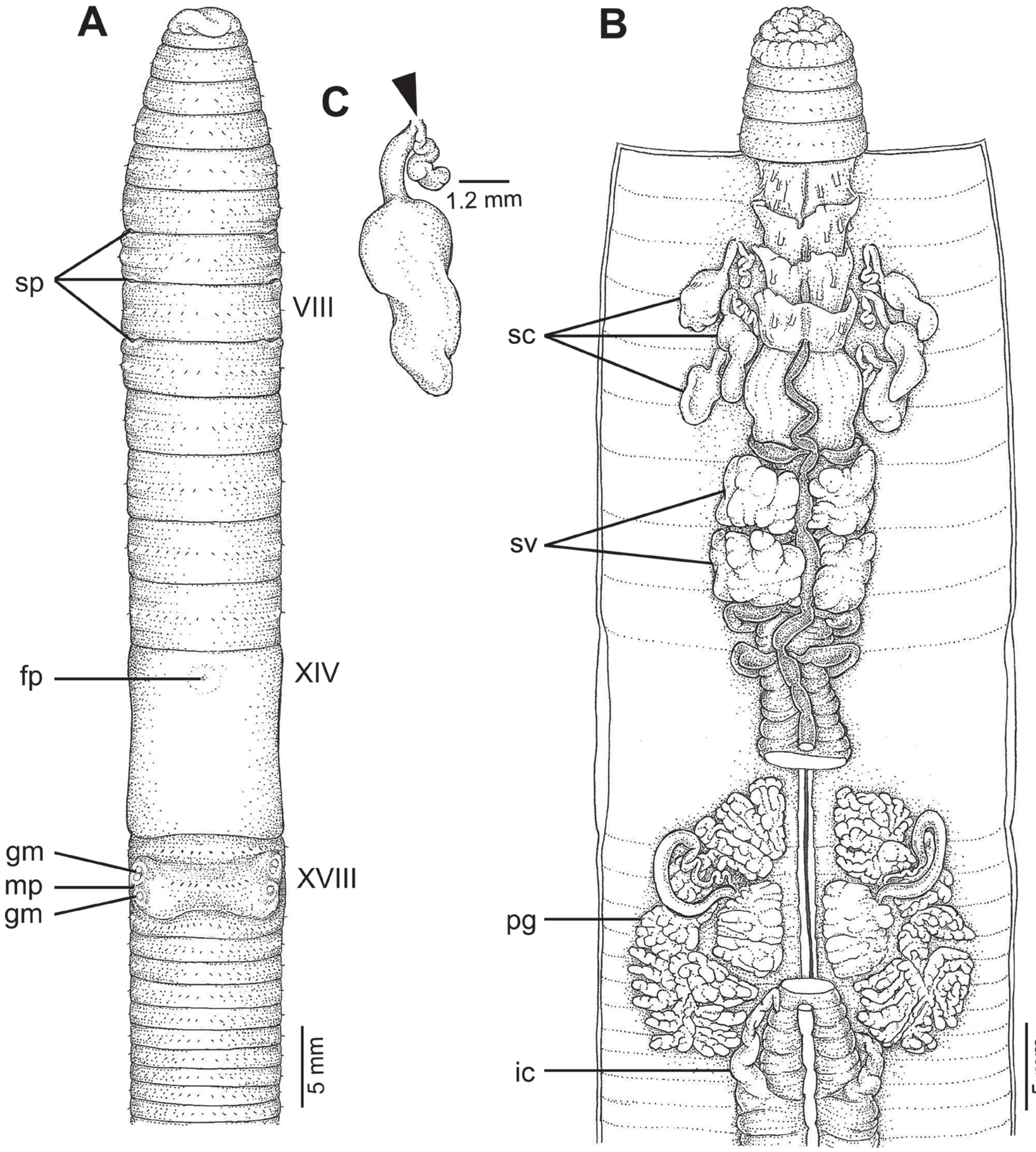

Figure 8. External and internal morphology of *Amynthas auriculus* sp. nov., holotype (CUMZ 3825) **A** external ventral view **B** internal dorsal view, and **C** spermatheca with location of spermathecal pore arrowed.

Etymology. The species was named after the region of the male pore that looks like an ear after being preserved.

Distribution. This species is known only from the type locality.

Remarks. *Amynthas auriculus* sp. nov. belongs to the *aelianus* species group. This new species is quite similar to *A. osmastoni* from the South Andaman Islands and *A. burchardi* from Sumatra in the body dimensions but differs in having genital markings in the male pore area and having large sessile glandular masses on the body wall in XVIII–XXI, while *A. osmastoni* has genital markings in the spermathecal pores region without glandular masses on the body wall, and *A. burchardi* has a group of circular papillae in mid-ventral XVIII with no glandular masses on the body wall. *Amynthas auriculus* sp. nov. can be distinguished from *A. monsoonus* and *A. huangi* from Taiwan in which the new species has a larger body size, *A. monsoonus* has a body size of 102 mm, a diameter of 3.6 mm, with 83 segments and genital markings in segments VII–IX, while the new species is absent. *Amynthas huangi* has a body size of 70 mm, a diameter of 3.5 mm, with 101 segments, and genital marking glands in the body wall in VI–IX, while the new species has no such character. *Amynthas monsoonus* and *A. huangi* have no genital markings in the male pores region, whereas the new species is present.

Amynthas auriculus sp. nov. is easily distinguished from the other four earthworms of the *aelianus* species group reported from Thailand, namely *A. fucosus* from Myanmar, *A. siam* from Thailand, by its size and other physical traits. *Amynthas fucosus* has a body size range of 120 mm with a diameter of 6 mm and 114 segments, *A. siam* has a body size range of 73 mm, diameter of 3 mm, while the new species has a body size that ranges from 184–267 mm with a diameter of 9–11 mm, and 95–151 segments. *Amynthas fucosus* has two pairs of genital markings at 17/18, 18/19, and *A. siam* has single pair between the male pores, while the new species has three genital markings in the male pores. This new species differs from *A. arenulus* in that *A. arenulus* has no genital markings. In contrast, the new species has them at segment XVIII, near the male pores area. The new species is relatively similar to *A. longicaeca* in body size; however, *A. longicaeca* differs in that it has an oval ampulla, a small ovate knob with nephridia on the diverticulum, and also has a crescent-shaped male pore region (Table 5).

Amynthas bantanensis **Chanabun & Panha, sp. nov.**

https://zoobank.org/10F71AA3-5917-4F80-846F-A0683B6063AC
Fig. 9, Tables 4, 5

Type material. ***Holotype*****:** Adult specimen (CUMZ 3827) Ban Tan, Nawah, Nakhon Phanom, northeast of Thailand, 17°30'28.0"N, 104°05'48.0"E, 149 m a.m.s.l., 28 September 2018, coll. R. Chanabun, A. Aoonkum. ***Paratypes*****:** 3 adults (CUMZ 3828), 2 adults (NHMUK); same collection data as for holotype.

Diagnosis. Medium-large size, length 159–213 mm, diameter 8–10 mm, 105–149 segments. Paired male pores in segment XVIII, surrounded by four genital papillae each. Paired spermathecal pores in intersegments 6/7–8/9. Spermathecae elongated sacs, with slender and long stalks of the ampulla, diverticulum slender, long at the beginning, zigzagged at the center, and slen-

Table 4. Holotype and paratype dimensions and other morphological characteristics of *Amynthas bantanensis* sp. nov.

Characters	Body length (mm)	Number of segments	Location of genital markings	Number of genital markings	First dorsal pore	Prostate glands	Intestinal caeca	Genital marking gland
Holotype CUZM 3827	191	127	XVIII	4	12/13	XVI–XXII	XXVII–XIX	absent
Paratype CUMZ 3828								
1	179	105	XVIII	4	12/13	XV–XXII	XXVII–XIX	absent
2	213	149	XVIII	4	12/13	XV–XXIII	XXVII–XIX	absent
3	159	124	XVIII	4	12/13	XV–XXII	XXVII–XIX	absent
4	164	119	XVIII	4	12/13	XV–XXII	XXVII–XX	absent
5	197	120	XVIII	4	12/13	XV–XXIII	XXVII–XIX	absent

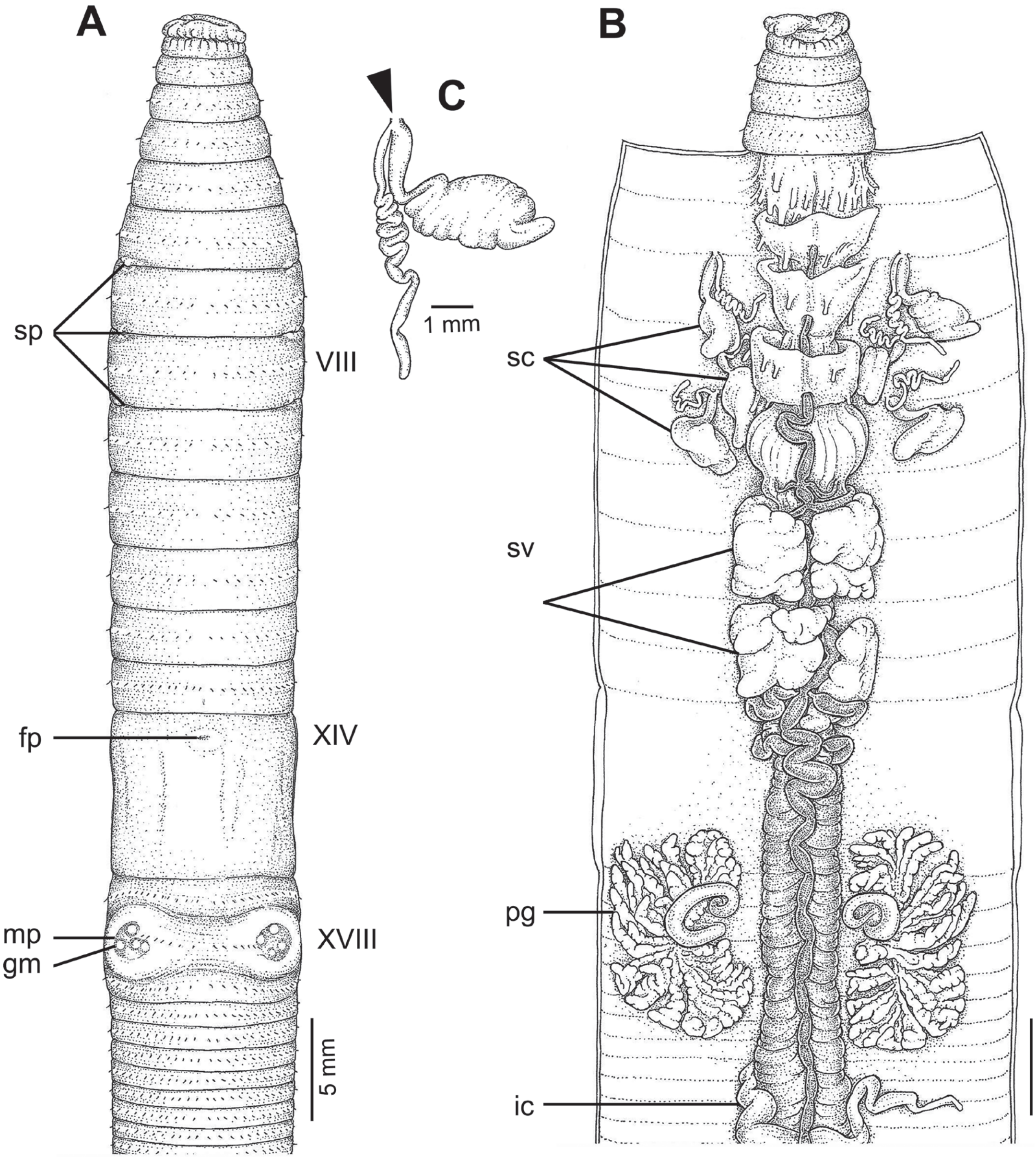

Figure 9. External and internal morphology of *Amynthas bantanensis* sp. nov., holotype (CUMZ 3827) **A** external ventral view **B** internal dorsal view, and **C** spermatheca with location of spermathecal pore arrowed.

Table 5. Morphological characteristics of *A. sakonnakhonensis* sp. nov., *A. auriculus* sp. nov., *A. bantanensis* sp. nov., and other *aelianus* species in Thailand, Burma, Sumatra. The comma is used to separate body length and width. Missing data are shown with a question mark (?). Data for *A. arenulus* Bantaowong & Panha, 2014, *A. longicaeca* Bantaowong & Panha, 2014, *A. burchardi* Michaelsen, 1899, *A. osmastoni* (Michaelsen, 1907), and *A. fucosus* (Gates, 1933) are from Bantaowong et al. (2014), and *A. siam* Blakemore, 2011 is from Blakemore (2011).

Characters	***A. sakonnakhonensis* sp. nov.**	***A. auriculus* sp. nov.**	***A. bantanensis* sp. nov.**	***A. arenulus***	***A. longicaeca***	***A. burchardi***	***A. osmastoni***	***A. fucosus***	***A. siam***
Body length (mm), width (mm)	134–238, 6–9	184–267, 9–11	159–213, 8–10	465, 13.3	278, 10.1	270, 9	250–320, 10–11	120, 6	73, 3
Segment number	85–162	95–151	105–149	133–176	115–160	126	126–148	114	?
First dorsal pore	12/13	12/13	12/13	12/13	12/13	13/14	12/13	12/13	12/13
Genital marking									
Pre-clitellum	absent	absent	absent	absent	absent	absent	median in VIII	absent	absent
Post-clitellum	four genital markings at XVIII	three genital markings at XVIII	four genital markings at XVIII	absent	crescent shape	mid-ventral group of small circular papillae on XVIII	absent	paired at 17/18, 18/19	pair at XVIII
Spermathecae	large oval sac	large elongated sacs	elongate sac	large sac	oval	oval	spherical	flatten	spherical
Diverticulum	long and zigzag, distal end a baton-like chamber	short and zigzag	slender, long, at the beginning, zigzag at the center and slender toward the end	slender	small ovate knob	long slender	tubular	tubular and coiled	convoluted
Prostate gland	XV–XXII	XVI–XXIV	XVI–XXII	XVII–XXII	XVI–XXI	XV–XX	XV–XXIV	XVII–XX	?
Intestinal caeca	XXVII–XXII	XXVII–XXI	XXVII–XIX	XXVII–XXIII	XXVII–XXIV	?	?	?	XXVII–?
Genital marking gland	large, long sessile glandular mass on segment XVII–XIX	large sessile glandular masses on segment XVIII–XXI	absent	large sessile glandular masses	large sessile glandular masses	absent	absent	absent	slight sessile genital glands
Type locality	Thailand	Thailand	Thailand	Thailand	Thailand	Sumatra, Indonesia	South Andaman Islands	Myanmar	Thailand

der towards the end. Holandric. Intestinal caeca are simple. First dorsal pore in 12/13. Prostate gland large and well developed in XVI–XXII; its duct is short, smooth and curled like a spiral.

Description of holotype. Length 191 mm, diameter 8 mm, body cylindrical, 127 segments. Preserved specimens are dark brownish on the dorsal part and pale gray on the ventral part. Setae are regularly distributed around the segmental equators, numbering 37 at segment VII, 57 at segment XX, and 12 between male pores at segment XVIII. Setal formula AA:AB:ZZ:ZY=1:1:2:1 at XIII. Single female pore mid-ventrally at segment XIV. Prostomium epilobic. First dorsal pore in 12/13. Clitellum annular in XIV–XVI with no dorsal pores or setae.

Male pores paired on the ventral side of XVIII, ~ 9 mm apart, distance 0.32× body circumference. Male pores superficial, convex, each surrounded by four genital markings. Spermathecal pores three pairs in 6/7–8/9. The distance between each pair is ~ 0.41× the body circumference ventrally apart. There are no genital markings in this area.

The septa in 5/6–7/8 thick, absent at 8/9–9/10, slightly thick in 10/11–11/12, thin in 12/13–14/15, and very thin behind 15/16. Gizzard large in IX. Intestine begins at segment XV. Vary long and simple intestinal caeca in XXVII–XIX. Esophageal hearts four pairs in segment X–XIII. Holandric; testes and funnels in X and XI. Seminal vesicles are paired, one in X–XI, the other one in XII–XIII. The prostate glands are large and well-developed, located in segments XVI–XXII and divided into several lobules. Prostate duct is relatively short, smooth, and spiraled.

Ovaries in segment XIII. Three pairs spermathecae in VII–IX. Spermathecae elongated sacs, with slender and long stalks of the ampulla, diverticulum slender, long at the beginning, zigzagged at the center, and slender towards the end.

Variation. Five paratypes range in size from 159–213 mm (183.83 ± 20.54 mm) body length with 105–149 segments (124 ± 14.39 mm) (Table 4).

Etymology. The name *bantanensis* is given to this species for its type locality at Ban Tan, Nawah, Nakhon Phanom.

Distribution. This species is known only from the type locality.

Remarks. *Amynthas bantanensis* sp. nov. is sexthecal with spermathecal pores in 6/7–8/9, belonging to the *aelianus* species group. The new species differs from other *aelianus* species group reported by body size, location of spermathecae and spermathecae, *A. bantanensis* sp. nov. is similar to *A. burchardi* from Sumatra, and *A. osmastoni* from the south Andaman Islands in body dimensions, but easily distinguished from *A. burchardi* which has a mid-ventral group of small circular papillae on segment XVIII, while they are absent in the new species. *Amynthas osmastoni* has genital markings at the pre-clitellum, which are absent in the new species. *Amynthas bantanensis* sp. nov. differs from *A. monsoonus* and *A. huangi* from Taiwan in that the new species has genital markings in the male pores region, whereas they are absent in *A. monsoonus* and *A. huangi*. *Amynthas monsoonus* has a smaller body size of 102 mm, a diameter of 3.6 mm, with 83 segments, and genital markings in segments VII–IX. *Amynthas huangi* has a smaller body size, a body range of 70 mm, a diameter of 3.5 mm, and 101 segments, and *A. huangi* has genital marking glands in the body wall in VI–IX, while the new species lacks them. The new species has genital markings in the male pores region, whereas they are absent in *A. monsoonus* and *A. huangi*.

Amynthas bantanensis sp. nov. differs from *A. arenulus* from Surin, Ubon Ratchathani, and Srisaket in that *A. arenulus* has a large transverse elliptical disc surrounded by an elevated rim with an indistinct male aperture located at the outer edge of each poropore and consists of a large sessile glandular mass on the body wall in this region, while the new species has genital markings at segment XVIII, near the male pore, but has no glandular mass on the body wall. *Amynthas bantanensis* sp. nov. is easily distinguished from *A. fucosus* from Burma and *A. siam* from Thailand because both previously reported species have smaller body sizes (*A. fucosus* = 6 mm, *A. siam* = 3 mm). *Amynthas fucosus* has two pairs of genital markings at 17/18, 18/19, and *A. siam* has a single pair between the male pores, while the new species has four genital markings surrounded by male pores at segment XVIII. The new species, *Amynthas bantanensis*, is similar body dimensions to *A. longicaeca* from Chaiyaphum, but is easily distinguished by *A. longicaeca* having crescent-shaped genital markings and a large sessile glandular mass on the body wall, while the new species lacks it. Considering the differences between *A. bantanensis* sp. nov., *A. auriculus* sp. nov., and *A. sakonnakhonensis* sp. nov. see the respective remarks sections above and Table 5.

Discussion

The benefits of earthworms have led to a variety of uses, such for decomposing organic waste from agriculture and households, as well as to enhance soil qualities and structure (Alegre et al. 1996; Sharma et al. 2017) and produce the best compost for plant growth and organic farming systems (Rajiv et al. 2009). Moreover, earthworms are used as food for animals. In some local areas, they serve as human food and a component of herbal medicine since the earthworm's tissue is rich in nutrients and enzymes that are very good for health (Grdiša et al. 2013; Sun and Jiang 2017), as mentioned in the introduction section.

In this paper, four new earthworm species are reported: one species of genus *Metaphire* and three species of genus *Amynthas*, of which three species were collected by locals and dried for sale (*Metaphire songkhramensis* sp. nov. from Sakon Nakhon, *Amynthas sakonnakhonensis* sp. nov. from Sakon Nakhon, and *Amynthas bantanensis* sp. nov. from Nakhon Phanom). *Metaphire songkhramensis* sp. nov. belongs to the *houlleti* species group. This new species has the longest and widest body size among the *houlleti* groups reported in Thailand. *Amynthas sakonnakhonensis* sp. nov., *A. auriculus* sp. nov., and *A. bantanensis* sp. nov. are classified in the *aelianus* species group. All of these new species were reported from northeast Thailand and collected from different habitat types.

Metaphire songkhramensis sp. nov. lives in the dark clay soil of the oxbow lake of the Songkhram River, which locals call Kud Klong Ngong. This habitat is covered with plenty of earthworm casts and with mainly small bamboo (*Bambusa* sp., *Bambusa arundinacae* Wild.), small shrubs (*Hymenocardia wallichii* Tul. and *Euryeoma longifolia* Jack.), and trees (*Syzygium cumini* L. and *Lagerstroemia calyculata* Kurz.). The earthworms move around on the soil, but, interestingly, all of them are the same species.

On the other hand, Nong Tuet, Sakon Nakon, from where *Amynthas sakonnakhonensis* sp. nov. was found, is a wetland. Water will flood in the rainy season and be covered by grass in the dry season. Villagers in the area use it as a pasture for livestock, such as cows and buffaloes. This habitat's soil is a mixture of clay and tiny pebbles, and the land could not be utilized for cultivation because shrubs predominate the area. The authors found that the new species of earthworms inhibit this area together with *M. peguana* (Rasa, 1890) and *Drawida* sp.

Amynthas auriculus sp. nov. lives in dark sandy loam soil covered with a mixed deciduous forest at Wut Tham-kham, Sakon Nakon. This area is a fertile forest of the Phu Phan Mountain range. Contrastingly, *Amynthas bantanensis* sp. nov. lives in the sandy loam of the soil covered with casts. This species was collected early in the morning from the field where rice and other economic plants are cultivated.

Furthermore, after interviewing the locals, the authors learned that every August to December of the year, from the end of the rainy and cold seasons (temperature ranges from 16–22.9 °C), earthworms come out from the soil at approximately 2:00–5:00 a.m. At this time the locals collect them for sale. If the weather is excellent, 30–80 kg/person of earthworms can be collected, but if it is otherwise rainy, very cold, or windy, the earthworms do not come out. Moreover, these earthworms also choose to stay underground during the full moon.

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Additional information

Conflict of interest

No conflict of interest was declared.

Ethical statement

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers): Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU) under approval number IACUC-KKU-32/65 approved the study.

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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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Research Article

A new species of the genus *Alainites* Waltz & McCafferty, 1994 (Ephemeroptera, Baetidae) from the north of Morocco

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Abstract

A new species of *Alainites* is described from northern of Morocco *Alainites albai* **sp. nov.** It can be separated from the other west Palearctic species by the gill number, the spination of the distal margin of tergites, the leg setation, and the paraproct shape and spination. This species is widespread in the study area but never abundant. It prefers small to medium streams with slow flow, and does not seem to be very sensitive to pollution and water logging activities.

Key words: COI, endemics, Maghreb, mayflies, Rif, systematics, West Palaearctic

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Introduction

The mayfly genus *Alainites* Waltz & McCafferty, 1994 was established to encompass species previously included in the *muticus* species group of the genus *Baetis* Leach, 1815 (Waltz et al. 1994). Currently, this genus encompasses 22 species (Barber-James et al. 2013; Kaltenbach and Gattolliat 2021; Phlai-ngam et al. 2022; Yanai et al. 2022). Also, the distribution of *Alainites* is nearly limited at the Palaearctic and Oriental regions; so far, the highest diversity is found in the West Palaearctic region with eight species. Except *Alainites muticus* (Linnaeus, 1758) which has a wide distribution in Europe (Bauernfeind and Soldán 2012) and has also been recently reported from western Asia (Armenia, Hrivniak et al. 2018) and Iran (Bojková et al. 2018), this genus has a high endemism rate in the Mediterranean basin. During the last decades, seven species with mostly restricted distributions have been described. These are *A. oukaimeden* (Thomas & Sartori, 1992) from the Moroccan High Atlas (Thomas and Gagneur 1994; Abdaoui et al. 2010; Zuedzang Abessolo et al. 2021), *A. sadati* Thomas, 1994 distributed from West Algeria to North Tunisia (Thomas and Gagneur 1994; Zrelli et al. 2012; Benhadji et al. 2020), *A. navasi* (Müller-Liebenau, 1974) located in the Iberian Peninsula (Müller-Liebenau 1974; Alba-Tercedor 1981, 1982; Puig 1983), *A. kars* (Thomas & Kazanci, 1989) found in Turkey (Kazanci and Thomas 1989; Novikova and Kluge 1994; Kazanci 2001; Kluge and Novikova 2014) and Armenia (Hrivniak et al. 2018; Sroka et al. 2021), *A. albinatii* (Sartori

& Thomas 1989) mentioned from Corsica (Sartori and Thomas 1989; Gattolliat et al. 2015; Tenchini et al. 2018), *A. bengunn* Yanai & Gattolliat, 2022 recorded from the sister island of Sardinia (Yanai et al. 2022), and *A. gasithi* Yanai & Gattolliat, 2022 found recently in Israel (Yanai et al. 2022).

The distinctive taxonomical characters of this genus at the nymphal stage were mentioned by Zrelli et al. (2012) and Sroka et al. (2021) and include a laterally compressed body, closely positioned antennae, an apical prolongation on the paraproct, and a reduced mandibular right prostheca (Müller-Liebenau 1969, 1974; Waltz et al. 1994; Fujitani et al. 2003). West Palearctic species are characterized by the presence of six pairs of gills, except for *A. muticus*, which has seven. Some larvae of *Alainites* discovered in the material collected from northern Morocco were different from the Moroccan endemic *A. oukaimeden* and from *A. muticus*. It represents the second West Palearctic species with seven pairs of gills. The specimens of this species were previously considered to belong to *A. muticus* (Dakki 1987; El Bazi et al. 2017; Khadri et al. 2017; Mabrouki et al. 2017; El Alami et al. 2022b). However, detailed morphological and genetic approaches proved that these specimens of *Alainites* noticeably differed from both the European and Asiatic lineages (Sroka 2012).

In the present study, we describe a new species of *Alainites* based on nymphs from the Rifean Mountains of Morocco. In addition, distinctive characters of western Palearctic *Alainites* species are provided. The holotype and part of the paratypes of the new species are housed in the Muséum cantonal des sciences naturelles, Lausanne, Switzerland (**MZL**); other paratypes are deposited in Laboratory of Ecology, Systematics, Conservation of Biodiversity Tetouan, Morocco (**LESCB**).

Materials and methods

The larvae of *Alainites albai* sp. nov. has a wide distribution and a wide altitudinal range in the Rif (Fig. 1). The sampling was performed by LESCB team between 1997 and 2022. They were subsequently preserved in 70% or 95% ethanol for description and DNA extraction. 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), using an Olympus SZM100 stereomicroscope.

To complement our morphological investigations, we sequenced a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI hereafter) for seven specimens of *Alainites albai* sp. nov., following the non-destructive DNA extraction procedure described in Vuataz et al. 2011. The Polymerase Chain Reaction (PCR), purification and sequencing steps were conducted according to the methodology described in El Alami et al 2022a. Forward and reverse sequencing reads were assembled and edited in CodonCode Aligner v. 10.0.2 (Codon-Code Corporation, Dedham, MA), and aligned using MAFFT (Katoh et al. 2019) with default settings as implemented in Jalview v. 2.11.2.6 (Waterhouse et al. 2009). The number of parsimony-informative sites of the alignment was calculated in Mega v. 10.2.4 (Kumar et al. 2018; Stecher et al. 2020).

To augment our molecular dataset, we initially downloaded all COI sequences associated with *Alainites* available on the GenBank database (identified among species of the genus *Takobia* Novikova & Kluge, 1987) as of 2 June

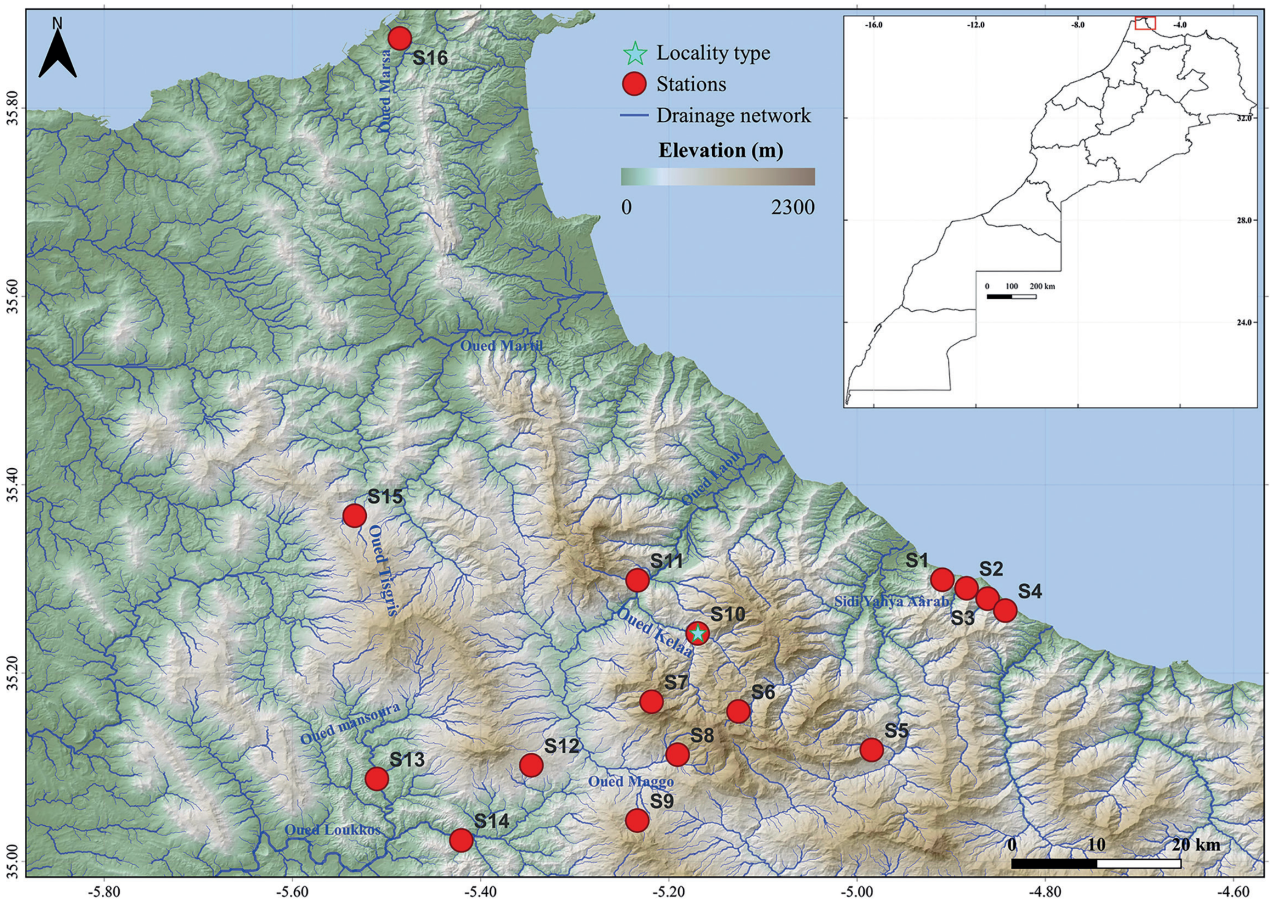

Figure 1. The sampling site localization of *Alainites albai* sp. nov., in the Rif domain.

2023, resulting in a total of 64 records. Additionally, we obtained the sequences associated with *Alainites/Takobia* accessible on the BOLDSYSTEMS data portal as of the same date and selectively retained only those that were not shared with GenBank, yielding an additional set of 5 sequences. We then manually excluded GenBank/BOLD sequences obtained from specimens collected outside the western Palearctic region. This selection process was conducted after confirming that the COI sequences of *Alainites albai* sp. nov. were clearly distinct from the removed sequences (data not shown). A total of 58 sequences remained for further analyses, comprising seven newly generated sequences (Table 1), 46 sequences from GenBank (four from Kjærstad et al. 2012; six from Sroka 2012; two from Cardoni et al. 2015; ten from Gattolliat et al. 2015; one from Corse et al. 2017; seven from Morinière et al. 2017; five from Tenchini et al. 2018; one from Behrens-Chapuis et al. 2021; two from Sroka et al. 2021; two from Roslin et al. 2022; five from Yanai et al 2022; one unpublished iBOL data release) and five from BOLD (DTNHM1449-21, TDAAT323-19, VMCOL019-20–VMCOL021-20). Two additional newly sequenced specimens, namely one *Nigrobaetis numidicus* (Soldán & Thomas, 1983) from Morocco and one *Nigrobaetis rhithralis* (Soldán & Thomas, 1983) from Algeria, were included in the study as outgroups (Table 1).

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

Table 1. Newly sequenced specimens for the study, with collection information, GenBank accessions and nomenclature details.

Specimen catalogue	Species	Country	Stage	Locality	GPS coordinates	Date	GenBank ID	GenSeq nomenclature
GBIFCH00980877	*Alainites albai* sp. nov.	Morocco	Nymph	Oued Kelâa	35.242222°N, 5.169444°W	3.iii.2021	OR126003	genseq-2 COI
GBIFCH01144254	*Alainites albai* sp. nov.	Morocco	Nymph	Oued Tisgris	35.367222°N, -5.533889°W	20.iv.2021	OR126002	genseq-2 COI
GBIFCH01144390	*Alainites albai* sp. nov.	Morocco	Nymph	Oued Ouara	35.035039°N, -5.237778°W	23.vi.2022	OR126001	genseq-2 COI
GBIFCH01144391	*Alainites albai* sp. nov.	Morocco	Nymph	Oued Afeska	35.169733°N, -5.185083°W	2. vi.2020	OR126000	genseq-2 COI
GBIFCH01144392	*Alainites albai* sp. nov.	Morocco	Nymph	Oued Amazithen	35.29924°N, -4.90937°W	27.ii.2021	OR125999	genseq-2 COI
GBIFCH01144393	*Alainites albai* sp. nov.	Morocco	Nymph	Oued Ouara	35.043567°N, -5.2336°W	23.vi.2022	OR125998	genseq-2 COI
GBIFCH01144394	*Alainites albai* sp. nov.	Morocco	Nymph	Oued Sidi Yahia Aarab	35.287056°N, -4.90185°W	28.v.2022	OR125997	genseq-2 COI
GBIFCH00673223	*Nigrobaetis rhithralis*	Algeria	Nymph	Oued Ftitich	36.900278°N, 8.618056°W	9.iii.2019	OR126004	genseq-4 COI
GBIFCH01144576	*Nigrobaetis numidicus*	Morocco	Nymph	Oued Brahim Ben Arrif	35.306111°N, -5.615833°W	21.vi.2013	OR125996	genseq-4 COI

Schliep 2019) in R 4.3.0 (R Core Team 2023), selecting the raw model and the pairwise.deletion option, corresponding to uncorrected p-distances (see Srivathsan 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 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 (HKY+Γ+I) was selected based on the second-order Akaike information criterion (AICc; Hurvich and Tsai 1989) implemented in JmodelTest v. 2.1.10 (Darriba et al. 2012) with three 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 v. 1.10.4 (Suchard et al. 2018) on the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The input BEAST file was generated in BEAUTi v. 1.10.4 (Suchard et al. 2018), incorporating the selected evolutionary model and partition scheme described above and enforcing a monophyletic constraint on the ingroup (the genus *Alainites*). 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 60 million generations, logging parameters every 1000 generations. Convergence of the MCMC runs was visually verified in Tracer v. 1.7.2 (Rambaut et al. 2018). The log and tree files from the independent runs were combined using LogCombiner v. 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 v. 1.10.4 (Suchard et al. 2018) with default settings. Visualization and editing of the tree were conducted in iTOL v. 6.7.5 (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://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) to estimate the most probable number of MOTUs based on our COI alignment. We calculated genetic distances using the Kimura 2-parameter substitution model (Kimura 1980) and selected the species delimitation hypothesis associated to the highest barcode gap width (W) among the two partitions sharing the same best asap-score. The GMYC model, which requires a time-calibrated ultrametric tree as input, implements a maximum likelihood 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 analysis 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 v. 4.6 (Chesters 2013). MCMC chains were run here for a total of 20 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. We conducted mPTP using the web service available at https://mptp.h-its.org, using the BEAST COI gene trees (full dataset) as input (see above).

Abbreviations

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

Results

Alainites albai El Alami, Vuataz & Gattolliat, sp. nov.

https://zoobank.org/0B24CF09-A193-42A2-9A9C-96266B5C0C4A
Figs 1–8

Type-material. *Holotype*. Morocco. Nymph; Chefchaouen Province, S10 Oued Kelâa; Loc. Akchour; 35°14'32"N, 05°10'10"W; alt. 460 m; 3.iii.2021; El Yaagoubi leg.; GBIFCH00763782; MZL.

Paratypes. Morocco. 1 nymph; same data as holotype; DNA; GBIFCH00980877; MZL • 2 nymphs; same data as holotype; 7.xi.2014; El Bazi leg.; in alcohol; LESCB • 1 nymph; Chefchaouen Province, S1 Oued Amazithen, Loc. El Ouesteyine; 35°17'57.264"N, 4°54'33.732"W; alt. 166 m; 27.ii.2021; El Yaagoubi leg.; DNA; GBIFCH01144392; MZL • 2 nymphs; Chefchaouen Province, S2 Oued Sidi Yahia Aarab, Loc. Sidi Yahia Aarab; 35°17'10.428"N, 4°53'37.644"W; alt. 114 m; 28.v.2022; El Yaagoubi leg.; one in alcohol; GBIFCH00763784 and other DNA; GBIFCH01144394; MZL; 1 nymph; 18.vi.2014; Khadri leg.; in Alcohol; GBIFCH00763781; MZL; 4 nymphs; 28/v/2022; El Yaagoubi leg.; LESCB • 6 nymphs ; Chefchaouen Province, S3 Oued Jenane Niche, Loc. Jenane Niche ; 35°16'44.904"N, 4°51'40.788"W ; alt. 93 m; 9.ix.2021 ; El Yaagoubi leg.; LESCB • 11 nymphs; Chefchaouen, Province, S4 Oued Aârkob, Loc. Arherarose; 35°15'59.4"N, 4°50'33.216"W; alt. 128 m; 9.xii.2021; El Yaagoubi leg.; in alcohol; LESCB • 22 nymphs; Chefchaouen Province, S5 Oued Assifane, Loc. Igourain; 35°7'6.7584"N, 4°59'3.9984"W; alt. 1405 m; 9.ix.2021; El Yaagoubi leg ; 1 on slide ; LESCB • 5 nymphs; Chefchaouen Province, S6 Oued Beni Mhammed, Loc. Beni Mhammed; 35°09'34.0812"N, 5°07'34.0212"W; alt. 1330 m; 29.v.2008; El Alami leg.; in alcohol; GBIFCH00763777; MZL • 2 nymphs; Chefchaouen Province, S7 Oued Afeska, Loc. Afeska; 35°10'11.0388"N, 5°13'6.2988"W; alt. 1293 m; 2.vi.2021; El Yaagoubi leg.; 1 nymph DNA; GBIFCH01144391; 2.vi.2020;2 in alcohol; GBIFCH00763777; GBIFCH00763783; MZL and 4 nymphs; 2.vi.2022; El Yaagoubi leg.; 3 in alcohol and 1 on slide; LESCB • 10 nymphs; Chefchaouen Province, S8 Oued Maggo, Loc. Maggo Nord village; 35°6'48.6"N, 5°11'26.7"W; alt. 905 m; 24.ii.2022; El Yaagoubi leg.; LESCB; 5 nymphs; 3.vi.2016; El Alami, leg.; in alcohol; GBIFCH00763780; MZL • 4 nymphs; Chefchaouen Province, S9 Oued Ouara, Loc. Khizana; 35°02'614"N, 5°14'016"W; alt. 930 m; 23.vi.2022; El Yaagoubi leg.; 2 on slide; LESCB; 2 for DNA; GBIFCH01144393; GBIFCH01144390; MZL • 3 nymphs; Chefchaouen Province, S11 Oued Laou, Loc. Abiyati; 35°17'55.14"N, 5°13'59.99"W; alt. 140 m; 11.iii.2001; El Alami leg.; in alcohol; GBIFCH00763779; MZL • 1 nymph; Chefchaouen Province, S12 Oued Harakat, Loc. Mezine village; 35°6'8"N, 5°20'46"W; alt. 740 m; 31.iii.2021; El Yaagoubi leg.; DNA, GBIFCH00980924; MZL • 20 nymphs; Chefchaouen Province, S13 Oued Mansoura, Loc. Tanaqoub; 35°5'16"N, 5°30'37"W; alt. 124 m; 01.vi.2021; El Yaagoubi leg.; 2 on slide; LESCB • 2 nymphs; Ouezzane Province, S14 Oued Loukkos, Loc. Souk El Had; 35°01'21"N, 5°25'14"W; alt. 140 m; 11.iv.2021; El Yaagoubi leg.; in alcohol; GBIFCH00763776; MZL • 1 nymph; Tetouan Province, S15 Oued Tisgris, Loc. Hammadesh; 35°22'02"N, 5°32'02"W; alt. 505 m; 20.iv.2021; El Yaagoubi leg.; DNA; GBIFCH01144254; MZL • 10 nymphs; Tetouan Province, S16 Oued Rmel, Loc. Ain Dchicha; 35°52'40"N, 5°28'24"W; alt. 49 m; 20.x.1997; El Alami leg.; in alcohol; LESCB and 2 nymphs in alcohol; GBIFCH00763778; MZL.

Differential diagnosis. *Alainites albai* sp. nov. can be distinguished from other West Palaearctic species of *Alainites* based on the combination of nymphal characters, summarized in Table 2: (a) seven pairs of abdominal gills, (b) paraproct prolongation covered with small spines on its entire surface with broad, triangular spines laterally, (c) small teeth between prostheca and mola on both mandibles, (d) low number of dorsal setae on its fore-femora (10–15).

Morphological description. *Nymph*. Length. Female body 6.0–7.9 mm; cerci 4.5–5.5 mm; median caudal filament 1.3–1.4 mm (ca 2/3 of cerci); Male body 6.0–6.7 mm; cerci 4.0–5.0 mm; median caudal filament ca 2/3 of cerci.

Table 2. Distinctive taxonomic criteria and distribution of West Palaearctic *Alainites* species.

Species	Distribution	Number of gill pairs	Right mandible: margin between prostheca and mola	Mandible lateral side	Fore-femur dorsal margin: setae number	Fore-tibia dorsal margin: setae number	Cuticle abdominal terga and sterna	Tergite IV: spines on distal margin	Prolongation of paraproct
Alainites albai sp. nov.	Morocco	7	10–16 small teeth	shagreened with scattered fine setae and deep scale bases	10–15	6–10	strongly shagreened	long triangular, pointed	covered by spines
Alainites albinatii (Sartori & Thomas, 1989)	Corsica	6	~ 10 small teeth	scale bases shagreened	15	6	slightly shagreened	long triangular, pointed	apically covered by spines
Alainites bengunn Yanai & Gattolliat, 2022	Sardinia	6	serrated	scale bases, slightly shagreened	14–20	9–17	shagreened	slightly lanceolate	covered by spines
Alainites gasithi Yanai & Gattolliat, 2022	Israel	6	serrated	no scale bases, almost not shagreened	10–20	~ 6 rarely 10–12	smooth	long triangular, pointed	spines only on border
Alainites kars (Thomas & Kazanci, 1989)	Turkey	6	teeth absent	no scale bases, almost not shagreened	> 40 in two rows	5–9	slightly shagreened	triangular pointed	spines on entire surface or just on apex
Alainites muticus (Linnaeus, 1758)	Palaearctic	7	~ 10 small teeth	rare scale bases	14	8	slightly shagreened	short triangular broad basally	spines only on border
Alainites navasi (Müller-Liebenau, 1974)	Iberian Peninsula	6	~ 10 small teeth	?	26	21	smooth	short triangular	covered by spines
Alainites oukaimeden (Thomas & Sartori, 1992)	Morocco (High Atlas)	6	~ 10 small teeth	shagreened	20	8	strongly shagreened	long, relatively narrow	covered by spines
Alainites sadati Thomas, 1994	Algeria, Tunisia	6	~ 10 small teeth	no scale bases, almost not shagreened	~ 25	6–9	slightly shagreened	medium triangular	covered by spines

Coloration. General coloration pale to medium brown (Figs 2, 3). Head uniformly pale brown to brown with yellow vermiform marks on vertex and frons (Fig. 3a). Turbinate eyes in male nymph's purple-brown. Legs ecru except upper side of femora brown (Fig. 2b). Thorax with some paler clear pattern (Fig. 3c). Abdominal tergites pale brown with a central, elongated, yellowish dot; distal part of tergite IX and whole tergite X pale brown to yellowish.

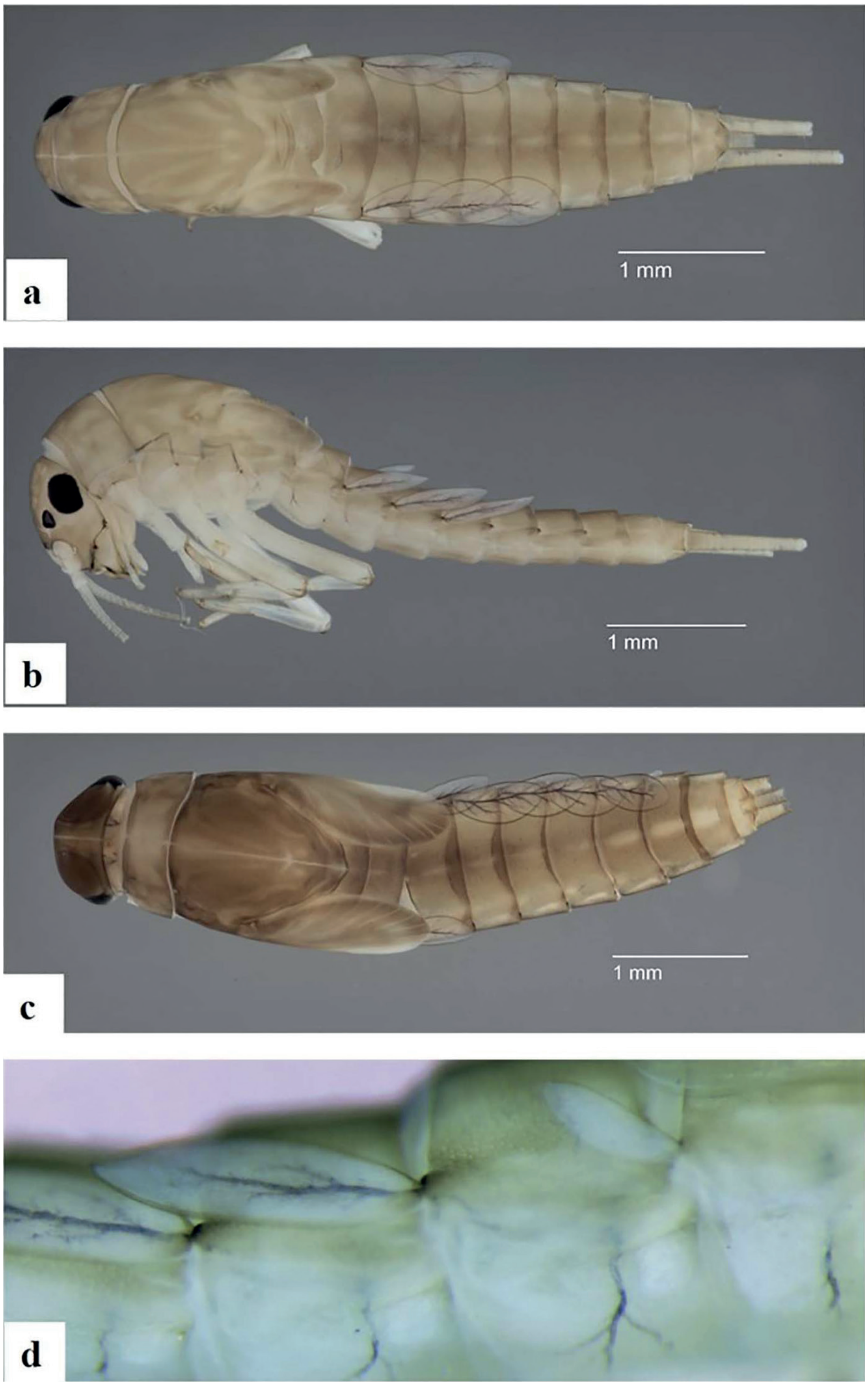

Figure 2. *Alainites albai* sp. nov., nymph habitus **a** female, dorsal view **b** female lateral view **c** male, dorsal view **d** lateral view of the first three gills.

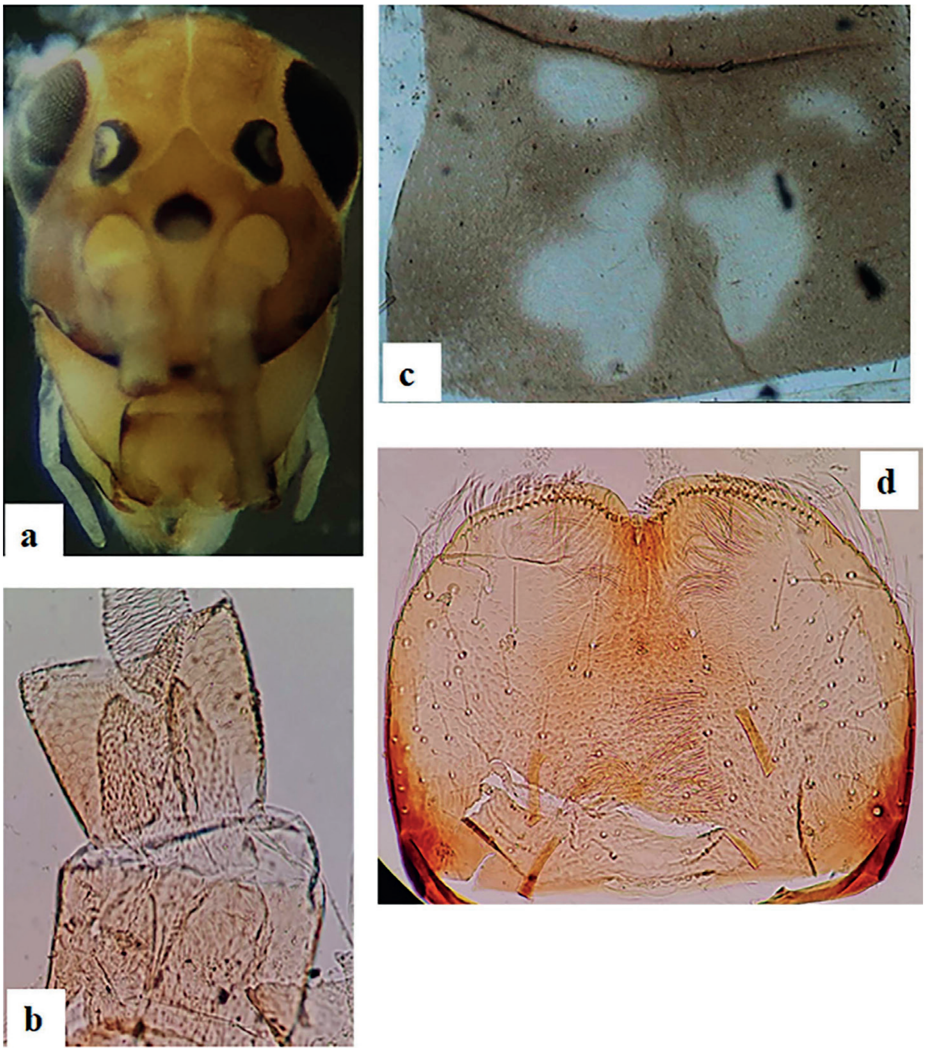

Figure 3. *Alainites albai* sp. nov., nymph morphology **a** head frontal view **b** scape and pedicel **c** pronotum **d** labrum.

Abdominal sternites yellowish to pale brown. Cerci ecru to pale brown without bands or pattern.

Head. ***Antennae*** (Fig. 3a) close to each other, with a narrow inter-antennal carina, scape with deep scale insertions, pedicel with deep scale insertions and few setae (Fig. 3b).

Dorsal surface of labrum (Fig. 3d) with one central long seta and distolateral arc of two long, simple, stout setae, and small fine setae scattered on surface; ventral surface with 5–8 submarginal small, pointed setae; distal margin fringed with ca 20 short, followed by eight or ten long, feathered setae.

Right mandible (Fig. 4a) shagreened, with scattered fine setae and deep scale insertions; incisors composed of eight blunt, distinct denticles, outer- and inner denticles notably smaller than others; prostheca reduced and bifid with numerous thin setae; 10–16 teeth in the space between prostheca and mola (Fig. 4b), tuft of setae absent.

Left mandible (Fig. 4c) slightly shagreened, with sparse fine setae and deep scale insertions; incisors composed of seven blunt, distinct denticles, outer denticle conspicuously smaller than others; prostheca with one row of 11–15 medium denticles and a comb-like structure (Fig. 4d); edge between prostheca and mola with sparse spines particularly in apical half, without setae.

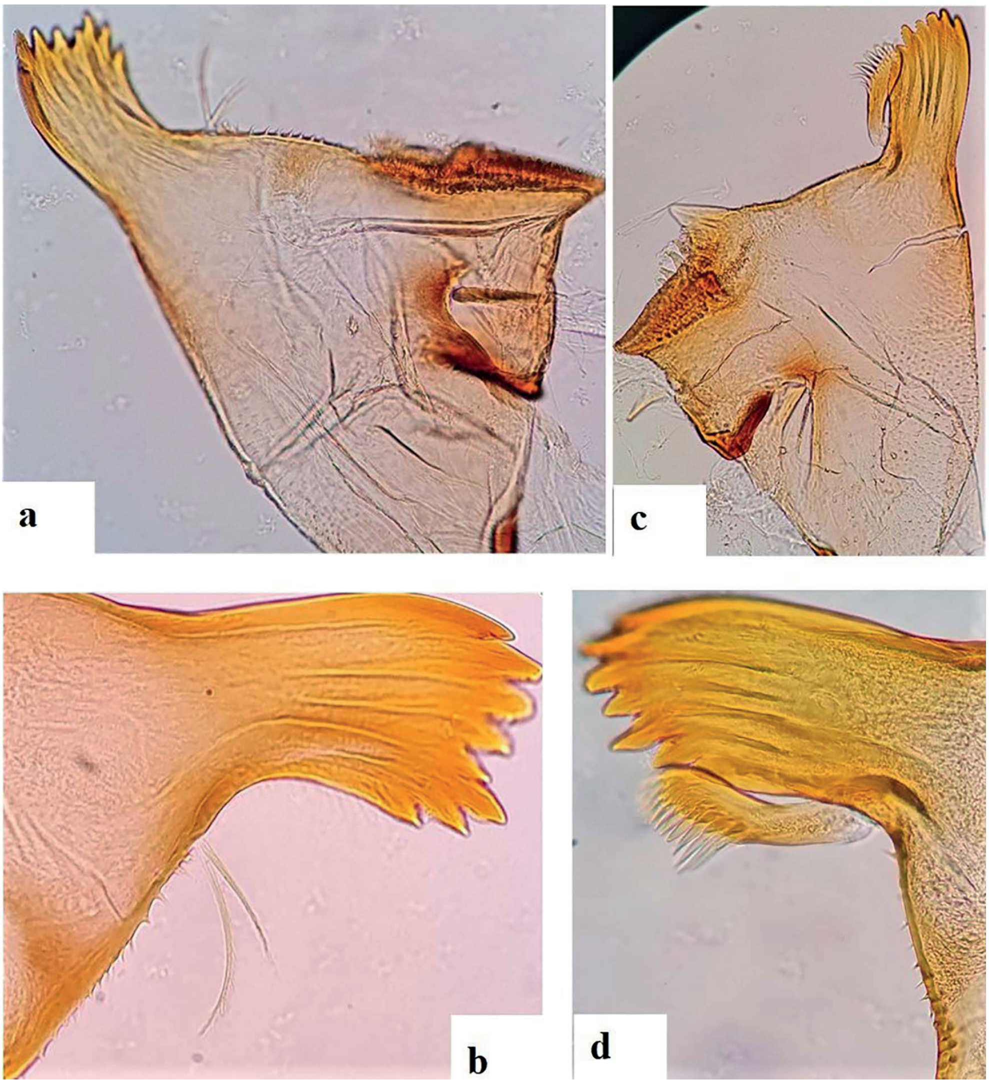

Figure 4. *Alainites albai* sp. nov., nymph morphology **a** right mandible **b** spines between mola and prostheca bifid **c** left mandible **d** spines between mola and prostheca.

Hypopharynx (Fig. 5a) trilobed, apically covered with thin setae; lingua with small central protuberance; superlingua longer than lingua.

Maxilla (Fig. 5b) apex with three elongated acute and curved teeth and a toothlike dentisetae; crown with one row of small setae ending with stouter and longer ones (Fig. 5c); palp two-segmented, extending behind apex of galea-lacinia, length of segment I approximately 0.65× segment II; segment II apically rounded, with few thin setae.

Labium (Fig. 5d) with glossae slightly shorter than paraglossae; inner margins of glossae with 7 or 9 stout medium setae, apical margin with ca 5–7 long stout setae, ventral surface with few thin scattered setae; dorsal surface with row of ca 6–9 medium setae; paraglossae of constant width, with three rows of 11 or 14 long, stout and feathery setae apically; labial palp three-segmented; segment I 0.8× length of segments II and III combined; segment II with dorsal oblique row of 4–6 medium setae; segment III conical, nearly symmetrical, slightly pointed apically, covered with few medium stout setae and small pointed setae apically.

Thorax. Forelegs (Fig. 6): Trochanter with seven marginal short stout pointed setae; femur dorsal margin with one regular row of 10–15 long, robust setae, and

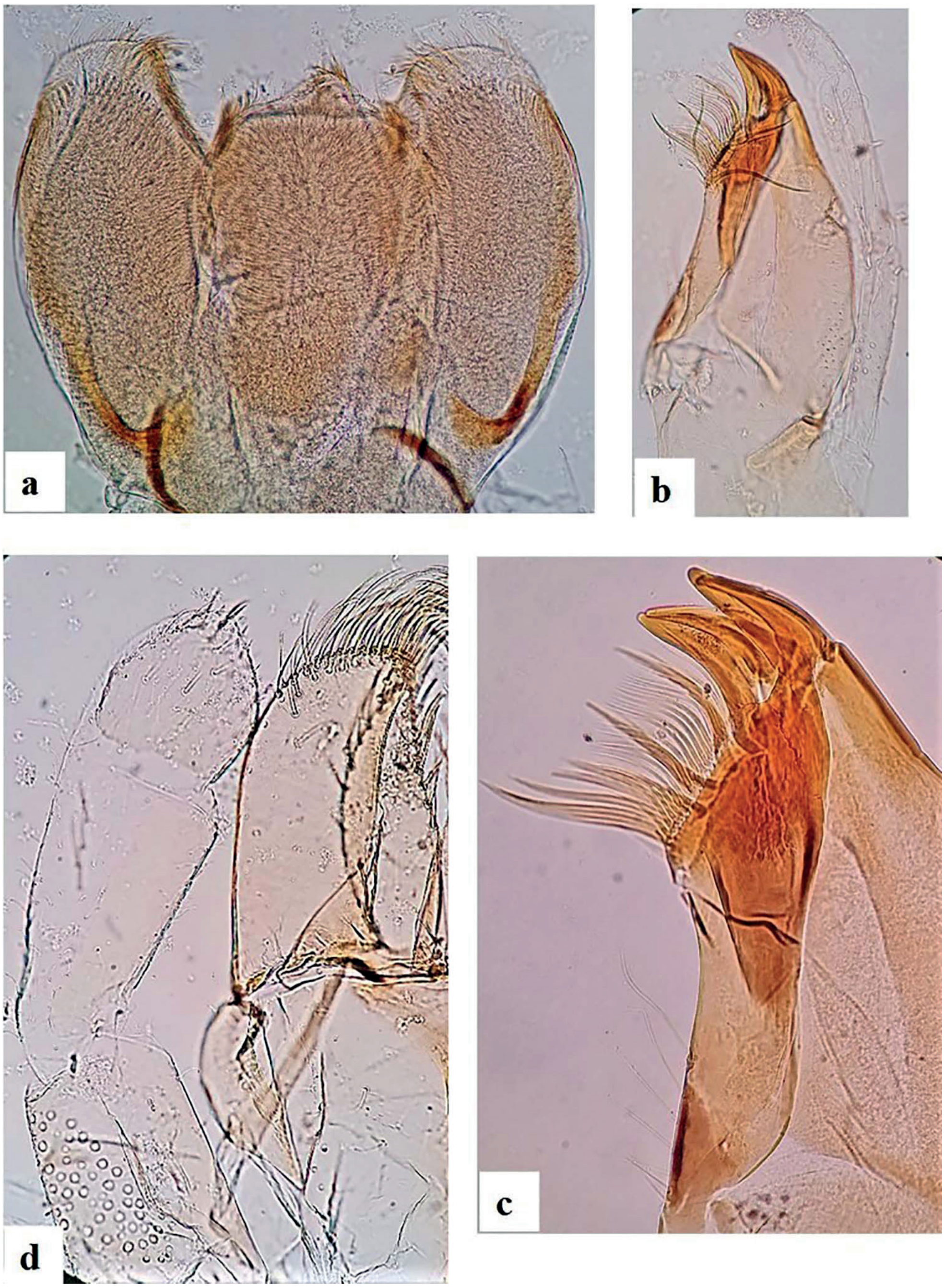

Figure 5. *Alainites albai* sp. nov., nymph morphology **a** hypopharynx **b** maxilla **c** details of dentisetae and maxilla crown of setae **d** labium.

two subapical stout and long setae; ventral margin with short stout pointed setae; lateral surface with sparse scale bases, mainly on apical half and along subdorsal area (Fig. 6a). Tibiae (Fig. 6b) dorsally with 6–10 short stout pointed setae and single apical seta; ventrally with marginal and submarginal short stout pointed setae, denser towards apical end; tibiopatellar suture absent; lateral surface with few short, stout, pointed setae and numerous scale bases. Tarsi bares dorsally; ventral margin with ca 15–24 pointed medium setae; lateral surface with numerous scale bases. Tarsal claws (Fig. 6c) hooked with one row of 11–13 medium teeth, apical setae absent (Fig. 6d). Mid and hindlegs similar to forelegs except femora dorsally with 13–17 pointed setae and tibiae with 21–24 setae dorsally.

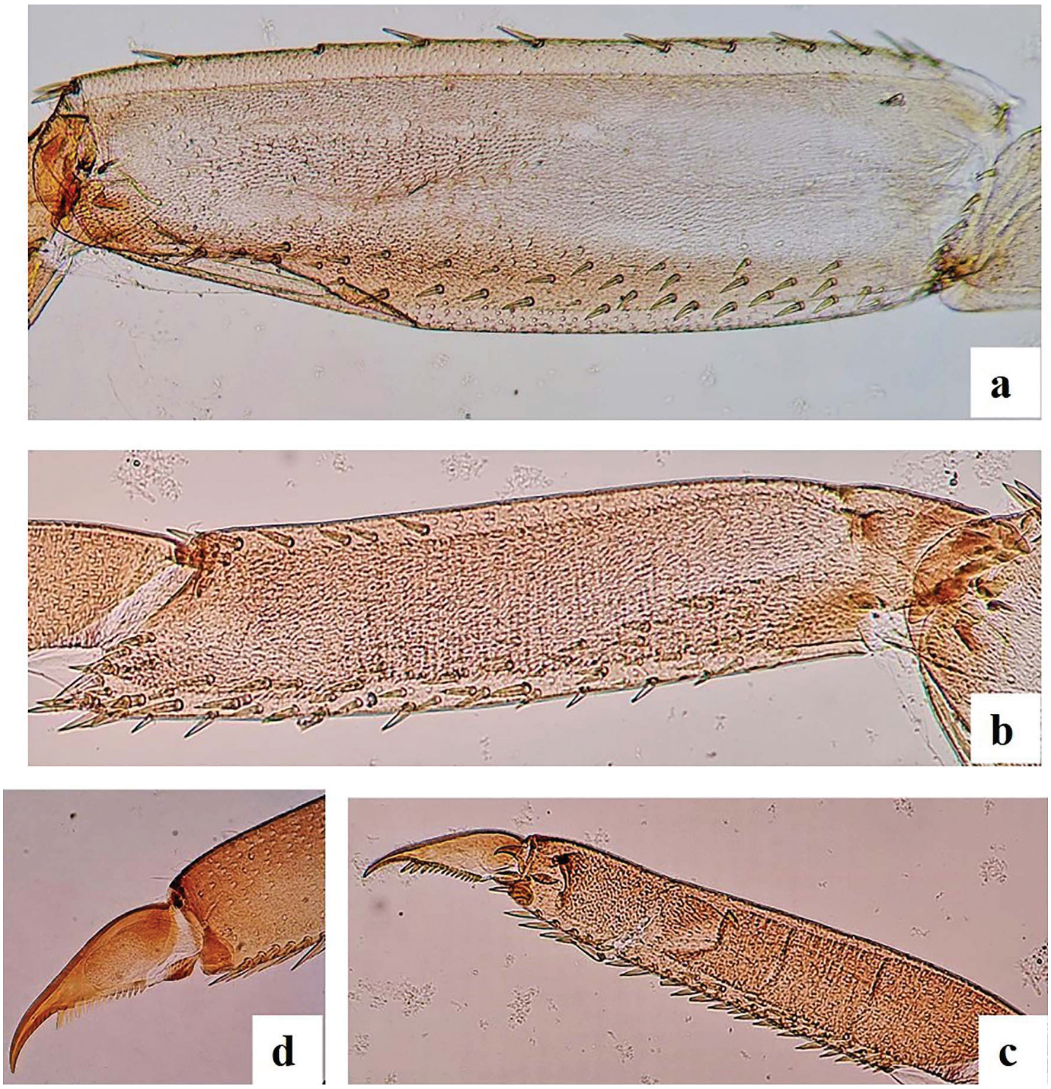

Figure 6. *Alainites albai* sp. nov., nymph morphology **a** foreleg femur **b** foreleg tibia **c** foreleg tarsal claw, foreleg claw.

Hindwing. Pads present.

Abdomen. Terga (Fig. 7a) shagreened, with numerous scale bases, distal margin of tergite IV with triangular spines ~ 2× longer than broad. Sternites with scale bases; posterior margin smooth without spination.

Gills (Fig. 2d) on segments I–VII, elliptic, almost symmetrical and serrated all along costal and distal margins except proximal part and with few setae, gill I smaller than others, length of gill I longer than half of tergite II; gill IV (Fig. 7b) and gill V larger than others, (Fig. 7c). Surface with pores and without any setae; tracheation well visible but with reduced ramification. Paraproct (Fig. 7d, e) shagreened with scale bases and setae; margin with seven broad, triangular spines inner to prolongation and numerous medium spines outer to prolongation; lateral margins of prolongation with numerous medium to broad spines, surface covered with spines; cercotractor with scattered scale bases, distal margin with ca 20 medium spines.

Imago. Unknown.

Molecular analysis. The COI data set was > 95% complete and included 35% of parsimony informative sites. Pairwise COI distances across all sequences ranged from 0% to 23.4%. The overall mean p-distance within MOTUs was 0.8% (mean range: 0.1%–2.3%), while the overall mean p-distance between MOTUs

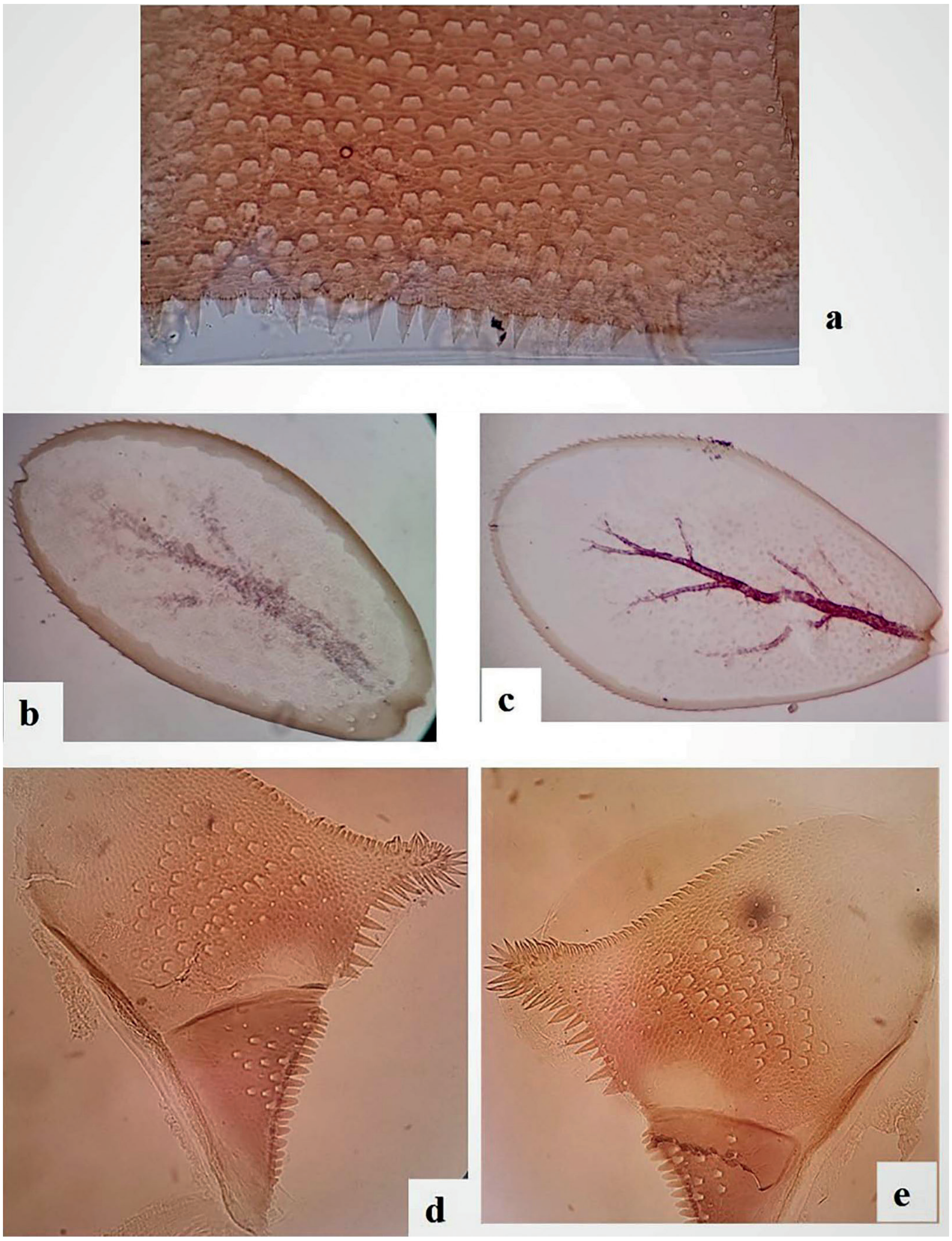

Figure 7. *Alainites albai* sp. nov., nymph morphology **a** distal margin of tergum IV **b** gill IV **c** gill V **d** left paraproct **e** right paraproct.

was 18.7% (mean range: 14.9%–22.6%). The maximum p-distance within MOTUs varied from 0.2% (*A. gasithi* and *A. kars*) to 2.8% (*A.* sp. 2). The minimum p-distance between MOTUs ranged from 14.3% (*A.* sp. 1–*A.* sp. 2) to 16.7% (*A. kars*–*A. gasithi* and *A. kars*–*A.* sp. 4). The seven sequences from *A. albai* sp. nov. formed a strongly supported monophyletic clade, identified as a distinct MOTU in the three species delimitation analyses (Fig. 8). All methods were fully congruent in delimitating the other MOTUs as well.

Distribution (Fig. 1). Morocco.

Etymology. The first author dedicates the name of this species to her former mentor, Professor Javier Alba-Tercedor, a specialist in the Ephemeroptera of the Iberian Peninsula. He contributed significantly to her training and specialization in the taxonomic study of the Ephemeroptera of Morocco.

Discussion

Morphology and genetic characteristics

The integrative approach, incorporating molecular, morphological, and biogeographic evidence, enabled a robust species delimitation within *Alainites*. Indeed, the molecular analyses not only support the monophyly of *Alainites albai* sp. nov. (Fig. 8), but also reveal an important genetic distance to all other included *Alainites* species. The minimum p-distance to the other MOTUs ranges from 16% to 20%, thereby solidifying *A. albai* sp. nov. as a distinct and valid new species. The findings from our molecular analyses also underline the need for further taxonomic research on *Alainites*, particularly in Europe.

The number of gills is a useful character to separate the different species of *Alainites*. This new species is assigned to *Alainites* because it has all the synapomorphic characters of the genus: a laterally compressed thorax, paraproct with postero-internal extension and the prostheca of the right mandible bifid (Waltz et al. 1994; Gattolliat et al. 2023). Within *Alainites*, the larva of the new species is the second western Palearctic species with seven pairs of gills. It differs from *A. muticus* by the size of the spines between prostheca and mola of the right mandible, by the reticulation degree of paraprocts, tergites and mandibles, by the spination of the paraproct extension surface and the presence of strong spines between the prolongation and the extension of the paraproct. *Alainites albai* sp. nov. can be easily separated from the other western Palearctic species (*A. albinatii*, *A. bengunn*, *A. gasithi*, *A. navasi*, *A. kars*), as well as the Maghreb endemic species (*A. oukaimeden* and *A. sadati*) by the number of pairs of gills (Table 2).

Hence, the discovery of this new species has increased the diversity of described *Alainites* species with seven pairs of gills to a total of six, with two found in the West Palaearctic and four in the Oriental realms. The latter are *A. siamensis* Phlai-ngam, Tungpairojwong & Gattolliat, 2022 from Thailand (Phlai-ngam et al. 2022), *A. lingulatus* Tong & Dudgeon, 2000 from Hong Kong (Tong and Dudgeon 2000), *A. yixiani* Gui & Lu, 1999 from the Chinese mainland (Gui and Lu 1999; Sroka et al. 2021; Phlai-ngam et al. 2022), and *A. clivosus* Chang & Yang, 1994 from Taiwan (Kang et al. 1994; Waltz et al. 1994; Kluge and Novikova 2014; Sroka et al. 2021).

Ecology

Alainites albai sp. nov. was first mentioned in the Rif and Middle Atlas by Dakki and El Agbani (1983) under the name *Baetis muticus*. This new species is confined preferentially to streams of northern Morocco, since it appears to be absent from the High Atlas (Bouzidi 1989; El Alami et al. 2022b), where it is replaced by its congener *A. oukaimeden*. Its absence in eastern Morocco (Berrahou et al. 2001; Mabrouki et al. 2019) and the Central Plateau (El Agbani et al. 1992) is probably related to excessive water heating.

In addition to the wide horizontal distribution of this species in northern Morocco, it has a fairly wide altitudinal distribution (5–1600 m) covering the three bioclimatic stages, the thermos-, meso-, and supra-Mediterranean. In fact, it shows a preference for the streams along the Mediterranean coastline with a semi-arid climate over regions with a sub-humid to humid climate. In certain

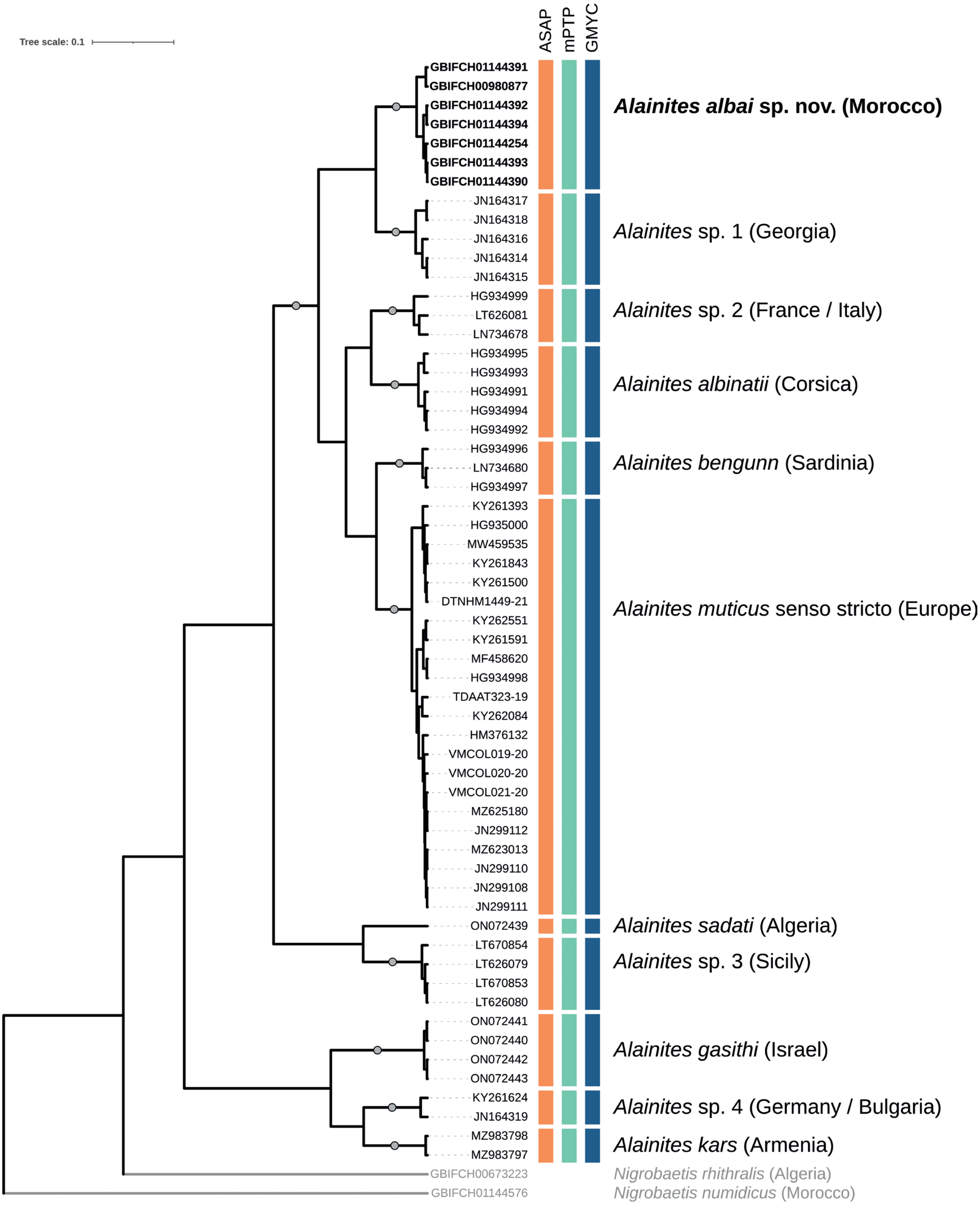

Figure 8. Bayesian (BEAST) maximum clade credibility COI tree of the genus Alainites in the West Palearctic: branch ends labelled with GBIF codes in bold indicate newly sequenced specimens; the DTNHM1449-21, TDAAT323-19, and VMCOL019-20–VMCOL021-20 codes are from BOLD; the other codes correspond to sequences obtained from GenBank. Colored vertical boxes indicate species delimitation hypothesis 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. Outgroup branches, labels, and species names are presented in grey.

mountainous regions it can thrive in conditions considered as perhumid. In the Rif, this species likes biotopes with a stony bottom rich in sand and submerged vegetation. In addition, it prefers the relatively cold waters of the upper and middle courses of wadis in which it reaches its ecological optimum during the summer period. In the Middle Atlas, its distribution is more restricted, as it has only been collected in two wadis at altitudes varying between 760 and 1500 m (Dakki 1987). However, recent surveys have shown the species to now be absent from these stations (Zerrouk et al. 2021).

Conclusions

The discovery of *A. albai* sp. nov. has increased the biodiversity of Morocco with a new endemic species of mayfly, highlighting the remarkable biodiversity and species richness of Ephemeroptera in the region. The recent identification of two new species, *Prosopistoma maroccanum* (El Alami et al., 2022a) and *Centroptilum alamiae* (Gattolliat et al., 2023), has significantly increased the proportion of Ephemeroptera endemism in Morocco to more than 33% (El Alami et al. 2022b). With the potential for further increases in the future, it is crucial to prioritize conservation and protection measures, particularly in sites hosting high species richness and endemic species.

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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: MEA. Investigation: SEY. Methodology: LV, SEY. Supervision: MEA, JLG. Writing – original draft: MEA. Writing – review and editing: LV, JLG.

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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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Research Article

Review of the *Lycocerus pallidulus* group (Coleoptera, Cantharidae), with descriptions of six new species from China

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Abstract

The *Lycocerus pallidulus* subgroup, originally placed in the *L. maculicollis* group, is suggested as an independent species group herein and its diagnosis is redefined. Ten previously known species of *Lycocerus* are attributed to this group, including *L. centrochinensis* (Švihla, 2004), *L. genaemaculatus* (Wittmer, 1951), *L. hubeiensis* (Švihla, 2004), *L. kubani* (Švihla, 2004), *L. zdeneki* (Švihla, 2004), *L. bilineatus* (Wittmer, 1995), *L. jelineki* (Švihla, 2004), *L. putzi* Švihla, 2011, *L. pictipennis* (Wittmer, 1995), and *L. curvatus* (Wittmer, 1995). Additionally, six new species of this group are described from China, including *L. laterophysus* **sp. nov.**, *L. flavipennis* **sp. nov.**, *L. putzimimus* **sp. nov.**, *L. maoershanensis* **sp. nov.**, *L. chongqingensis* **sp. nov.**, and *L. bispermathecus* **sp. nov.** These species are illustrated with photographs of habitus, aedeagi, abdominal sternites VIII, and reproductive systems of female. In addition, an identification key and a distribution map of the *L. pallidulus* group are provided.

Key words: Alpha taxonomy, *Lycocerus*, Oriental region, soldier beetles

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Introduction

Lycocerus Gorham, 1889 sensu Okushima (2005) is one of the largest genera in Cantharidae, with more than 300 species worldwide (Liu et al. 2022). No subgenera could be defined in the genus (Okushima 2005), but 16 species-groups have been proposed to date (Okushima 2005; Okushima and Brancucci 2008; Okushima and Yang 2013; Yang et al. 2014; Hsiao and Okushima 2015, 2016; Hsiao et al. 2016; Okushima and Hsiao 2017, 2021; Xi et al. 2021a, b, 2022; Wang et al. 2022). Among these species-groups, *L. maculicollis* group was proposed by Okushima (2005), originally contained three subgroups, including *L. ryukyuanus* subgroup, *L. maculicollis* subgroup and *L. pallidulus* subgroup. This species-group was characterized by median lobe of male genitalia without dorsal process, short spermathecal duct, spermatheca with one spiral tube which is relatively short and stout and gradually expanded to the base (Okushima 2005). However, the monophyly of *L. maculicollis* group was frequently not recovered, with the *L. pallidulus* subgroup in a distant position from *L. maculicollis* subgroup in the morphological phylogenetic trees (Hsiao 2021; Wang et al. 2022; Xi et al. 2022).

Prior to this study, three taxa were included in *L. pallidulus* subgroup, including *L. pallidulus* (Wittmer, 1995), *L. guerryi* (Pic, 1906), and *L. guerryi atroapicipennis* (Pic, 1914) (Okushima 2005). In our taxonomic study on the Chinese *Lycocerus*, we found some previously known species should be placed in this group, meanwhile, we discovered some hitherto unknown species. With more species added, we discovered that some species did not agree perfectly with the conventional characteristics, also referred to in the previous phylogenetic results and these need further work.

Materials and methods

The studied material is preserved in the following collections:

CWNU	China West Normal University, Nanchong, China;
IZAS	Institute of Zoology, Chinese Academy of Sciences, Beijing, China;
MHBU	Museum of Hebei University, Baoding, China;
MNHN	Muséum national d'Histoire naturelle, Paris, France;
NHMB	Naturhistorisches Museum Basel, Switzerland;
NMPC	National Museum, Prague, Czech Republic;
NWAFU	Northwest Agriculture & Forestry University, Yangling, China;
ZFMK	Zoological Research Museum Alexander Koenig, Bonn, Germany.

We identified specimens based on examination of the type material and reference to the relevant publications (Wittmer 1995; Švihla 2004, 2011; Okushima 2005), from which we also derived our taxon concepts and taxonomic classification. Morphological terminology used in this study mainly follows that of Okushima (2005) and Kazantsev (2023).

Genitalia of both sexes and abdominal sternites VIII of females were dissected and cleared in 10% NaOH solution, and female genitalia was dyed with hematoxylin. Habitus photos were taken by a Leica M205A stereomicroscope. Line drawings were made using a camera lucida attached to a Nikon SMZ1500 stereomicroscope, then edited in CorelDraw 12 and Adobe Photoshop CS6.13.0.

The label of the specimens in Chinese are transliterated, and the complete label data are cited for the type specimens. The distribution information was collected from the publications (Wittmer 1951, 1995; Švihla 2004, 2011) and the examined material of the present study. The distribution map was prepared by ArcMap 10.8 and edited in Adobe Photoshop CS6.13.0.

Results

Lycocerus pallidulus group

Common characteristics. Body middle-sized (8.0–11.5 mm), slender. Antennae filiform, present or absent with impressions on middle antennomeres in male. Pronotum subquadrate, longer than or nearly as long as wide. Elytra pale yellow or even transparent, sometimes with black longitudinal bands or markings on disc, subparallel-sided. Tarsal claws diverse: if all simple in male, fore and mid-anterior and or posterior claws each with a digitiform tooth at base in female; when fore and mid-anterior claws each with a tooth in male, same

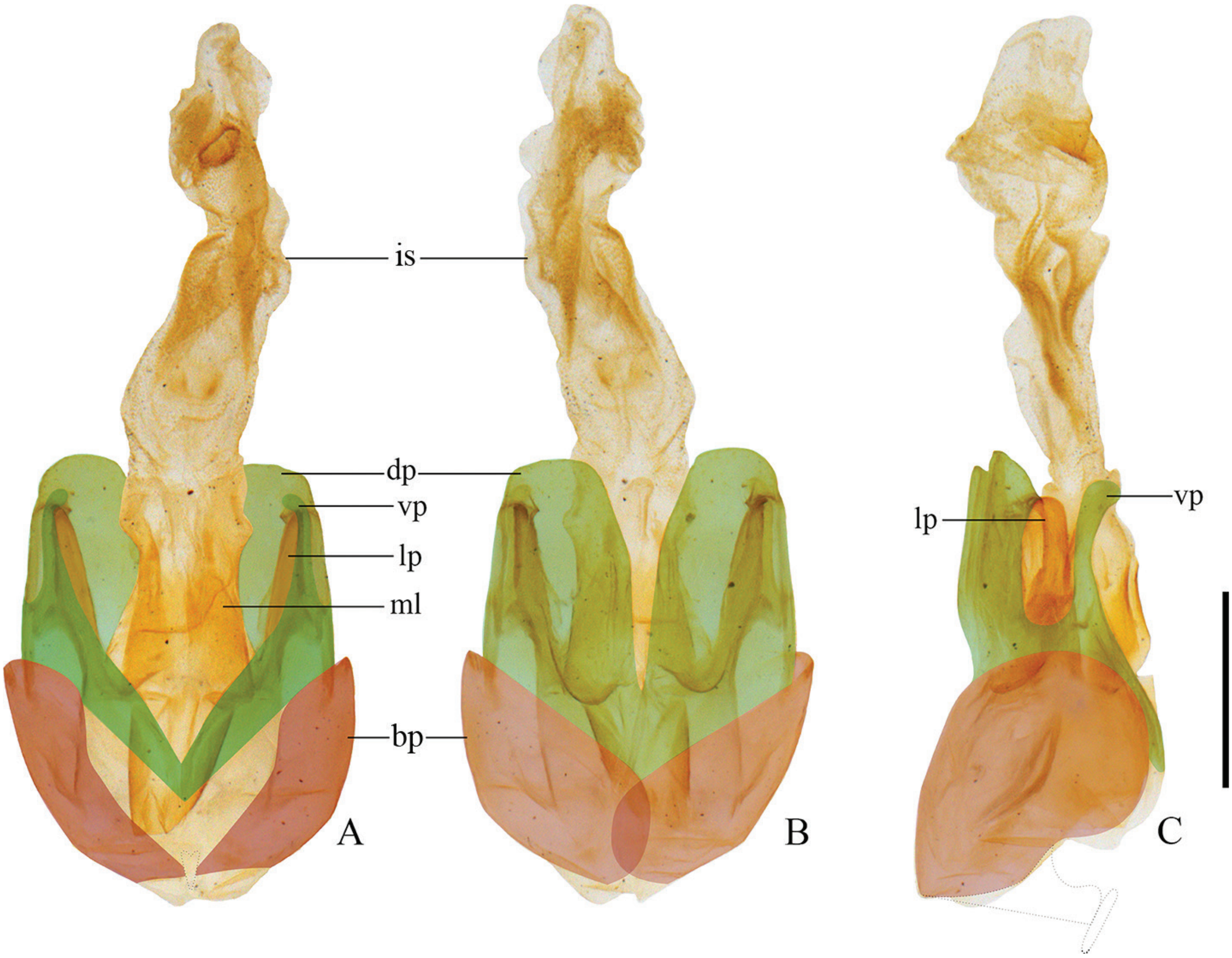

Figure 1. Aedeagus of *Lycocerus curvatus* (Wittmer, 1995): **A** ventral view **B** dorsal view **C** lateral view. Abbreviations: bp – basal piece; dp – dorsal plate of each paramere; lp – laterophyse; ml – median lobe; vp – ventral process of each paramere; is – inner sac. Scale bar: 1.0 mm. The red hues show basal pieces (with middle nodule in ventral part missing), green for parameres, and yellow represents median lobe exhibiting with inner sac and laterophyses.

in female or both anterior and posterior claws each with a tooth; otherwise, fore and mid-anterior and posterior claws each with a tooth in both sexes; hind claws always simple. Aedeagus (Fig. 1): dorsal plates of parameres separate, each with a keel near lateral margin and located on inner surface, laterophyses well-developed and nearly as long as ventral processes, with apices opposite to the keels of dorsal plates, inner sac of median lobe lengthened apically and nearly as long as tegmen, without dorsal process. Female internal genitalia: vagina stout and abruptly thinned at ventroapical portion into a stout tube, where diverticulum and spermathecal duct arising separately; diverticulum moderately long, thin, and spiral; spermathecal duct short and stout; spermatheca with a spiral tube, often abruptly thinned apically near base, basal portion of spermatheca extended into a short tube, where accessory gland opening, accessory gland longer than spermatheca.

Included species. *L. pallidulus* (Wittmer, 1995), *L. guerryi* (Pic, 1906), *L. guerryi atroapicipennis* (Pic, 1914), *L. centrochinensis* (Švihla, 2004), *L. genaemaculatus* (Wittmer, 1951), *L. hubeiensis* (Švihla, 2004), *L. jelineki* (Švihla, 2004), *L. putzi* Švihla, 2011, *L. bilineatus* (Wittmer, 1995), *L. zdeneki* (Švihla, 2004), *L. kubani* (Švihla, 2004), *L. curvatus* (Wittmer, 1995), *L. pictipennis* (Wittmer, 1995), *L. laterophysus* sp. nov., *L. flavipennis* sp. nov., *L. putzimimus* sp. nov., *L. maoershanensis* sp. nov., *L. chongqingensis* sp. nov. and *L. bispermathecus* sp. nov.

Distribution (Fig. 2). China (Yunnan, Sichuan, Fujian, Jiangsu, Shanghai, Jiangxi, Hubei, Shaanxi, Ningxia, Guangxi, Zhejiang); Vietnam; Myanmar.

Lycocerus pallidulus **(Wittmer, 1995)**

Athemus (*Isathemus*) *pallidulus* Wittmer, 1995: 261, figs 119, 189.
Lycocerus pallidulus: Okushima 2005: 48, figs 11b, 12c, 14b, 22, 74.

Type material examined. ***Holotype*****:** 1♂ (NHMB), Yunnan, Lijiang, 1800 m, 26°53'N, 100°18'E, 23.VI–21.VII.1992, lgt. S. Becvar.

Distribution. China (Yunnan).

Remarks. This species was omitted from the Palaearctic Catalogue by Kazantsev and Brancucci (2007). Both the aedeagus and female internal genitalia of this species have been well illustrated by Okushima (2005).

Lycocerus guerryi **(Pic, 1906)**

Figs 3A–C, 12C, 16C

Cantharis guerryi Pic, 1906: 83.
Athemus (*Isathemus*) *guerryi*: Wittmer 1995: 261.
Lycocerus guerryi: Okushima 2005: 48; Kazantsev and Brancucci 2007: 250.

Type material examined. ***Holotype*****:** 1♂ (MNHN), China, P. Guerry.

Non-type material examined. China: 4♂1♀ (IZAS), Yunnan, Dali, 2100 m, 31.V.1955, leg. L. Wu; 1♀ (IZAS), Yunnan, Dali, 2100 m, 30.V.1955, leg. Bussik; 1♀ (IZAS), Yunnan, Xiaguan, 2050 m, 30.V.1955, leg. S. C. Ha.

Descriptive notes. Male. Aedeagus: basal piece nearly as long as dorsal plate of each paramere (Fig. 3A–C); ventral process of each paramere stout and abruptly narrowed near apex, obviously bent inwards in ventral view (Fig. 3A), slightly bent dorsally in lateral view (Fig. 3C); dorsal plates of parameres obviously longer than ventral processes, with lateral margins slightly sinuate in middle, apical margins slightly arcuate and descending outwards in lateral view (Fig. 3B); laterophyse feebly shorter than ventral process, with apex acute and directing dorso-outwards (Fig. 3A); inner sac of median lobe with a stout tube extruding near base (Fig. 3A, B).

Female. Internal organ of reproductive system (Fig. 12C): spermatheca nearly as long as diverticulum; accessory gland ~ 2.5× longer than spermatheca.

Abdominal sternite VIII (Fig. 16C): triangular emarginations on both sides and in middle of posterior margin, lateral emarginations slightly deeper and much wider than the middle one, the portions between lateral and middle emarginations wide and obtuse-triangular at apices, slightly extending over apices of latero-apical angles, which are nearly truncated.

Distribution. China (Yunnan).

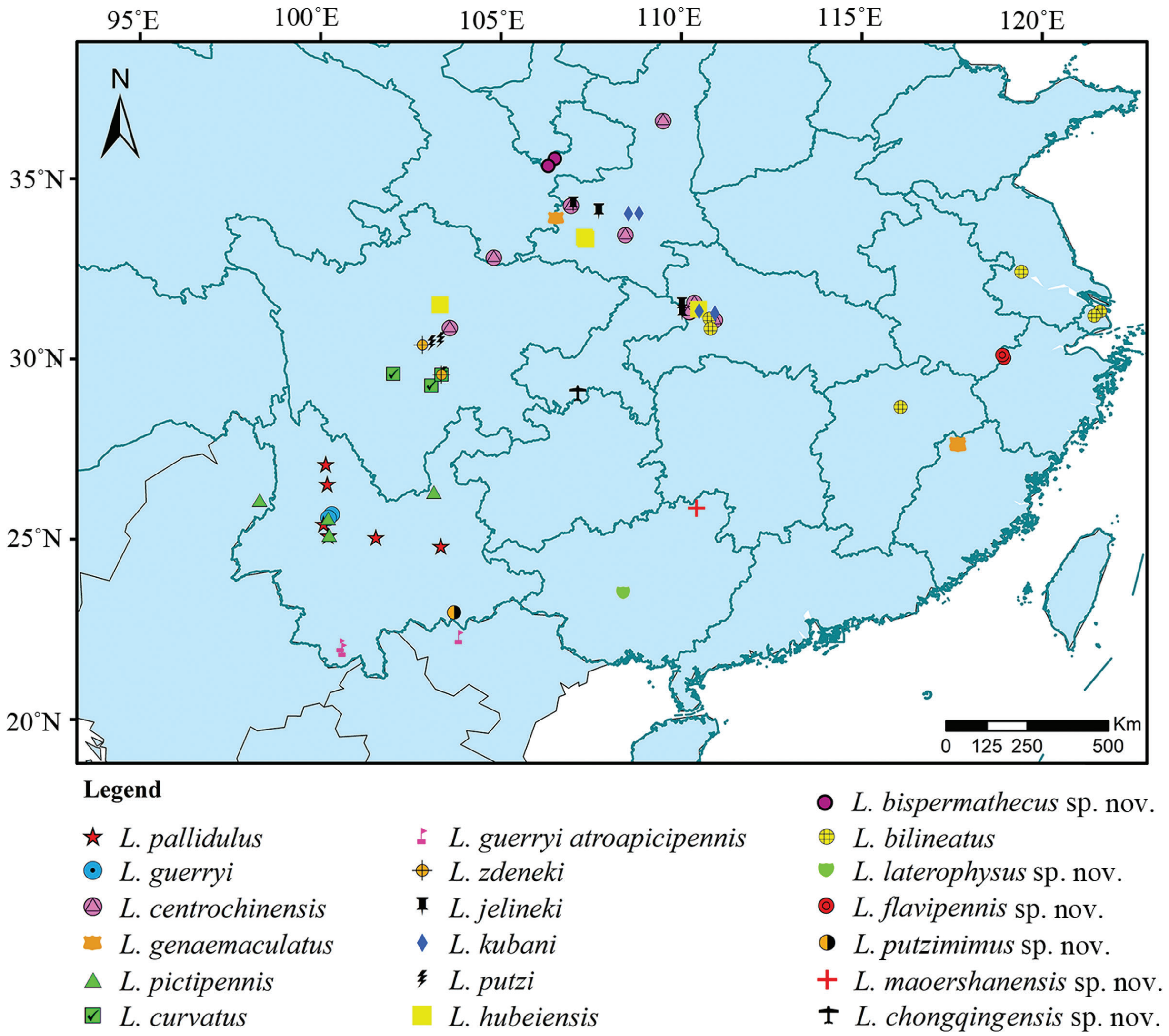

Figure 2. Distribution map of *Lycocerus pallidulus* group.

Remarks. *Cantharis jeanvoinei* Pic, 1927 was listed as a synonym of *L. guerryi* by Kazantsev and Brancucci (2007), which is an obvious error. In fact, the former was synonymized with *L. guerryi atroapicipennis* (Pic, 1914) by Wittmer (1995), but was missing in the Palaearctic Catalogue (Kazantsev and Brancucci 2007).

Lycocerus guerryi atroapicipennis (Pic, 1914)

Cantharis atroapicipennis Pic, 1914:8.
Athemus (*Isathemus*) *guerryi atroapicipennis*: Wittmer 1995: 274, fig. 139.
Cantharis jeanvoinei Pic, 1927: 2. Synonymized by Wittmer 1995: 274.
Lycocerus guerryi atroapicipennis: Okushima 2005: 48.

Type material examined. *Holotype* of *Cantharis atroapicipennis*: 1♂ (MNHN), Lao Kay.

Holotype of *Cantharis jeanvoinei:* 1♀ (MNHN), Tonkin, Chapa, 7.V.1918, Eanvoine.

Non-type material examined. China: 1♂ (IZAS), Yunnan, Menlongbanna, Mengsong, 1600 m, 22.IV.1958, leg. C. B. Hong; 1♂ (IZAS), same data as the preceding, 23.IV.1958; 1♂(IZAS), Yunnan, Menghai, Nannuoshan, 1100 m, 28.IV.1957, leg. F. J. Pu.

Distribution. China (new record: Yunnan); Vietnam.

Remarks. The aedeagus of this subspecies is very similar to that of *L. guerryi guerryi*, but its elytra are darkened at apices enabling external identification. The body coloration is constant, and the elytra of the individuals from northern Yunnan are uniformly pale yellow (*L. guerryi guerryi*), while those from southern Yunnan and adjacent area (northern Vietnam) are always darkened at apices (*L. guerryi atroapicipennis*), so they are kept as two subspecies at the moment. Here, *L. guerryi atroapicipennis* is recorded in the Chinese fauna for the first time.

Lycocerus centrochinensis **(Švihla, 2004)**

Figs 3D–F, 12B, 16B

Athemus (s.str.) *centrochinensis* Švihla, 2004: 182, figs 83–85, 202.
Lycocerus centrochinensis: Kazantsev and Brancucci 2007: 250.

Type material examined. ***Holotype***: 1♂ (NMPC), China, Shaanxi, Quing Ling Shan mts, m rd. Baoji-Taibai, 35 km S Baoji, 21–23.VI.1998, lgt. O. Šafránek & M. Trýzna.

Non-type material examined. China: 2♂1♀ (MHBU), Sichuan, Wolong, 6–7.VIII.2004, leg. X. J. Yang & H. R. Hua; 1♂1♀ (MHBU), Gansu, Wenxian, Huangtulling, 2350 m, 8.VII.2003, leg. Y. B. Ba & Y. Yu; 1♂ (MHBU), Hubei, Dalaoling Nature Reserve, 1200 m, 9.VII.2011, leg. X. L. Liang; 1♀ (MHBU), same locality as the preceding, 11.VII.2011, leg. X. Liao; 1♂ (MHBU), Hubei, Shennongjia, Muyuzhen, 1200 m, 12.VII.2004, leg. S. Q. Xu; 1♀ (MHBU), same data as the preceding, leg. F. L. Zou; 1♀ (MHBU), Hubei, Shennongjia, Wenshui Forestry, 1700–2000 m, 16.VII.2003, leg. C. Gui; 1♂2♀ (MHBU), Shaanxi, Fengxian, Jialing, Jiangyuan, 13.VII.2012, leg. G. D. Ren; 1♂4♀ (MHBU), Shaanxi, Ningshaan, Huoditang, 1505 m, 33.434126°N, 108.448091°E (DD), 15.VIII.2013, leg. X. C. Zhu & Y. Tian.

Descriptive notes. Male. Aedeagus: basal piece slightly longer than dorsal plate of each paramere (Fig. 3D–F); ventral process of each paramere thin and straight, approaching to each other in ventral view (Fig. 3D) and inclining ventrally in lateral view (Fig. 3F); dorsal plates of parameres slightly longer than ventral processes (Fig. 3D, F), with inner margins abruptly diverging near middle, apical margins slightly arcuate and descending outwards in dorsal view (Fig. 3E); laterophyse feebly longer than ventral process, with apices acute and directing dorso-outwards (Fig. 3D, F).

Female. Internal organ of reproductive system (Fig. 12B): spermatheca nearly as long as diverticulum; accessory gland ~ 3.0× longer than spermatheca.

Abdominal sternite VIII (Fig. 16B): rounded emargination in middle and triangular emarginations on both sides of posterior margin, lateral emarginations obviously deeper than and nearly as wide as the middle one, the portions between lateral and middle emarginations moderately wide and right-angled at apices, obviously extending over apices of latero-apical angles, which are narrowly triangular.

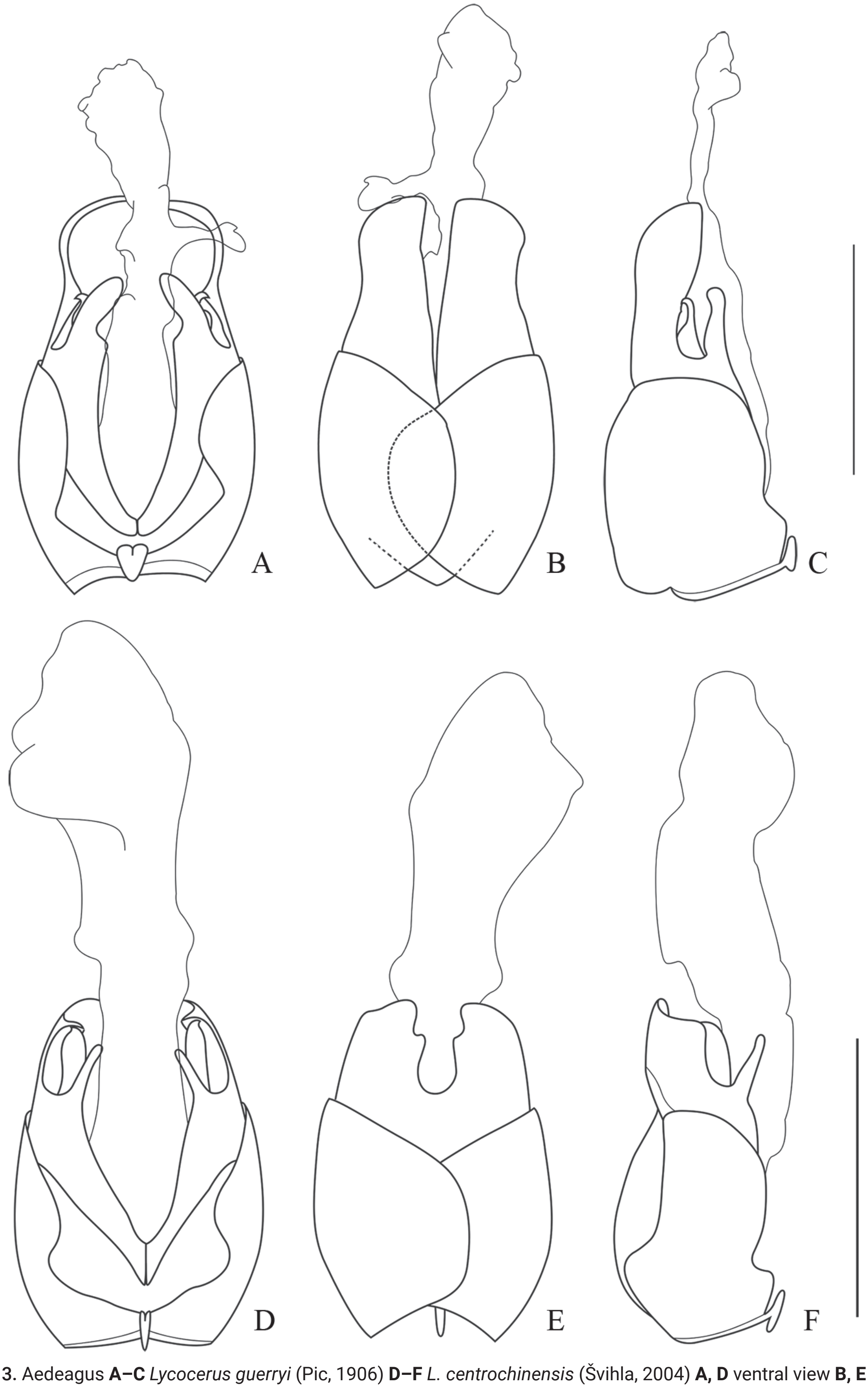

Figure 3. Aedeagus **A–C** *Lycocerus guerryi* (Pic, 1906) **D–F** *L. centrochinensis* (Švihla, 2004) **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

Distribution. China (Shaanxi, Hubei, Sichuan, Gansu).

Remarks. Li et al. (2015) provided an illustration of female internal genitalia for *L. centrochinensis*, which is of an unknown species. Additionally, the distribution range of this species is expanded, with geographic records added from Sichuan and Gansu provinces.

Lycocerus genaemaculatus (Wittmer, 1951)

Figs 4A–C, 12D, 16D

Athemus genaemaculatus Wittmer, 1951: 100.
Athemus (*Isathemus*) *genaemaculatus*: Wittmer 1995: 259.
Lycocerus genaemaculatus: Kazantsev and Brancucci 2007: 250.

Type material examined. ***Holotype*:** 1♂ (ZFMK), Kuatun (Fukien), 2300 m, 27.40n, Br. 117.40ö, 20.V.1938, L. J. Klapperich. ***Paratypes*:** 1♀ (NHMB), Fukien (Fujian), Kuatun, 21.IV.1946, lgt. Tschung Sen; 1♀ (NHMB), Fukien (Fujian), Kuatun, 2300 m, 27°40'N, 117°40'E (DDM), 13.V.1938, lgt. J. Klapperich.

Non-type material examined. China: 1♂ (IZAS), Fujian, Jianyang, Huangkeng, Aotou, 950 m, 2.V.1960, leg. Y. R. Zhang; 1♀ (IZAS), Fujian, Jianyang, Huangkeng, Guilin, 270–340 m, 8.IV.1960, leg. Y. R. Zhang; 1♀ (IZAS), same locality as the preceding, 290–320 m, 12.IV.1960, leg. F. J. Pu.

Descriptive notes. Male. Aedeagus: basal piece distinctly longer than dorsal plate of each paramere (Fig. 4A–C); ventral process of each paramere short and thin, nearly straight and approaching to each other in ventral view (Fig. 4A), slightly bent dorsally in lateral view (Fig. 4C); dorsal plates of parameres as long as ventral process (Fig. 4B, C), with outer margins abruptly converging apically, apical margins subrounded in dorsal view (Fig. 4B); laterophyse feebly shorter than ventral process, with apices acute and markedly directing dorso-outwards (Fig. 4C).

Female. Internal organ of reproductive system (Fig. 12D): spermatheca nearly as long as diverticulum; accessory gland ~ 3.0× longer than spermatheca.

Abdominal sternite VIII (Fig. 16D): triangular emargination in middle and subrounded emarginations on both sides of posterior margin, lateral emarginations slightly deeper and obviously wider than the middle one, the portions between lateral and middle emarginations narrow and acute at apices, obviously extending over apices of latero-apical angles, which are widely triangular.

Distribution. China (Fujian).

Remarks. The aedeagus, abdominal sternite VIII, and internal genitalia of female are illustrated for the first time for this species herein.

Lycocerus hubeiensis (Švihla, 2004)

Figs 4D–F, 12A, 16A

Athemus (*Isathemus*) *hubeiensis* Švihla, 2004: 191, figs 124, 125.
Lycocerus hubeiensis: Kazantsev and Brancucci 2007: 250.

Type material examined. ***Holotype*:** 1♂ (NMPC), China, Hubei, Dashennongjia mts., 2000–3000 m, 31°05'N, 103°03'E (DDM), 21–24.VI.2001, lgt. O. Šafánek.

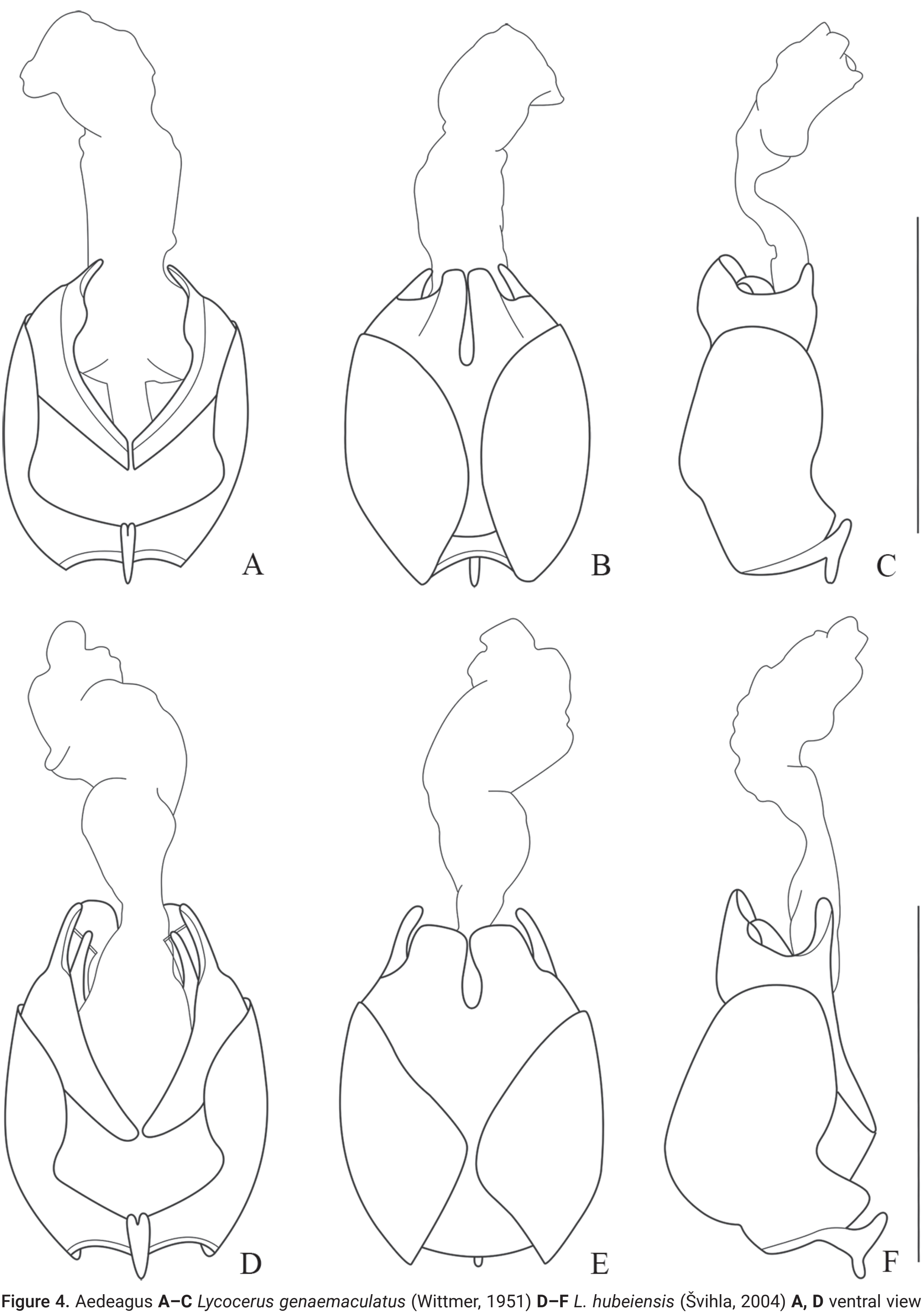

Figure 4. Aedeagus **A–C** *Lycocerus genaemaculatus* (Wittmer, 1951) **D–F** *L. hubeiensis* (Švihla, 2004) **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

Non-type material examined. China: 1♂ (MHBU), Shaanxi, Yangxian, Huayanghanba, 1014 m, 33°32'52"N, 107°35'5"E (DMS), 6.V.2017, leg. H. Y. L iu; 1♀ (MHBU), Shaanxi, Yangxian, Huayangzhen, Yantou, 1206 m, 33°38'29"N, 107°31'6.9"E (DMS), 7.VIII.2017, leg. H. Y. Liu & X. D. Zhang; 1♀ (MHBU), Hubei, Chaoshuihe, 23.V. 2019, leg. P. Wang.

Descriptive notes. Male. Aedeagus: basal piece distinctly longer than dorsal plate of each paramere (Fig. 4D–F); ventral process of each paramere nearly straight, feebly bent inwards in ventral view (Fig. 4D) and vertical in lateral view (Fig. 4F); dorsal plates of parameres feebly longer than ventral processes (Fig. 4D, F), with outer margin obviously abruptly converging apically, apical margins truncate in dorsal view (Fig. 4E); laterophyse slightly shorter than ventral process, with apices acute and appreciably directing dorso-inwards (Fig. 4F).

Female. Internal organ of reproductive system (Fig. 12A): spermatheca nearly as long as diverticulum; accessory gland ~ 2.5× longer than spermatheca.

Abdominal sternite VIII (Fig. 16A): triangular emargination in middle and narrowly rounded emarginations on both sides of posterior margin, lateral emarginations obviously deeper and feebly wider than the middle one, the portions between lateral and middle emarginations moderately wide and right-angled at apices, obviously extending over apices of latero-apical angles, which are narrowly rounded.

Distribution. China (Hubei, Shaanxi).

Remarks. The aedeagus of this species was illustrated only in ventral and lateral views by Švihla (2004). Here, the aedeagus is illustrated in ventral, dorsal, and lateral views, and the abdominal sternite VIII and internal genitalia of the female are illustrated for the first time. Additionally, the distribution range of this species is expanded, with geographic records added from Shaanxi Province.

Lycocerus kubani **(Švihla, 2004)**

Figs 5A–C, 14C, 16G

Athemus (*Isathemus*) *kubani* Švihla, 2004: 190, figs 122, 123, 207.
Lycocerus kubani: Kazantsev and Brancucci 2007: 251.

Type material examined. ***Holotype***: 1♂ (NMPC), China, Shaanxi, Haozhenzi env., 1350–2000m, 14–24.VI.1999, lgt. S. Murzin.

Non-type material examined. China: 1♂, 1♀ (IZAS), Hubei, Xingshan, Xiaohekou, 700 m, 11.V.1994, leg. X. K. Yang; 1♀ (IZAS), Hubei, Xingshan, Longmenhe, 1400 m, 16.VI.1993, leg. Z. R. Huang; 1♀ (IZAS), same locality as the preceding, 1310 m, 15.VI.1993, leg. J. Yao.

Descriptive notes. Male. Aedeagus: basal piece obviously longer than dorsal plate of each paramere (Fig. 5A–C); ventral process of each paramere thin and nearly straight, approaching to each other in ventral view (Fig. 5A) and inclining dorsally in lateral view (Fig. 5C); dorsal plates of parameres feebly longer than ventral processes (Fig. 5A, C), with outer margins converging apically in dorsal view, apical margins truncate at apices (Fig. 5B); laterophyse feebly shorter than ventral process, with apices acute and directing dorso-outwards (Fig. 5C).

Female. Internal organ of reproductive system (Fig. 14C): spermatheca feebly longer than diverticulum; accessory gland ~ 1.2× longer than spermatheca.

Abdominal sternite VIII (Fig. 16G): triangular emargination in middle and rounded emarginations on both sides of posterior margin, lateral emarginations obviously deeper and wider than the middle one, the portion between lateral and middle emarginations narrow and acute at apices, obviously extending over apices of latero-apical angles, which are rounded.

Distribution. China (Shaanxi, Hubei).

Remarks. The aedeagus of this species was illustrated only in ventral and lateral views by Švihla (2004). Here, the aedeagus is illustrated in ventral, dorsal, and lateral views, and female internal genitalia is illustrated for the first time. Additionally, the distribution range of this species is expanded, with geographic records added from Hubei province.

Lycocerus zdeneki **(Švihla, 2004)**

Figs 5D–F, 14B, 16F

Athemus (*Isathemus*) *zdeneki* Švihla, 2004: 192, figs 126–128.
Lycocerus zdeneki: Kazantsev and Brancucci 2007: 254.

Type material examined. ***Holotype***: 1♂ (NMPC), China, Sichuan, Baoxing, 100 km N of Yaan, 12–14.VII.1995, lgt. Zd. Jindra.

Non-type material examined. China: 1♂ (IZAS), Sichuan, Emeishan, Xixiangdi, 1800–2000 m, 25.VI.1957, leg. F. X. Zhu; 1♀ (IZAS), same locality as the preceding, 12.VII.1957, leg. F. X. Zhu.

Descriptive notes. Male. Aedeagus: basal piece slightly longer than dorsal plate of each paramere (Fig. 5D–F); ventral process of each paramere slender and slightly expanded at apices, nearly straightly and approaching to each other in ventral view (Fig. 5D), inclining ventrally in lateral view (Fig. 5F); dorsal plates of parameres feebly longer than ventral process (Fig. 5D, F), with inner margins feebly emarginate at inner apical angles, outer margins slightly converging apically in dorsal view (Fig. 5E); laterophyse slightly longer than ventral process, with apices acute and appreciably directing dorso-outwards (Fig. 5F).

Female. Internal organ of reproductive system (Fig. 14B): spermatheca slightly longer than diverticulum; accessory gland slightly longer than spermatheca.

Abdominal sternite VIII (Fig. 16F): right-triangular emargination in middle and subrounded emarginations on both sides of posterior margin, lateral emarginations feebly deeper and wider than the middle one, the portions between lateral and middle emarginations wide and wide-triangular at apices, feebly extending over apices of latero-apical angles, which are truncated.

Distribution. China (Sichuan).

Remarks. Only the apical parts of the aedeagus of this species was illustrated by Švihla (2004). In this work, the aedeagus is illustrated in general views, and the abdominal sternite VIII and internal genitalia of the female are illustrated for the first time.

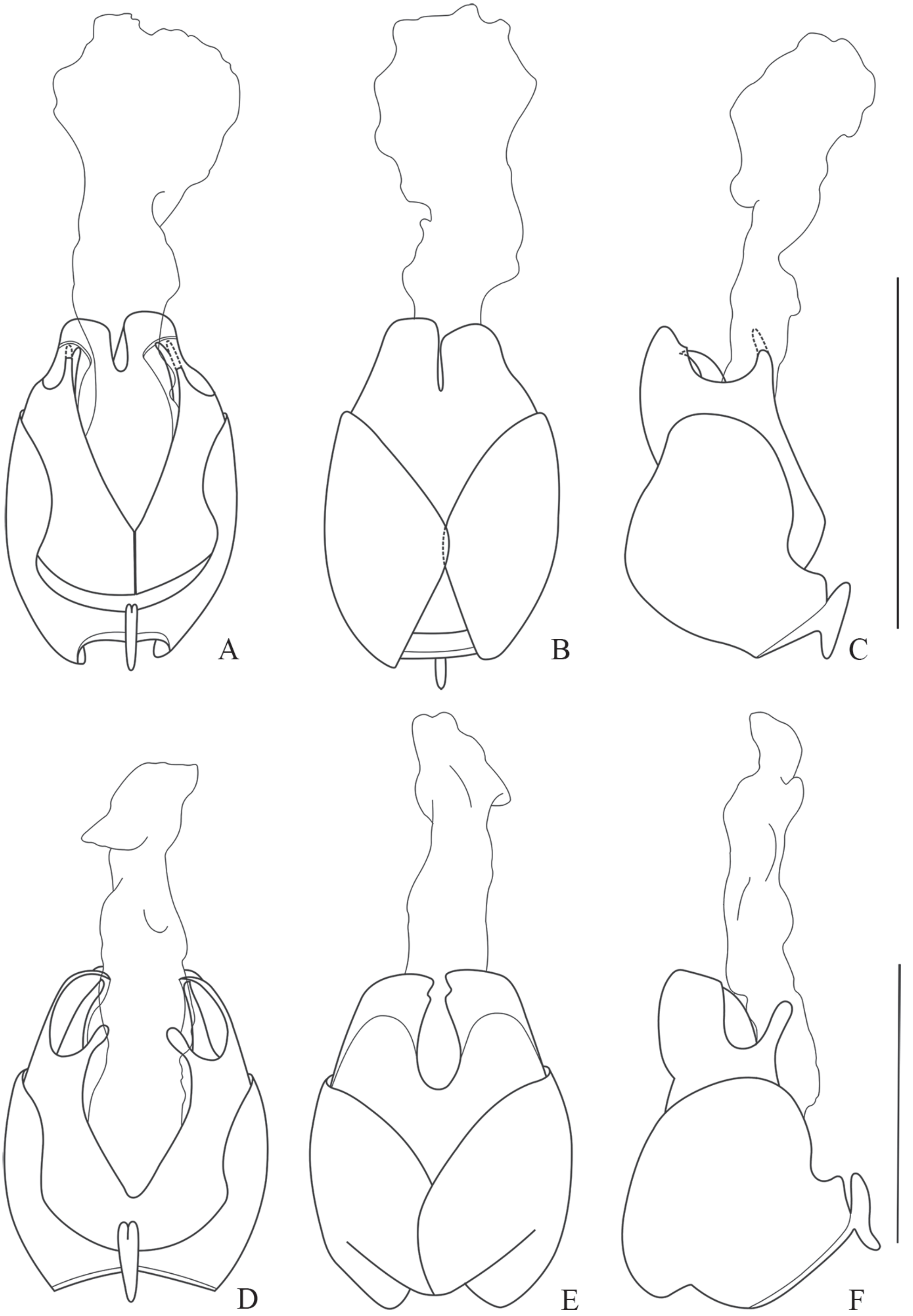

Figure 5. Aedeagus **A–C** *Lycocerus kubani* (Švihla, 2004) **D–F** *L. zdeneki* (Švihla, 2004) **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

Lycocerus bilineatus **(Wittmer, 1995)**

Figs 6A–C, 13A, 17A

Athemus (*Isathemus*) *bilineatus* Wittmer, 1995: 275, figs 140, 141.
Athemus (s.str.) *amplus* Wittmer, 1995: 278, figs 146, 147, 203.
Lycocerus amplus: Kazantsev and Brancucci 2007: 249. Synonymized by Yang et al. 2013: 10, fig. 8.
Lycocerus bilineatus: Kazantsev and Brancucci 2007: 249; Yang et al. 2013: 10, fig. 7.

Type material examined. See Yang et al. (2013).

Non-type material examined. China: 2♂, 1♀ (IZAS), Jiangxi, date and collector unknown; 1♀ (IZAS), Shanghai, 1947, leg. Marist Brothers.

Descriptive notes. Male. Aedeagus: basal piece feebly longer than dorsal plate of each paramere (Fig. 6A–C); ventral process of each paramere stout and obviously bent inwards apically in ventral view (Fig. 6A), inclining ventrally in lateral view (Fig. 5C); dorsal plates of parameres obviously longer than ventral processes (Fig. 5A, C), with apical margins rounded (Fig. 5B); laterophyse feebly longer than ventral process, with apices acute and directing dorso-outwards (Fig. 5A, C).

Female. Internal organ of reproductive system (Fig. 13A): spermatheca feebly longer than diverticulum; accessory gland nearly as long as spermatheca.

Abdominal sternite VIII (Fig. 17A): triangular emarginations in middle and on both sides of posterior margin, lateral emarginations obviously deeper and wider than the middle one, the portion between lateral and middle emarginations wide and right-angled at apices, obviously extending over apices of latero-apical angles, which are narrowly rounded.

Distribution. China (Jiangsu, Shanghai, Jiangxi, Hubei).

Remarks. The aedeagus of this species was illustrated only in ventral and lateral views by Wittmer (1995). Herein, the aedeagus is illustrated in ventral, dorsal, and lateral views, and the female internal genitalia is illustrated for the first time.

Lycocerus jelineki **(Švihla, 2004)**

Figs 6D–F, 13B, 17B

Athemus (*Andrathemus*) *jelineki* Švihla, 2004: 189, figs 109–111, 204.
Lycocerus jelineki: Kazantsev and Brancucci 2007: 251.

Type material examined. ***Holotype***: 1♂ (NMPC), China, Shaanxi, Qinling mts. m rd. Baoji-Taibai, 35 km S Baoji, 21–23.VI.1998, lgt. O. Šafránek & M. Trýzna.

Non-type material examined. China: 1♂, W Hubei prov. Dashennongjia Nat. Res. Muyu, E slope, 2000 m, 12–15.VI.1997, leg. Bolm; 1♂ (MHBU), Hubei, Shennongjia, Pingqian, 1576 m, 31°28'08.0"N, 110°02'23.4"E (DMS), 4–7.VII.2014, leg. Y.B. Ba & S. Y. Tang; 1♂ (NWAFU), Shaanxi, Taibaishan, Haopingsi, 1200 m, 31.V.1981, collector unknown; 1♂ (NWAFU), Shaanxi, Taibaishan, Zhongshansi, 1500 m, 9.VI.1981, collector unknown; 1♀ (NWAFU), Shaanxi, Taibaishan, Haopingsi, 25.VI.1982, collector unknown; 1♀ (NWAFU), Shaanxi, Taibaishan, Zhongshansi, 400 m, 11.VI.1981, collector unknown.

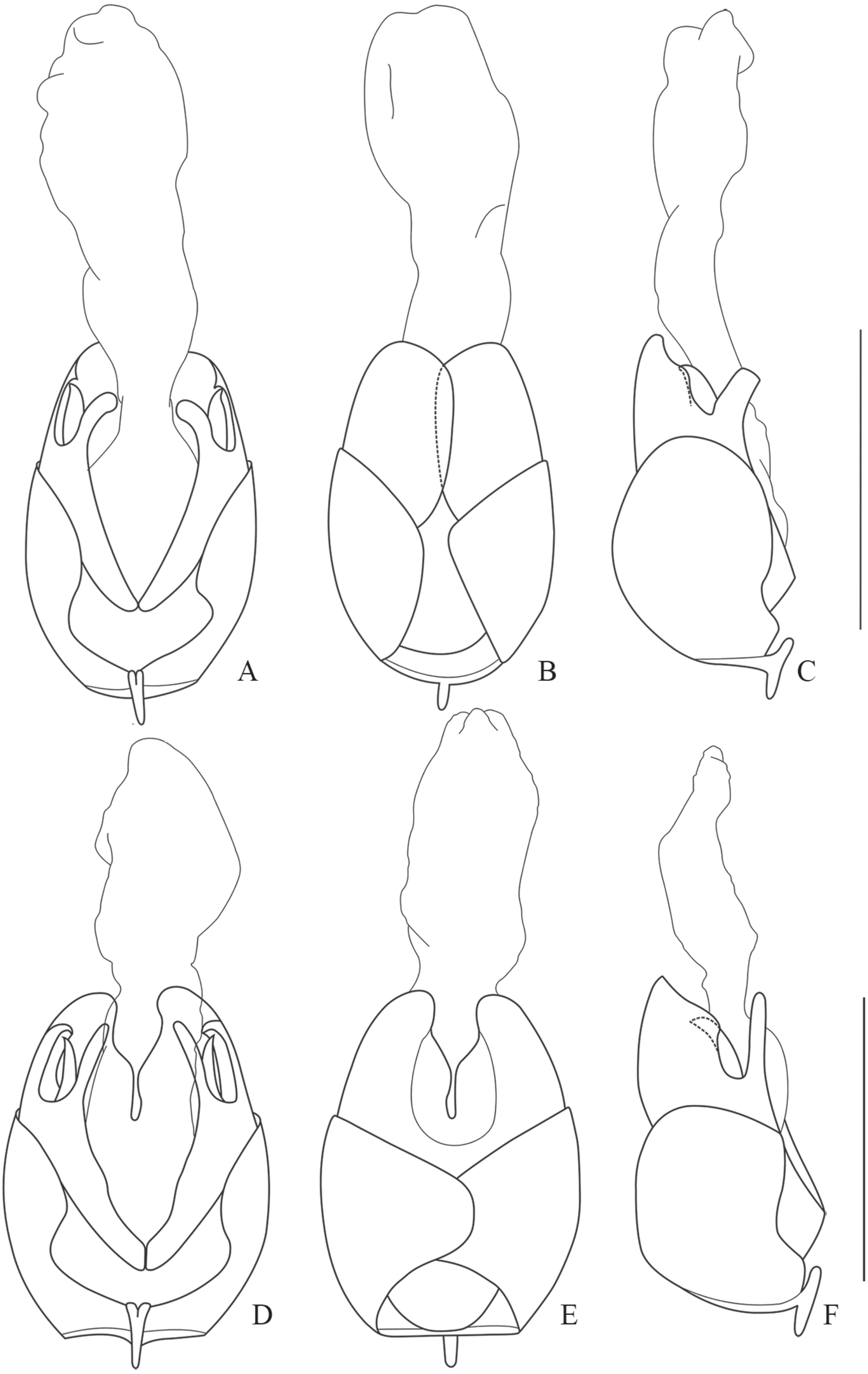

Figure 6. Aedeagus **A–C** *Lycocerus bilineatus* (Wittmer, 1995) **D–F** *L. jelineki* (Švihla, 2004) **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

Descriptive notes. Male. Aedeagus: basal piece feebly longer than dorsal plate of each paramere (Fig. 6D–F); ventral process of each paramere nearly slender and approaching to each other in ventral view (Fig. 6D), nearly vertical in lateral view (Fig. 6F); dorsal plates feebly longer than ventral process (Fig. 6D, F), with inner margins abruptly diverging near middle, outer margins slightly converging apically, apical margins subrounded (Fig. 6E); laterophyse slightly shorter than ventral process, with apices acute and appreciably directing dorso-outwards (Fig. 6D, F).

Female. Internal organ of reproductive system (Fig. 13B): spermatheca nearly as long as diverticulum; accessory gland ~ 2× longer than spermatheca.

Abdominal sternite VIII (Fig. 17B): rounded emargination in middle and subtriangular emargination on both sides of posterior margin, lateral emarginations deeper and feebly narrower than the middle one, the portion between lateral and middle emarginations wide and widely triangular at apices, extending over apices of latero-apical angles, which are subrounded.

Distribution. China (Shaanxi, Hubei).

Remarks. Sometimes the pronotum and vertex have dark brown spots, in both sexes. Only the apical parts of the aedeagus were illustrated by Švihla (2004). Here, the aedeagus is illustrated in general views, and the female internal genitalia is illustrated for the first time.

Lycocerus putzi **Švihla, 2011**

Figs 7A–C, 13D, 17D

Lycocerus putzi Švihla, 2011: 11, figs 12, 60, 61–63.

Non-type material examined. China: 1♂1♀(NHMB), Sichuan, Chengdu, Qingchengshan, 1360 m, 30°44'N, 103°08'E (DDM), 28.VIII.2004, leg. S. Murzin; 1♂ (IZAS), Sichuan, Emeishan, Qingyinge, 800–1000 m, 25.IV.1957, lg. K. R. Huang.

Descriptive notes. Male. Aedeagus: basal piece feebly longer than dorsal plate of each paramere (Fig. 7A–C); ventral process of each paramere nearly straight and approaching to each other in ventral view (Fig. 7A), nearly vertical in lateral view (Fig. 7C); dorsal plates of parameres obviously longer than ventral processes (Fig. 7A, C), outer margins obviously converging apically, apical margins rounded in dorsal view (Fig. 7B); laterophyse feebly longer than ventral process, with apices acute and appreciably directing dorso-outwards (Fig. 7A, C).

Female. Internal organ of reproductive system (Fig. 13D): spermatheca obviously longer than diverticulum; accessory gland ~ 2.5× longer than spermatheca.

Abdominal sternite VIII (Fig. 17D): triangular emargination in middle and subrounded emarginations on both sides of posterior margin, lateral emarginations feebly deeper and obviously wider than the middle one, the portion between lateral and middle emarginations narrow and acute at apices, obviously extending over apices of latero-apical angles, which are rounded.

Distribution. China (Sichuan).

Remarks. The aedeagus of this species was illustrated only for apical parts by Švihla (2011). Here, the aedeagus is illustrated with general views, and the female internal genitalia is illustrated for the first time.

Lycocerus pictipennis **(Wittmer, 1995)**

Fig. 7D–F

Athemus (*Isathemus*) *pictipennis* Wittmer, 1995: 276, figs 142, 143.

Type material examined. ***Holotype*****:** 1♂ (NHMB), China, Yunnan, Dongchuan, 26°31'07'N, 103°14'E (DDM), 28.VI–3.VII.1994, leg. Vit Kubáň.

Non-type material examined. 1♂ (MHBU), Myanmar, Prov. Kachin Mt Emaw Bum, 2358 m road of Kanphant, 26°09'N, 98°31'E (DDM), 28.V.2006, leg. L. Langer.

Descriptive notes. Male. Aedeagus: basal piece as long as dorsal plate of each paramere (Fig. 7D–F); ventral process of each paramere thin and bent inwards in ventral view (Fig. 7D), inclining ventrally in lateral view (Fig. 7F); dorsal plates of parameres obviously longer than ventral processes (Fig. 7D, F), with apical margins rounded in lateral view (Fig. 7E); laterophyse slightly shorter than ventral process, with apices acute and directing dorso-outwards (Fig. 7D, F).

Distribution. China (Yunnan); Myanmar (new record).

Remarks. This species was missing in the Palaearctic Catalogue by Kazantsev and Brancucci (2007). It is recorded to Myanmar for the first time herein. The aedeagus of this species was illustrated only in ventral and lateral views by Wittmer (1995). Here, the aedeagus is illustrated in ventral, dorsal, and lateral views, and the abdominal sternite VIII and internal genitalia of female are illustrated for the first time.

Lycocerus curvatus **(Wittmer, 1995)**

Fig. 11A–C

Athemus (*Isathemus*) *curvatus* Wittmer, 1995: 260, figs 117, 118, 188.
Lycocerus curvatus: Kazantsev and Brancucci 2007: 250.

Type material examined. ***Holotype*****:** 1♂ (NHMB), CHINA, Sichuan, Liziping, 28.VI–3.VII.1991, lgt. R. Dunda.

Non-type material examined. CHINA: 1♂ (IZAS), Sichuan, Emeishan, 2100 m, 25.VI.1955, leg. X. K Bu; 1♂ (IZAS), same locality as the preceding, 2100–3100 m, 25.VI.1955, leg. X. C. Yang.

Descriptive notes. Male. Aedeagus: basal piece nearly as long as dorsal plate of each paramere (Fig. 11A–C); ventral process of each paramere slender and bent inwards apically in ventral view (Fig. 11A), inclining ventrally in lateral view (Fig. 11C); dorsal plates of parameres feebly longer than ventral processes (Fig. 11A, C), with apical margins rounded in dorsal view (Fig. 11B); laterophyse slightly shorter than ventral process, with apices acute and appreciably directing dorso-outwards (Fig. 11A, C).

Distribution. China (Sichuan).

Remarks. The aedeagus of this species was illustrated only in ventral and lateral views by Wittmer (1995). Here, the aedeagus is illustrated in ventral, dorsal, and lateral views, and the female internal genitalia is illustrated for the first time.

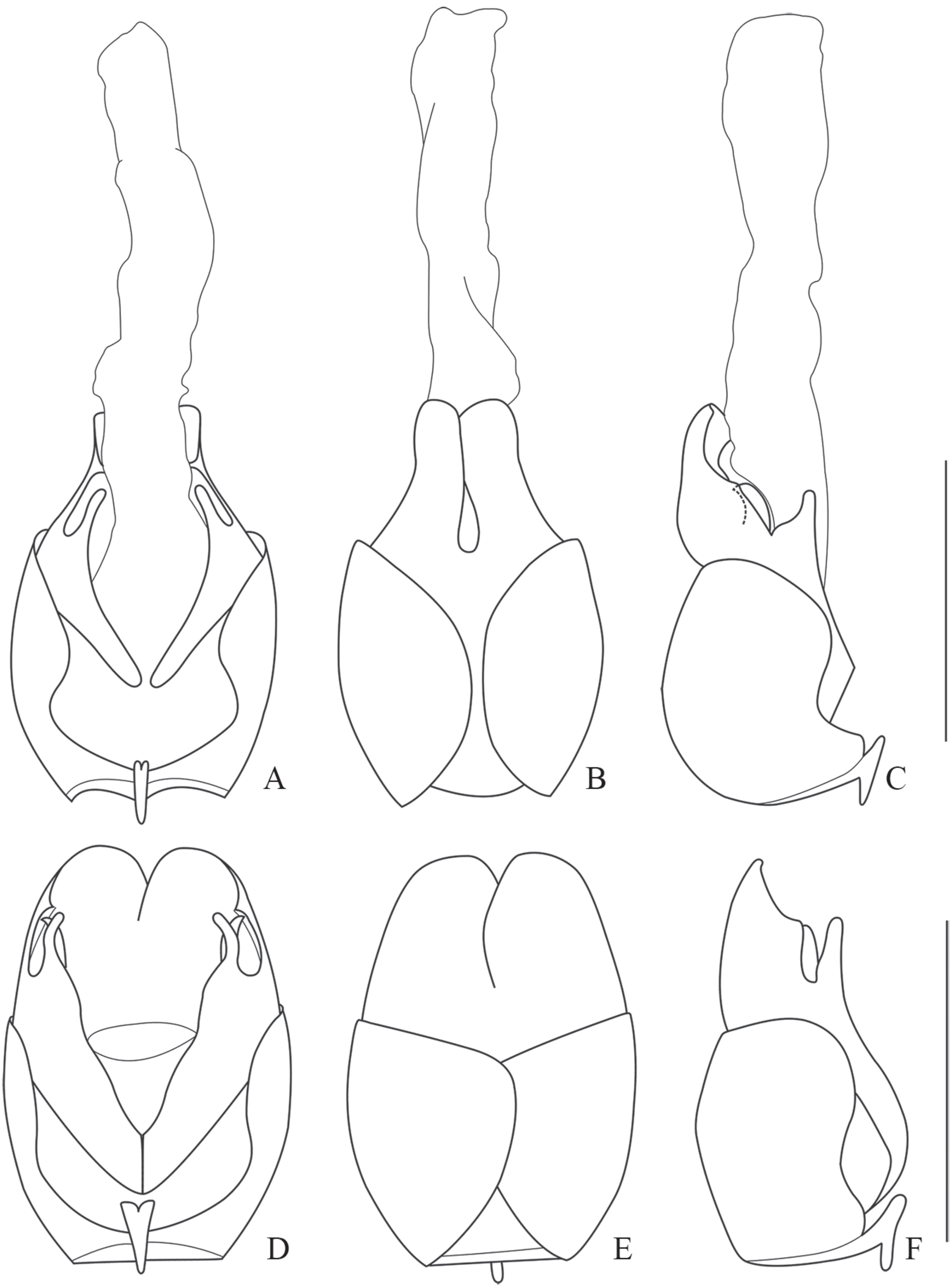

Figure 7. Aedeagus **A–C** *Lycocerus putzi* Švihla, 2011 **D–F** *L. pictipennis* (Wittmer, 1995) **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

Lycocerus laterophysus **Y. Yang, Wang & Liu, sp. nov.**

https://zoobank.org/D433A884-731B-41D2-ABA2-3F813CF640C1
Figs 8A–C, 14D, 16H, 18A, B

Type material. ***Holotype*****:** ♂ (MHBU), China, Guangxi, Wuming, Damingshan, 1230–1423 m, 20.V.2011, leg. H. Y. Liu. ***Paratypes*****:** China: 4♀1♂ (MHBU), same data as holotype; 2♀ (MHBU), same locality as holotype, 1100 m, 27.V.2011, leg. H. Y. Liu; 1♀ (MHBU), same locality as holotype, 600–900 m, 25.V.2011, leg. H. Y. Liu.

Diagnosis. The new species can be easily distinguished from all others by its body coloration, head and pronotum bicolored, with vertex black and clypeus yellow, pronotum with a black wide longitudinal median band (Fig. 18A, B). Also, its aedeagus is unique and differs from all others in the ventral process of each paramere expanded near base in lateral view (Fig. 8C), and broad laterophyse in ventral view (Fig. 8A).

Description. Male (Fig. 18A). Head, prothorax and legs orange, vertex black, mandibles dark brown, antennomeres III–XI black, pronotum with a wide black longitudinal median band extending from posterior to anterior margins, scutellum black, elytra pale yellow, tarsi darkened, meso- and metasterna and abdomen black. Body densely covered with yellow recumbent pubescence.

Head feebly narrowed behind eyes, surface densely and finely punctate; eyes moderately large and protruding, head width across eyes feebly wider than anterior margin of pronotum; antennae filiform, extending to elytral mid-length when reclined, antennomere II shortest, ~ 2.5× longer than wide at apex, III–V feebly widened apically, IV–X each with a short smooth impression near apical part of outer margin, V longest, VI–XI nearly parallel-sided, XI acute at apex.

Pronotum distinctly longer than wide, anterior margin feebly arcuate, lateral margins subparallel, posterior margin nearly straight, anterior angles rounded, posterior angles right-angled, disc convex on posterolateral parts, surface finely and feebly sparsely punctate than that on head.

Elytra ~ 4.5× longer than pronotum, 5.4× longer than width across humeri, outer margins nearly parallel, disc semi-lustrous, coarsely and densely punctate.

Legs slender, all tarsal claws simple.

Aedeagus: basal piece nearly as long as dorsal plate of each paramere (Fig. 8A–C); ventral process of each paramere slender, feebly bent inwards and approaching each other in ventral view (Fig. 8A), slightly expanded near base and bent ventrally in lateral view (Fig. 8C); dorsal plates of parameres obviously longer than ventral process (Fig. 8A, C), with apical margins arcuate and descending inwards in dorsal view (Fig. 8B); laterophyse nearly as long as ventral process and broad in ventral view, with apices acute and directing dorso-outwards (Fig. 8A, C).

Female (Fig. 18B). Similar to the males, but eyes less protruding, antennae thinner and shorter, extending to basal one-third length of elytra when reclined, middle antennomeres without impressions, fore and middle legs with a digitiform tooth on each anterior claw, pronotum feebly longer than wide.

Internal organ of reproductive system (Fig. 14D): spermatheca feebly shorter than diverticulum; accessory gland ~ 2.5× longer than spermatheca.

Abdominal sternite VIII (Fig. 16H): hardly emarginate in middle and subtriangular emarginations on both sides of posterior margin, lateral emarginations obviously deeper than the middle one, the portion between lateral and middle emarginations wide and rounded at apices, obviously extending over apices of latero-apical angles, which are subrounded.

Body length: 9.3–9.7 mm (9.3 mm in holotype); width: 1.8–2.0 mm (1.8 mm in holotype).

Distribution. China (Guangxi).

Etymology. The new species is named after the quite wide laterophyse of its aedeagus.

Lycocerus flavipennis Y. Yang, Wang & Liu, sp. nov.

https://zoobank.org/A565BCEE-4AF7-454A-8F7E-D0650A58A1FB
Figs 8D–F, 14A, 16E, 18C, D

Type material. ***Holotype*:** ♂ (MHBU), China, Zhejiang, Lin'an, Qingliangfeng, 16–22.V.2012, leg. G. L. Xie. ***Paratypes*:** China: 2♀ (MHBU), same data as holotype; 1♀ (MHBU), Zhejiang, Lin'an, Qingliangfeng, Shunxiwu, 15–18.V.2012, leg. J. S. Xu & L. X. Chang; 1♀ (MHBU), Zhejiang, Lin'an, Qingliangfeng, Longtangshan, 19.V.2011, leg. G. L. Xie.

Diagnosis. The new species resembles *L. pictipennis* in the coloration and characteristics of tarsal claws, but differs from the latter in the aedeagus: basal piece very large, ~ 3× longer than dorsal plate of each paramere in lateral view (Fig. 8F), dorsal plate narrow (Fig. 8E), laterophyse slightly bent inwards in ventral view (Fig. 8D). In comparison, in the aedeagus of *L. pictipennis*, basal piece is nearly as long as dorsal plate of each paramere in lateral view (Fig. 7F), dorsal plate wide (Fig. 7E), laterophyse obviously bent outwards in ventral view (Fig. 7D).

Also, it is similar to *L. kubani* in the structure of tarsal claws, genitalia of both sexes and female abdominal sternite VIII, but which are different in each component part in detail, and can be easily distinguished from the latter in the uniformly yellow elytra, while elytra black at apices in *L. kubani*. The coloration of elytra is stable and a good character to recognize the species in *L. pallidulus* group.

Description. Male (Fig. 18C). Head, prothorax and legs yellowish orange, mandibles dark brown at apices, antennomeres III–XI black, scutellum orange and darkened along margins, elytra pale yellow, tibiae and tarsi black, except for protibiae yellow ventrally, meso- and metasterna and abdomen yellowish brown. Body densely covered with yellow recumbent pubescence.

Head feebly narrowed behind eyes, surface densely and finely punctate; eyes moderately large and protruding, head width across eyes distinctly wider than anterior margin of pronotum; antennae filiform, extending to elytral mid-length when reclined, antennomere II shortest, ~ 2× longer than wide at apex, III–IX feebly widened apically, IV–IX each with a short smooth impression near basal part of outer margin (X–XI missing), VII longest.

Pronotum distinctly longer than wide, anterior margin feebly arcuate, lateral margins subparallel, posterior margin nearly straight, anterior angles rounded, posterior angles right-angled, disc convex on posterolateral parts, surface finely and feebly sparsely punctate than that on head.

Elytra ~ 3.5× longer than pronotum, 4.4× longer than width across humeri, outer margins nearly parallel, disc semi-lustrous, coarsely and densely punctate.

Legs slender, fore and middle legs with a digitiform tooth on each anterior and posterior claw, and hind claws simple.

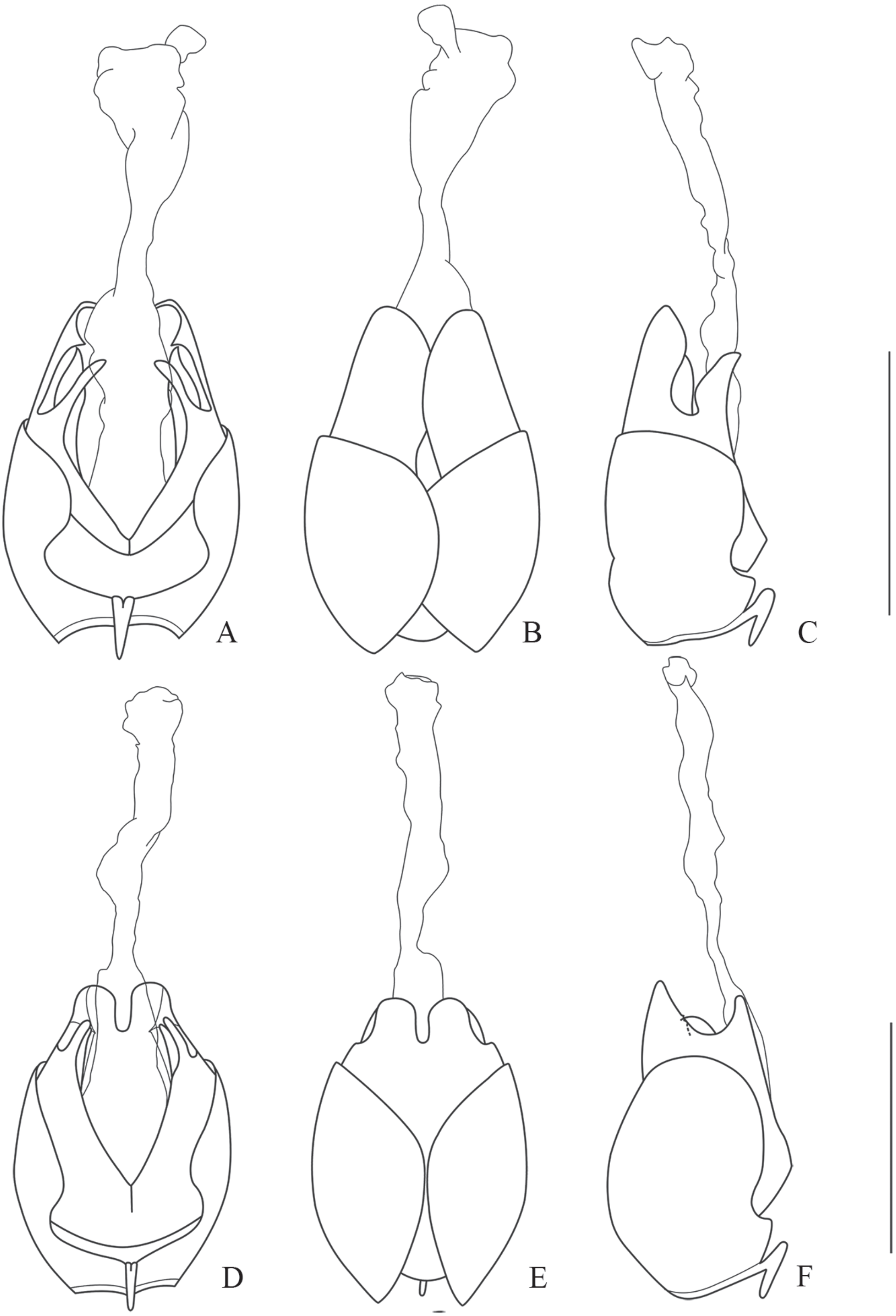

Figure 8. Aedeagus **A–C** *Lycocerus laterophysus* sp. nov. **D–F** *L. flavipennis* sp. nov. **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

Aedeagus: basal piece obviously longer than dorsal plate of each paramere (Fig. 8D–F); ventral process of each paramere thin and short, feebly bent inwards and approaching to each other in ventral view (Fig. 8D), nearly straight in lateral view (Fig. 8F); dorsal plates of parameres obviously longer than ventral processes (Fig. 8D, F), with inner margins parallel, outer margins abruptly converging apically in the middle, apical margins rounded (Fig. 8E); laterophyse feebly shorter than ventral process, bent dorsally, with apices acute and directing dorso-inwards in ventral view (Fig. 8D, F).

Female (Fig. 18D). Similar to the males, but eyes less protruding, antennae thinner and shorter, extending to basal one-third length of elytra when reclined, middle antennomeres without impressions, pronotum nearly as long as wide.

Internal organ of reproductive system (Fig. 14A): spermatheca slightly longer than diverticulum; accessory gland ~ 1.5× longer than spermatheca.

Abdominal sternite VIII (Fig. 16E): triangular emargination in middle and rounded emarginations on both sides of posterior margin, lateral emarginations wider and feebly deeper than the middle one, the portions between lateral and middle emarginations narrow and acute at apices, obviously extending over apices of latero-apical angles, which are widely triangular.

Body length: 11.0–12.0 mm (11.0 mm in holotype); width: 2.3–3.0 mm (2.3 mm in holotype).

Distribution. China (Zhejiang).

Etymology. The specific name is derived from the Latin *flavus* (golden-yellow) and *pinna* (wing), referring to its yellow elytra.

Lycocerus maoershanensis **Y. Yang, Liu & X. Yang, sp. nov.**

https://zoobank.org/482BA762-7D56-4D21-9EA8-FF1CAD1998C7
Figs 9A–C, 13C, 17C, 19A, B

Type material. ***Holotype*:** ♂ (IZAS), CHINA, Guangxi, Maoershan, 1900 m, 14.VII.1985, leg. S. B. Liao. ***Paratypes*:** CHINA: 1♂, 1♀ (IZAS), same data as holotype; 1♀ (IZAS), same locality as holotype, 1950 m, 14.VII.1985, leg. S. M. Song.

Diagnosis. Although the new species is similar to *L. laterophysus* sp. nov. in the bicolored head, it is more related to *L. zdeneki* on basis of the structure of aedeagus. Further, *L. maoershanensis* sp. nov. can be distinguished from the latter in the following characters: scutellum dark brown; female abdominal sternite VIII (Fig. 17C) with the portion between lateral and middle emarginations rounded at apices, which obviously extending over latero-apical angles. In comparison, *L. zdeneki* has yellow scutellum; female abdominal sternite VIII (Fig. 16F) with the portion between lateral and middle emarginations feebly extending over latero-apical angles.

Description. Male (Fig. 19A). Head, prothorax and legs yellowish orange, vertex with a small triangular dark brown marking, mandibles dark brown at apices, antennomeres III–XI black, pronotum with two dark brown irregular markings near middle of anterior and posterior margins, scutellum black, elytra pale yellow and almost transparent, legs more or less darkened at tarsi, apices of tibiae and femora, meso- and metasterna and abdomen black. Body densely covered with yellow recumbent pubescence.

Head feebly narrowed behind eyes, surface densely and finely punctate; eyes moderately large and protruding, head width across eyes slightly wider than anterior margin of pronotum; antennae filiform, extending to three-fifths of elytra when reclined, antennomere II shortest, ~ 1.5× longer than wide at apex, III–VI feebly widened apically, IV–XI each with a short smooth impression near basal part of outer margin, VII–XI nearly parallel-sided, VIII longest.

Pronotum subquadrate, slightly longer than wide, anterior margin feebly arcuate, lateral margins subparallel, posterior margin nearly straight, anterior angles rounded, posterior angles obtuse-angled, disc convex on posterolateral parts, surface finely and feebly sparsely punctate than that on head.

Elytra ~ 3.75× longer than pronotum, 5.0× longer than width across humeri, outer margins nearly parallel, disc semi-lustrous, coarsely and densely punctate.

Legs slender, fore and middle legs with a digitiform tooth on each anterior claw, and hind claws simple.

Aedeagus: basal piece slightly longer than dorsal plate of each paramere (Fig. 9A–C); ventral process of each paramere slender, feebly bent inwards and approaching to each other in ventral view (Fig. 9A), inclining ventrally in lateral view (Fig. 9C); dorsal plates of parameres slightly longer than ventral processes (Fig. 9A, C), with inner margins emarginate at apical parts, outer margins slightly converging apically, apical margins slightly arcuate and descending inwards, inner apical angle acute angled and outer angle rounded in dorsal view (Fig. 9B); laterophyse feebly longer than ventral process, with apices acute and directing dorso-inwards in ventral view (Fig. 9A).

Female (Fig. 19B). Similar to the males, but eyes less protruding, antennae shorter and extending to basal third length of elytra when reclined, middle antennomeres without impressions, pronotum nearly as long as wide, fore and middle legs with a digitiform tooth on each anterior and posterior claw.

Internal organ of reproductive system (Fig. 13C): spermatheca nearly as long as diverticulum; accessory gland nearly as long as spermatheca.

Abdominal sternite VIII (Fig. 17C): subrounded emarginations in middle and on both sides of posterior margin, lateral emarginations deeper than and as wide as the middle one, the portions between lateral and middle emarginations moderately wide and rounded at apices, obviously extending over apices of latero-apical angles, which are narrowly triangular.

Body length: 9.0–10.0 mm (9.1 mm in holotype); width: 1.7–2.3 mm (1.8 mm in holotype).

Distribution. China (Guangxi).

Etymology. The specific name is derived from the name of the type locality, Maoershan, Guangxi, China.

Lycocerus putzimimus **Y. Yang, Wang & Liu, sp. nov.**

https://zoobank.org/C42DA1C1-F720-470F-BEB2-C0DA470121CE
Figs 9D–F, 19C

Type material. ***Holotype***: ♂ (MHBU), CHINA, Yunnan, Pingbian, Daweishan, 1900 m, 24.V.1996, leg. L. Y. Zheng.

Diagnosis. The new species seems similar to *L. putzi* in the coloration, but differs in the following characters: fore and mid legs with a digitiform tooth on

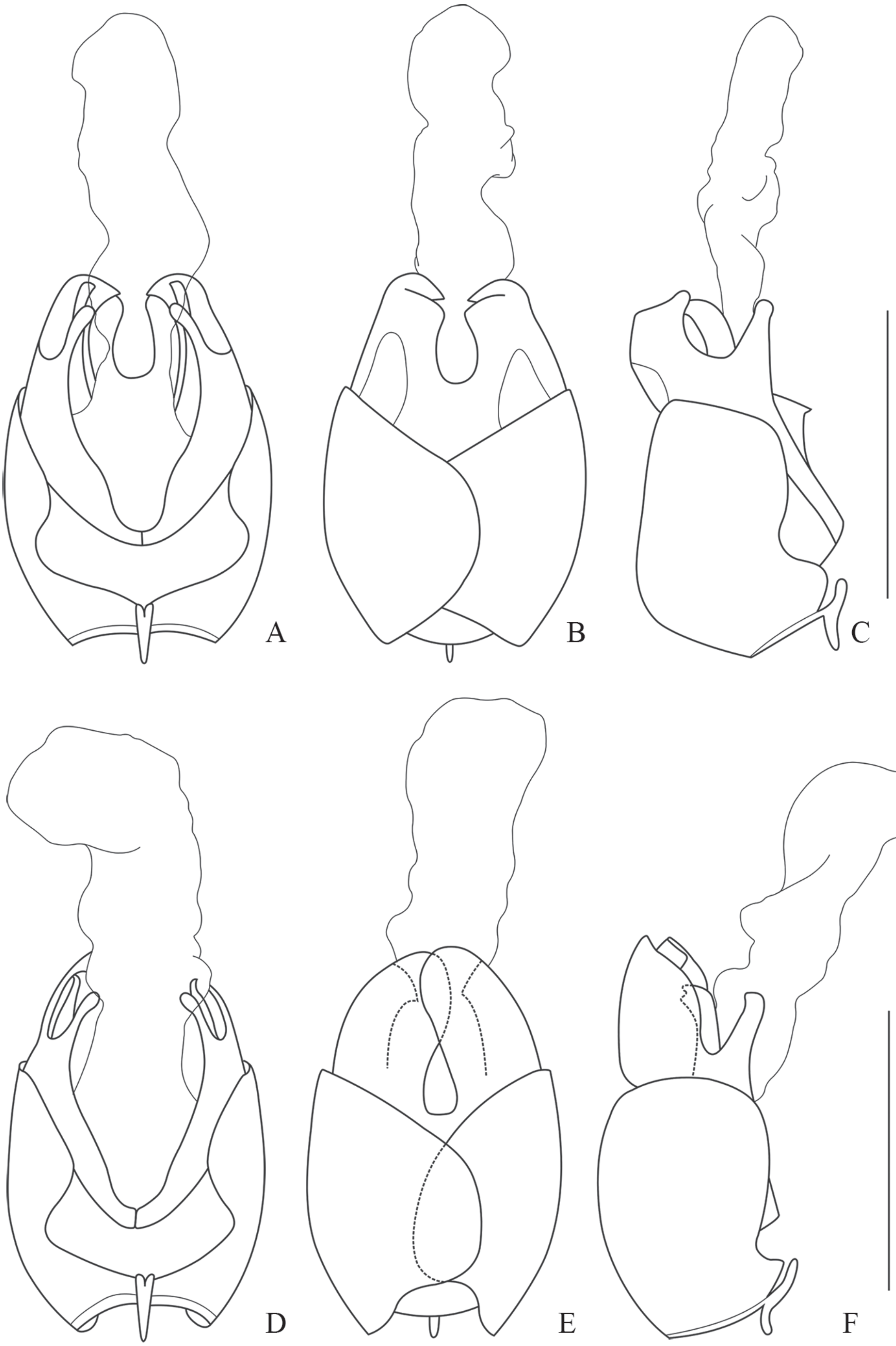

Figure 9. Aedeagus **A–C** *Lycocerus maoershanensis* sp. nov. **D–F** *L. putzimimus* sp. nov. **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

each anterior claw in male, while on both anterior and posterior claws in the latter; aedeagus with dorsal plate of each paramere wide (Fig. 9E) and moderately longer than ventral process (Fig. 9F), while narrower (Fig. 7B) and distinctly long (Fig. 7C) in *L. putzi*; laterophyse feebly furcate at apices (Fig. 9F), while acute in the latter (Fig. 7C).

Description. Male (Fig. 19C). Head, prothorax and legs orange, mandibles dark brown at apices, antennae black, except for bases of antennomeres I yellow, pronotum with a small and round black marking in center of disc, scutellum yellow, elytra pale yellow and almost transparent, each with a black longitudinal band along apical two-thirds part, leaving a long triangular area pale yellow along suture, legs darkened at tarsi, meso- and metasterna and abdomen black, terminal two abdominal ventrites and sternites pale yellow. Body densely covered with yellow recumbent pubescence.

Head feebly narrowed behind eyes, surface densely and finely punctate; eyes moderately large and protruding, head width across eyes slightly wider than anterior margin of pronotum; antennae filiform, extending to apical third length of elytra when reclined, antennomeres II shortest, ~ 2× longer than wide at apices, IV–XI nearly parallel-sided, each with a short smooth impression near apical part of outer margin, IV longest.

Pronotum subquadrate, feebly longer than wide, anterior margin feebly arcuate, lateral margins subparallel, posterior margin nearly straight, anterior angles obtuse-rounded, posterior angles nearly right-angled, disc convex on posterolateral parts, surface finely and feebly sparsely punctate than that on head.

Elytra ~ 4.4× longer than pronotum, 3.12× longer than width across humeri, outer margins nearly parallel, disc semi-lustrous, coarsely and densely punctate.

Legs slender, fore and middle legs with a digitiform tooth on each anterior claw, and hind claws simple.

Aedeagus: basal piece slightly longer than dorsal plate of each paramere (Fig. 9D–F); ventral process of each paramere slender, slightly expanded at apices, slightly bent inwards and approaching to each other in ventral view (Fig. 9D), inclining ventrally in lateral view, with apices slightly curved dorsally (Fig. 9F); dorsal plate obviously longer than ventral process of each paramere, with apical margins rounded (Fig. 9E); laterophyse feebly longer than ventral process, with apices bifurcate and directing dorso-outwards (Fig. 9D, F).

Female. Unknown.

Body length: 10.2 mm; width: 2.2 mm.

Distribution. China (Yunnan).

Etymology. The specific name is derived from the Latin *mimus* (imitator), referring to its similarity to *L. putzi* Švihla, 2011.

Lycocerus chongqingensis **Y. Yang, Wang & Liu, sp. nov.**

https://zoobank.org/74057CE9-07BD-4C2E-9F51-55584FDCDC9D
Figs 10A–C, 15A, 17E, 20A, B

Type material. ***Holotype*:** ♂ (MHBU), China, Chongqing, Nanchuan, Jinfoshan, 23–24.VII.2003, leg. Y. S. Liu & C. X. Yuan. ***Paratypes*:** 1♂2♀ (MHBU), same data as holotype.

Diagnosis. The new species seems most similar to *L. centrochinensis* in both coloration and characteristics of tarsal claws, but differs in the following characters: body size is smaller; aedeagus: dorsal plates of parameres with inner margins feebly protuberant near base (Fig. 10B); abdominal sternite VIII (Fig. 17E) moderately narrowed posteriorly, the portion between lateral and middle emarginations rounded at apices, which slightly extending over apices of latero-apical angles. In comparison, *L. centrochinensis* has larger body; aedeagus: dorsal plates of parameres with inner margins abruptly diverging near middle (Fig. 3E); abdominal sternite VIII (Fig. 16B) strongly narrowed posteriorly, the portion between lateral and middle emarginations triangular at apices, which distinctly extending over apices of latero-apical angles.

Description. Male (Fig. 20A). Head, prothorax, scutellum and legs orange, mandibles dark brown at apices, antennomeres III–XI black, elytra pale yellow, legs darkened at tarsi, meso- and metasterna and abdomen black. Body densely covered with pale yellow recumbent pubescence.

Head feebly narrowed behind eyes, surface densely and finely punctate; eyes moderately large and protruding, head width across eyes wider than anterior margin of pronotum; antennae filiform, extending to apical third of elytra when reclined, antennomere II shortest, ~ 2× longer than wide at apex, III–IV feebly expanded at apex, IV–X each with a short smooth impression near apical part of outer margin, V–XI nearly parallel-sided, VIII longest.

Pronotum subquadrate, feebly longer than wide, anterior margin feebly arcuate, lateral margins subparallel, posterior margin nearly straight, anterior angles obtuse-rounded, posterior angles nearly right-angled, disc convex on posterolateral parts, surface finely and feebly sparsely punctate than that on head.

Elytra ~ 4.6× longer than pronotum, 3.3× longer than width across humeri, outer margins nearly parallel, disc semi-lustrous, coarsely and densely punctate.

Legs slender, all claws simple.

Aedeagus: basal piece nearly as long as dorsal plate of each paramere (Fig. 10A–C); ventral process of each paramere slender and feebly bent inwards and approaching to each other in ventral view (Fig. 10A), inclining ventrally in lateral view (Fig. 10C); dorsal plates of parameres obviously longer than ventral processes (Fig. 10A, C), with inner margins nearly parallel, feebly triangularly protuberant near base, outer margins slightly converging apically, apical margins slightly descending outwards, inner and outer apical angles rounded in dorsal view (Fig. 10B); laterophyse nearly as long as ventral process, with apices acute and directing dorso-outwards (Fig. 10A, C).

Female (Fig. 20B). Similar to the males, but eyes less protruding, antennae shorter, not reaching elytral mid-length when reclined, middle antennomeres without impressions, pronotum nearly as long as wide, fore and middle legs with a digitiform tooth on each anterior and posterior claw.

Internal organ of reproductive system (Fig. 15A): spermatheca nearly as long as diverticulum; accessory gland ~ 2.2× longer than spermatheca.

Abdominal sternite VIII (Fig. 17E): hardly emarginate in middle and subrounded emarginations on both sides of posterior margin, lateral emarginations obviously deeper than the middle one, the portion between lateral and middle emarginations wide and rounded at apices, slightly extending over apices of latero-apical angles, which are truncated.

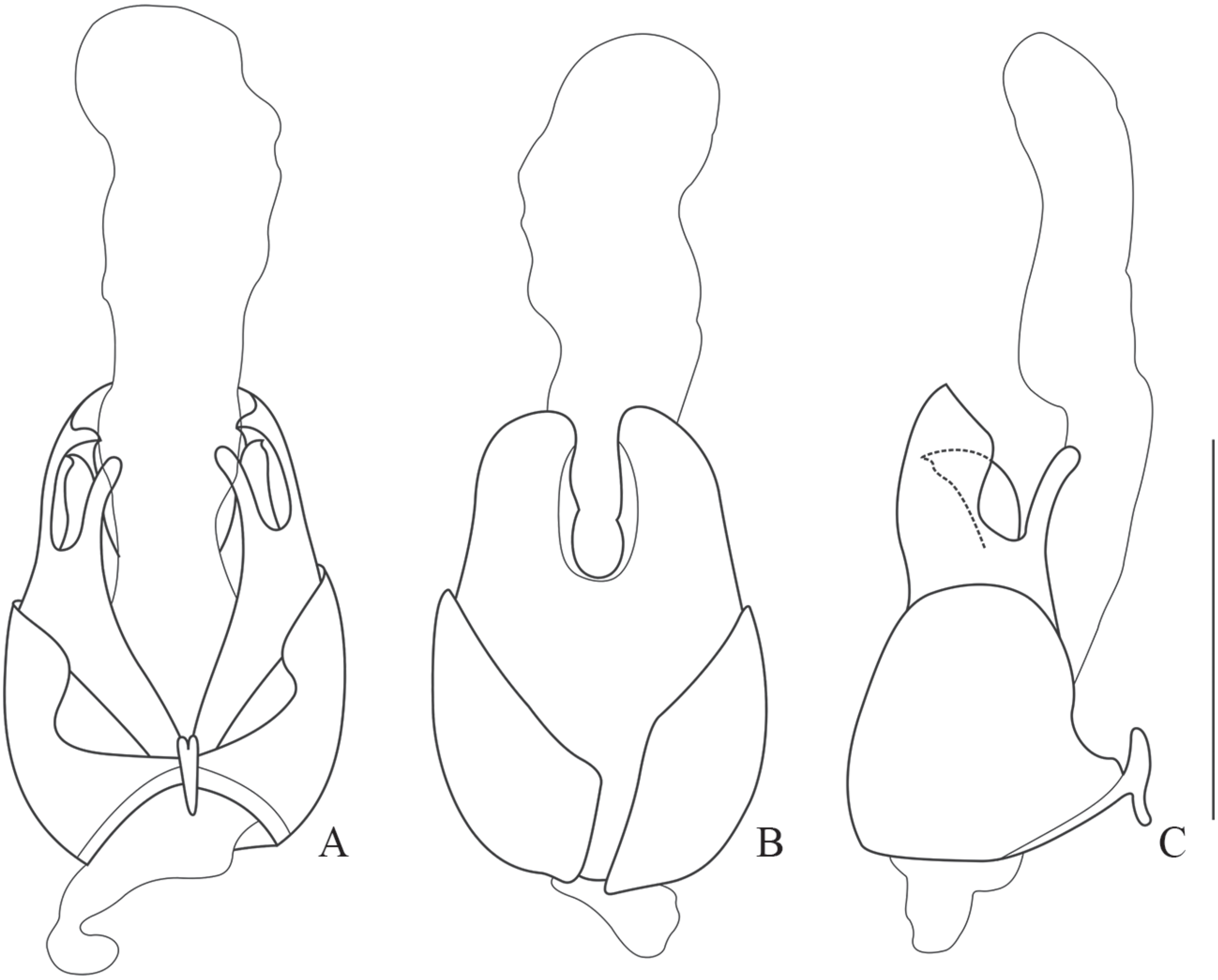

Figure 10. Aedeagus of *Lycocerus chongqingensis* sp. nov. **A** ventral view **B** dorsal view **C** lateral view. Scale bars: 1.0 mm.

Body length: 7.8–9.0 mm (8.0 mm in holotype); width: 1.7–2.1 mm (1.8 mm in holotype).

Distribution. China (Chongqing).

Etymology. The specific name is derived from the type locality, Chongqing, China.

Lycocerus bispermathecus Y. Yang, Wang & Liu, sp. nov.

https://zoobank.org/372D5287-5C6F-484C-8EAC-C8CC9DD0972E
Figs 11D–F, 15B, 17F, 20C, D

Lycocerus centrochinensis (Švihla, 2004): Li et al. 2015: 300, fig. 1A [misidentification].

Type material. ***Holotype***: ♂ (MHBU), China, Ningxia, Kongtongshan, 6.VI.1992, leg. J. L. Ding. ***Paratypes***: China: 1♀ (MHBU), Ningxia, Jingyuan, Liupanshan, 13.VI.1995, Collectors Group III of Forestry; 1♀ (MHBU), same data as the preceding, 8.VI.1995, Collectors Group III of Forestry; 1♀ (MHBU), same data as the preceding, 17.VI.1995, Collectors Group III of Forestry.

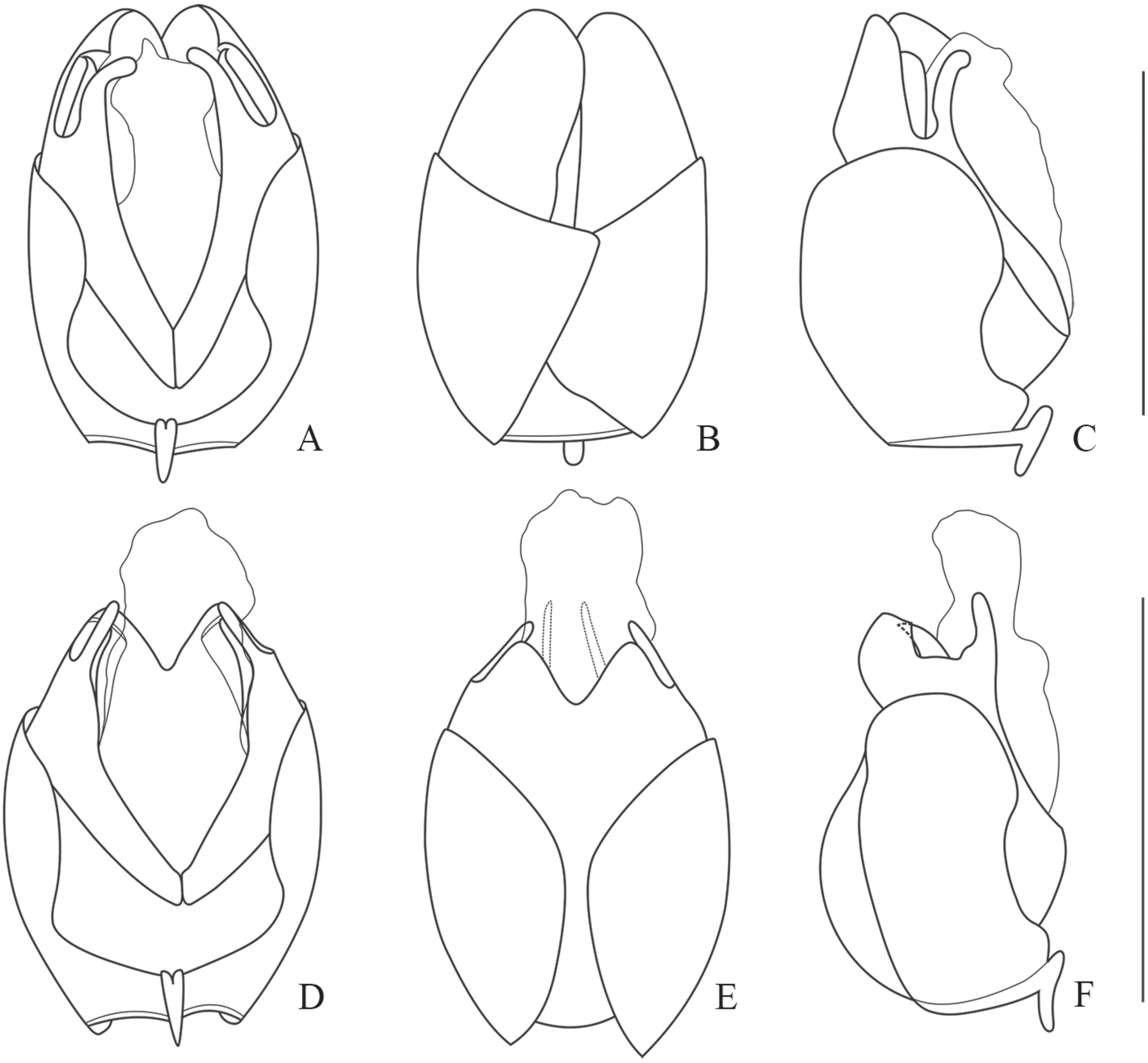

Figure 11. Aedeagus **A–C** *Lycocerus curvatus* (Wittmer, 1995) **D–F** *L. bispermathecus* sp. nov. **A, D** ventral view **B, E** dorsal view **C, F** lateral view. Scale bars: 1.0 mm.

Diagnosis. The new species seems similar to *L. hubeiensis* in the coloration, but differs in the following characters: tarsal claws simple in males, while fore and mid-anterior and posterior claws each with a digitiform tooth at base in the latter; aedeagus: dorsal plates of parameres triangular at apices (Fig. 11E), while truncated in the latter (Fig. 4E); spermatheca with two spiral tubes (Fig. 15B), while only one in the latter.

Description. Male (Fig. 20C). Head, prothorax, scutellum and legs yellowish orange, mandibles dark brown at apices, antennomeres III–XI black, elytra pale yellow and almost transparent, black at apices, legs darkened at tarsi, meso- and metasterna and abdomen yellowish brown. Body densely covered with yellow recumbent pubescence.

Head feebly narrowed behind eyes, surface densely and finely punctate; eyes moderately large and protruding, head width across eyes nearly wider than anterior margin of pronotum; antennae filiform, extending to apical third length of elytra when reclined, antennomere II shortest, ~ 2× longer than wide at apices, IV–XI nearly parallel-sided, each with a short smooth impression near apical part of outer margin, IV longest.

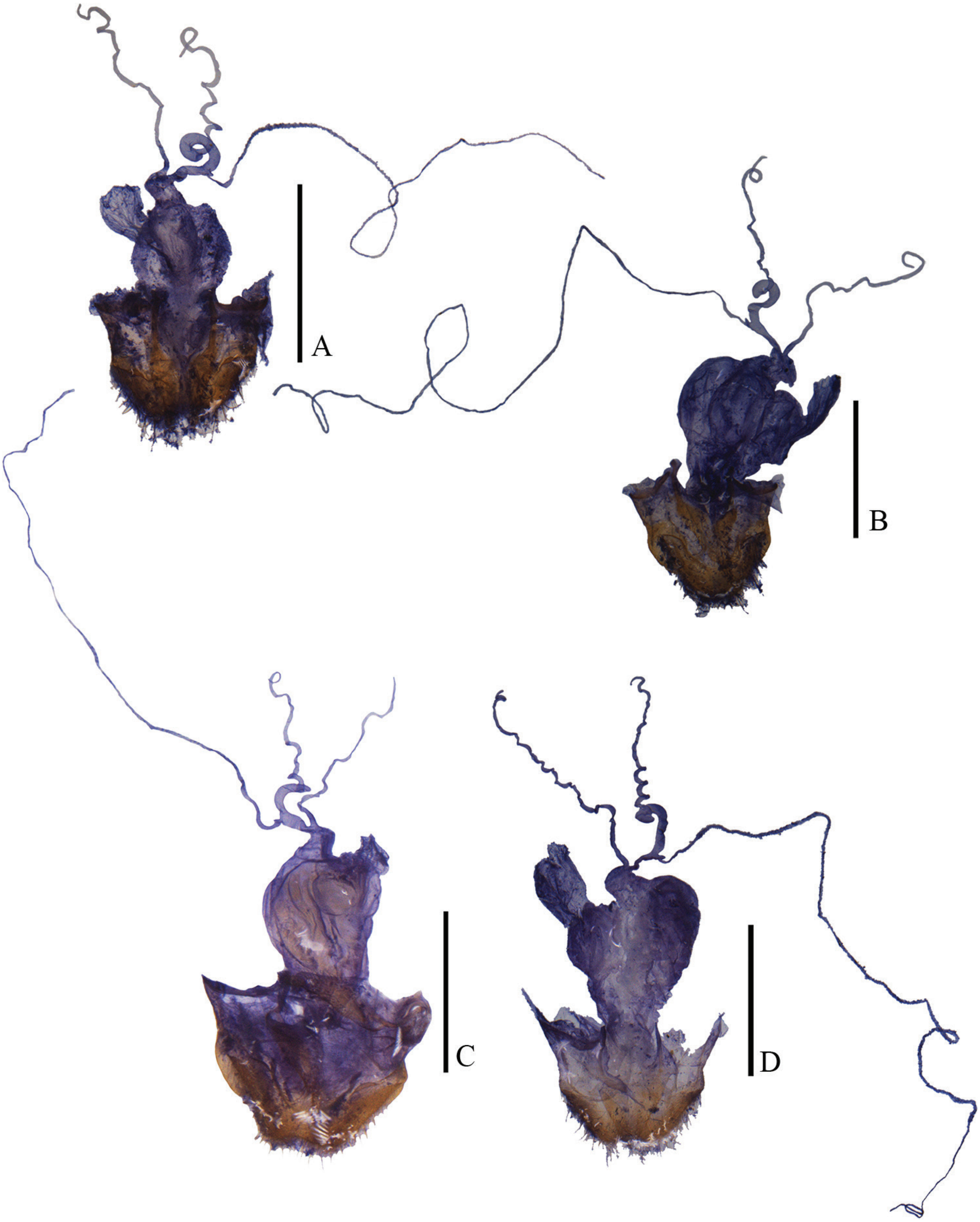

Figure 12. Internal organ of female reproductive system, lateral view **A** *Lycocerus hubeiensis* (Švihla, 2004) **B** *L. centrochinensis* (Švihla, 2004) **C** *L. guerryi* (Pic, 1906) **D** *L. genaemaculatus* (Wittmer, 1951). Scale bars: 1.0 mm.

Figure 13. Internal organ of female reproductive system, lateral view **A** *Lycocerus bilineatus* (Wittmer, 1995) **B** *L. jelineki* (Švihla, 2004) **C** *L. maoershanensis* sp. nov. **D** *L. putzi* Švihla, 2011. Scale bars: 1.0 mm.

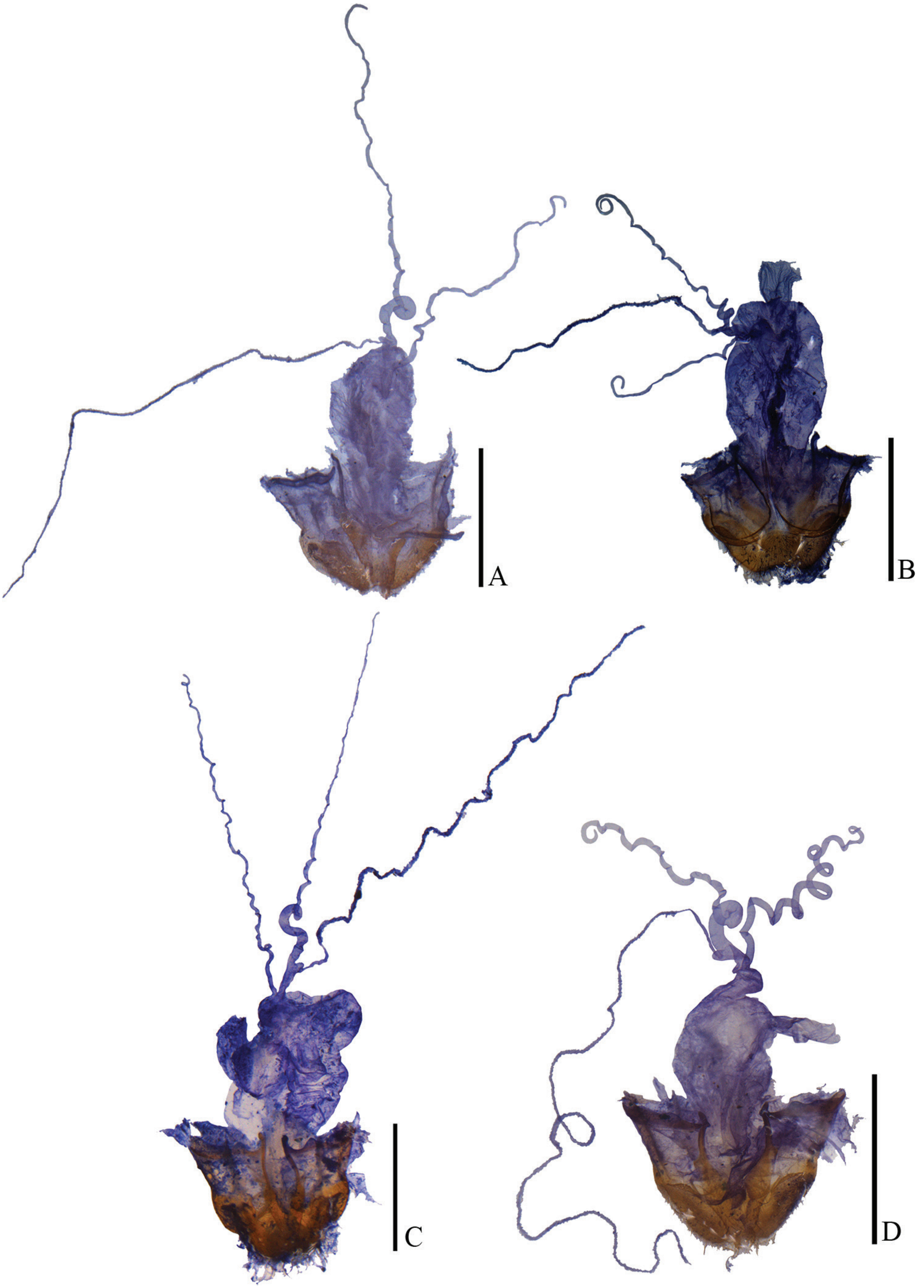

Figure 14. Internal organ of female reproductive system, lateral view **A** *L. flavipennis* sp. nov. **B** *L. zdeneki* (Švihla, 2004) **C** *L. kubani* (Švihla, 2004) **D** *L. laterophysus* sp. nov. Scale bars: 1.0 mm.

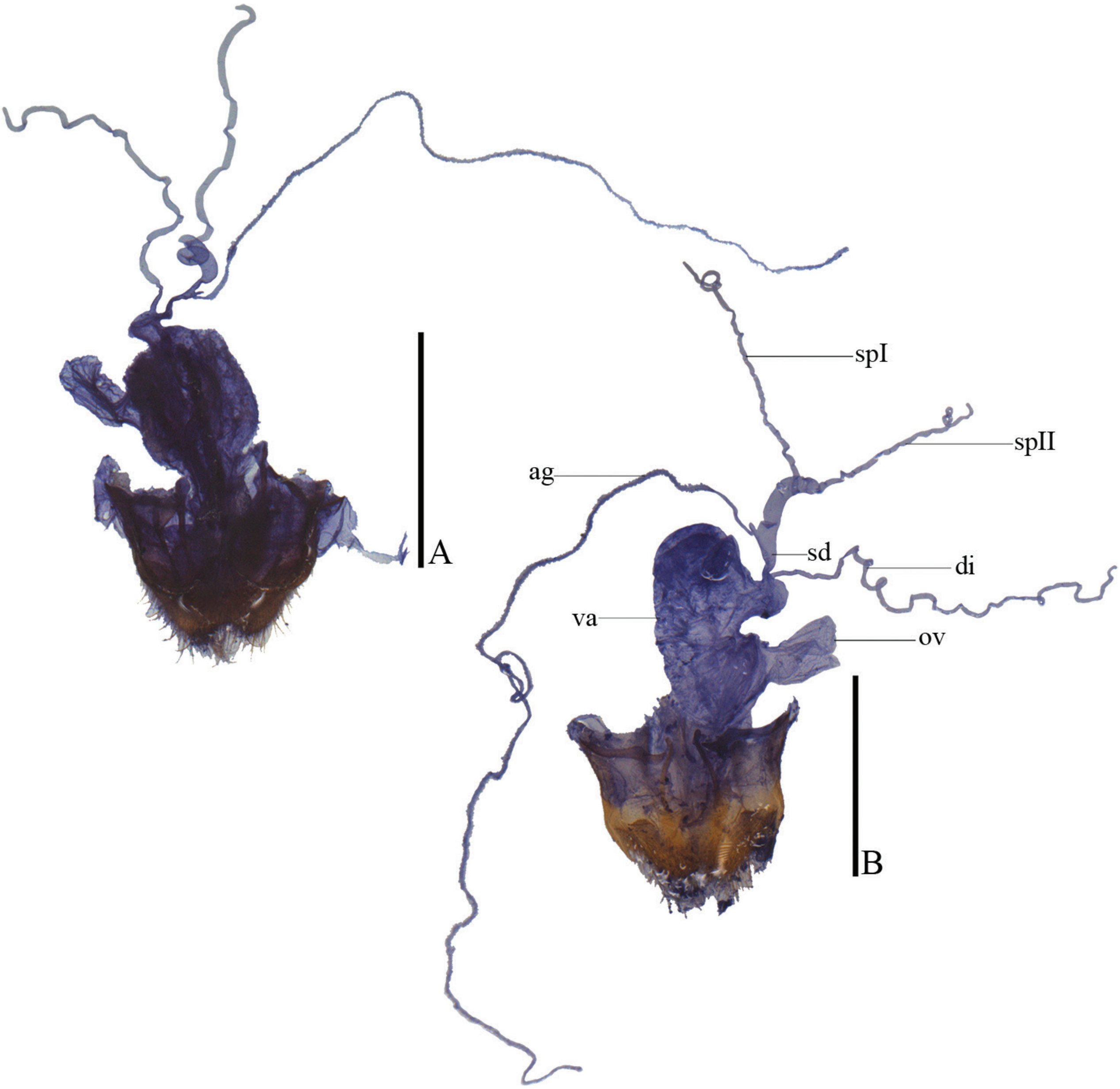

Figure 15. Internal organ of female reproductive system, lateral view **A** *L. chongqingensis* sp. nov. **B** *L. bispermathecus* sp. nov. Abbreviations: ag – accessory gland; di – diverticulum; sd – spermathecal duct; sp – spermatheca; ov – median oviduct; va – vagina. Scale bars: 1.0 mm.

Pronotum subquadrate, slightly longer than wide, anterior margin feebly arcuate, lateral margins subparallel, posterior margin nearly straight, anterior angles obtuse-rounded, posterior angles nearly right-angled, disc convex on posterolateral parts, surface finely and feebly sparsely punctate than that on head.

Elytra ~ 4.4× longer than pronotum, 3.12× longer than width across humeri, outer margins nearly parallel, disc semi-lustrous, coarsely and densely punctate.

Legs slender, all claws simple.

Aedeagus: basal piece obviously longer than dorsal plate of each paramere (Fig. 11D–F); ventral process of each paramere slender and straight, approaching to each other in ventral view (Fig. 11D) and feebly inclining dorsally in lateral view (Fig. 11F); dorsal plates of parameres feebly shorter than ventral processes (Fig. 11D, F), with inner margins diverging and outer margins converging

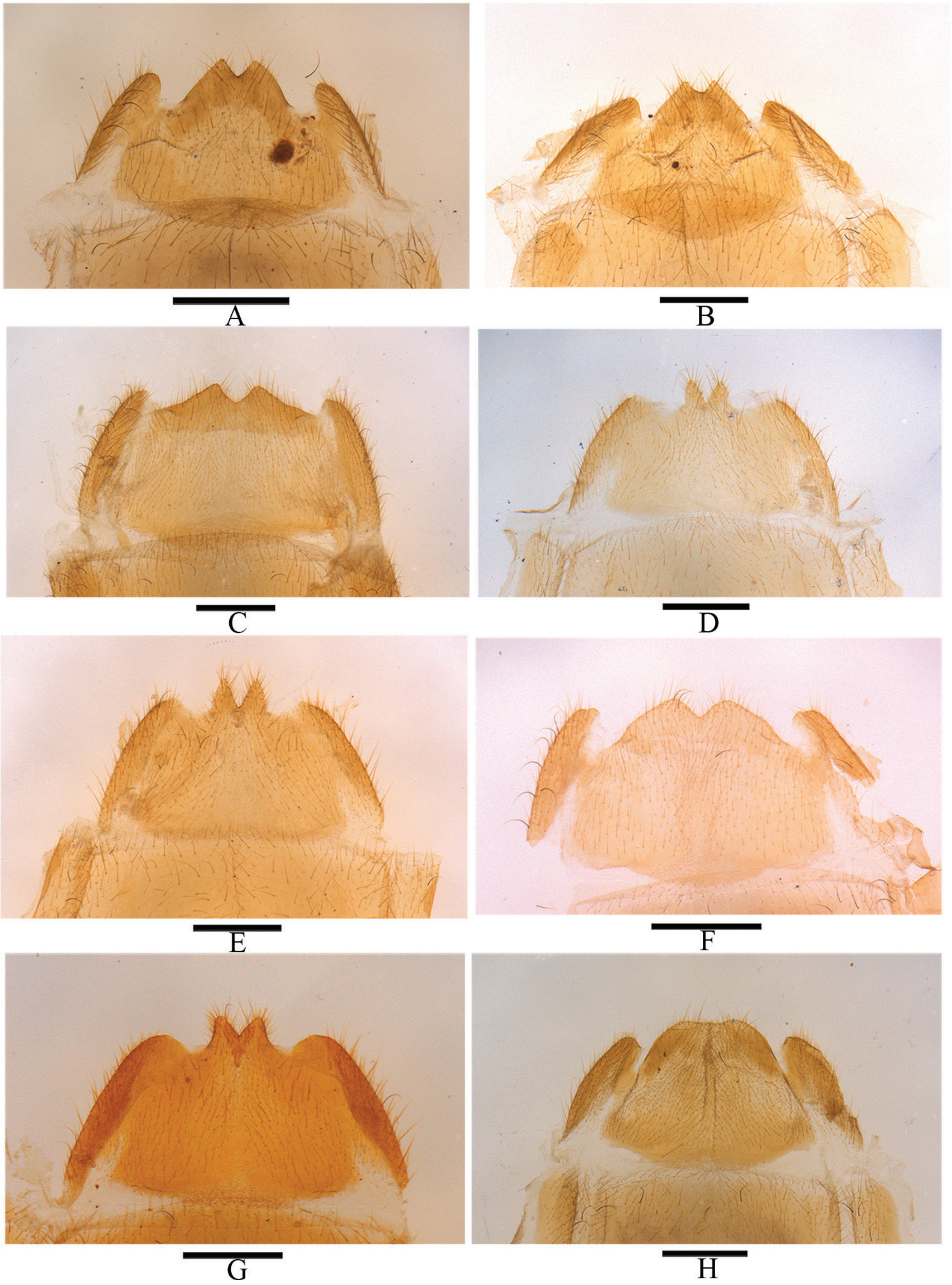

Figure 16. Abdominal sterite VIII of female, ventral view **A** *Lycocerus hubeiensis* (Švihla, 2004) **B** *L. centrochinensis* (Švihla, 2004) **C** *L. guerryi* (Pic, 1906) **D** *L. genaemaculatus* (Wittmer, 1951) **E** *L. flavipennis* sp. nov. **F** *L. zdeneki* (Švihla, 2004) **G** *L. kubani* (Švihla, 2004) **H** *L. laterophysus* sp. nov. Scale bars: 0.5 mm.

Figure 17. Abdominal sterite VIII of female, ventral view **A** *Lycocerus bilineatus* (Wittmer, 1995) **B** *L. jelineki* (Švihla, 2004) **C** *L. maoershanensis* sp. nov. **D** *L. putzi* Švihla, 2011 **E** *L. chongqingensis* sp. nov. **F** *L. bispermathecus* sp. nov. Scale bars: 0.5 mm.

apically, apical margins widely triangular in dorsal view (Fig. 11E); laterophyse obviously shorter than ventral process, with apices acutely hooked, directing dorso-inwards in ventral view (Fig. 11D); inner sac with a pair of longitudinal sclerites on dorsal side (Fig. 11E).

Female (Fig. 20D). Similar to the males, but eyes less protruding, antennae shorter and extending to elytral mid-length when reclined, middle antennomeres without impressions, pronotum nearly as long as wide, fore and middle legs with a digitiform tooth on each anterior and posterior claw.

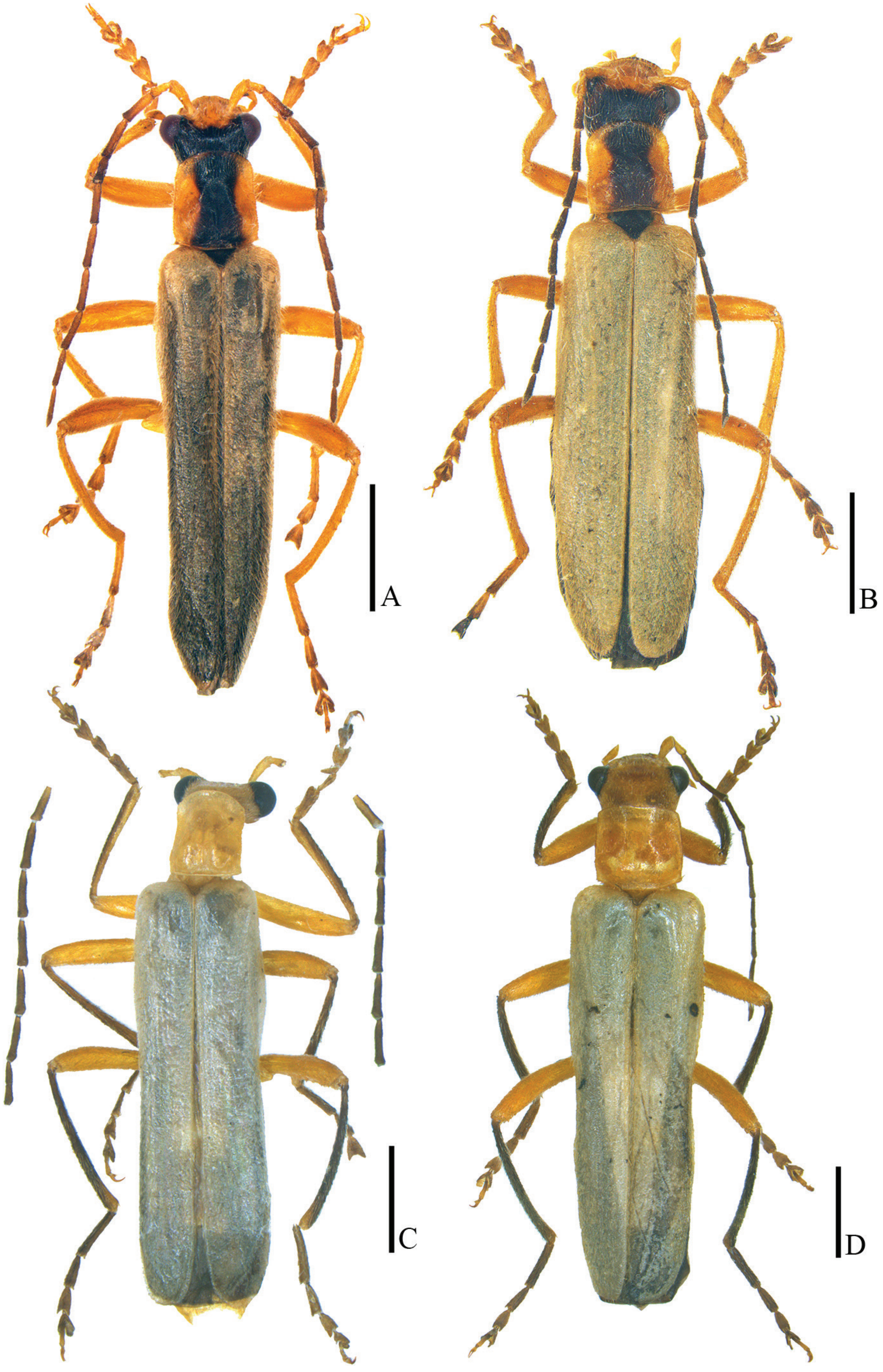

Figure 18. Habitus, dorsal view **A, B** *Lycocerus laterophysus* sp. nov. **C, D** *L. flavipennis* sp. nov. **A, C** male **B, D** female. Scale bars: 2.0 mm.

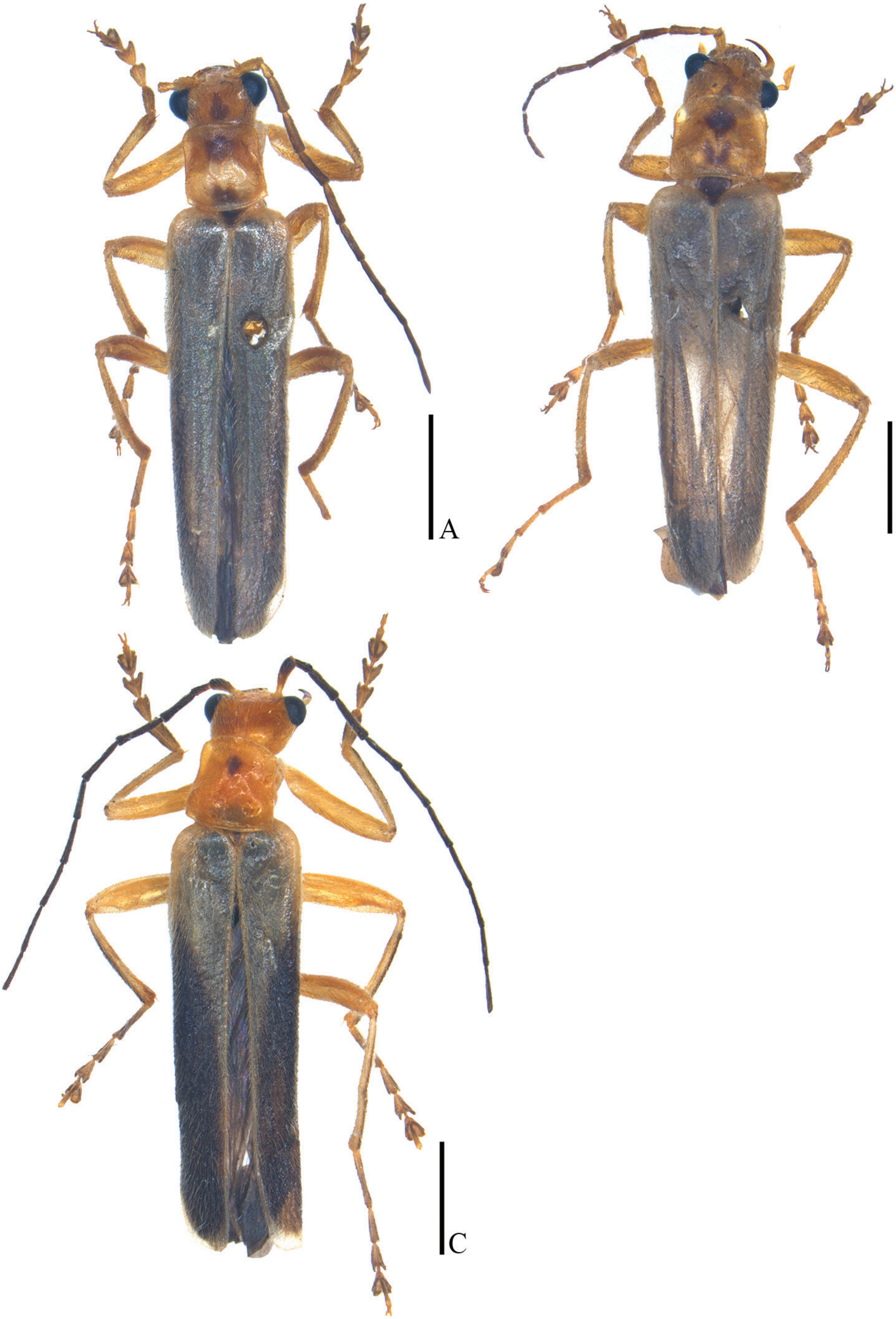

Figure 19. Habitus, dorsal view **A, B** *Lycocerus maoershanensis* sp. nov. **C** *L. putzimimus* sp. nov. **A, C** male **B** female. Scale bars: 2.0 mm.

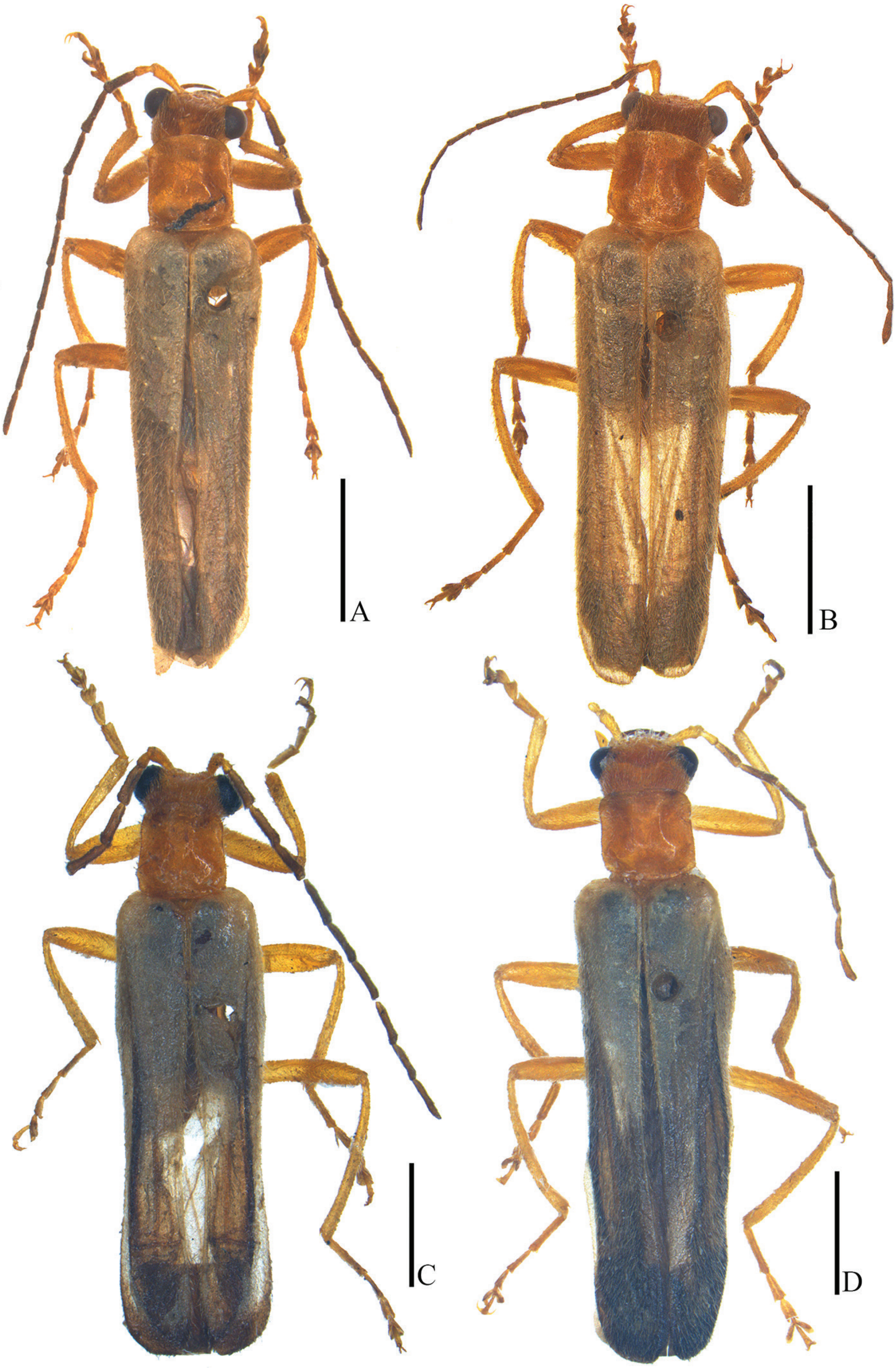

Figure 20. Habitus, dorsal view **A, B** *Lycocerus chongqingensis* sp. nov. **C, D** *L. bispermathecus* sp. nov. **A, C** male **B, D** female. Scale bars: 2.0 mm.

Internal organ of reproductive system (Fig. 15B): spermathecal duct stout, spermatheca with two spiral tubes, which are subequal in length, both of them shorter than diverticulum; accessory gland ~ 2.5× longer than spermatheca.

Abdominal sternite VIII (Fig. 17F): triangular emarginations in middle and on both sides of posterior margin, lateral emarginations feebly deeper than the middle one, the portion between lateral and middle emarginations narrow and acute at apices, obviously extending over apices of latero-apical angles, which are widely triangular.

Body length: 9.0–10.0 mm (9.3 mm in holotype); width: 2.0–2.3 mm (2.0 mm in holotype).

Distribution. China (Ningxia).

Etymology. The specific name is derived from the Latin *spermatike* (sperm-carrying), referring to its distinctive spermatheca, which has two spiral tubes.

Discussion

The characters of tarsal claws, which was emphasized by Wittmer (1995) to define the subgenera of former *Athemus* Lewis, 1895 (now a junior synonym of *Lycocerus*), is proven again to be variable even within a species group (Table 1), as noted by Okushima and Hsiao (2017). Since no character was considered valuable enough to define the subgenera, Okushima (2005) proposed to define species groups to subdivide the large genus *Lycocerus* sensu lato.

At the beginning, Okushima (2005) defined the species groups of *Lycocerus* based on the genitalia of both sexes. Especially, he highlighted the characters of the female internal organ of reproductive system, including the length of spermathecal duct, and shape, length, and number of tubes of spermatheca. However, in the present study, we discovered that the number of tubes of the spermatheca could be variable within the species group. In the *L. pallidulus* group, the spermatheca of *L. bispermathecus* sp. nov. has two spiral tubes, while all others have only one tube. Also, it is related to *L. centrochinensis* and *L. chongqingensis* sp. nov. on basis of the shape of aedeagus and tarsal claws, as well as the body size and coloration, so they probably belong to a natural species group. In this case, we suggest integrating the characters of both appearance and genitalia to define the species groups of *Lycocerus*, also we should take the distribution range into account.

All species of *L. pallidulus* group are distributed in the southern China, located between 21.94–36.60°N and 98.31–21.60°E (Fig. 2). China is located in East Asia and lies in the transitional zone between Palaearctic and Oriental Regions (Zhang 1999). It is a region where some cantharid lineages occur only there and adjacent areas, like other insects (e.g., Bocak and Bocakova 2008). Many lineages with the highest diversity in the Chinese fauna would expand their ranges southwards to the Oriental Region, and *L. pallidulus* group is this case.

Originally, *L. pallidulus* group was regarded as a subgroup and placed in *L. maculicollis* group (Okushima 2005). Based on our studies and Wang et al. (2023), we found that *L. pallidulus* group definitely differs from *L. maculicollis* group in the shape of aedeagus, which is unique in the latter group noted previously by Kazantsev (1999). In addition, the former *L. maculicollis* group has already been suggested as a non-monophyletic (Hsiao 2021; Xi et al. 2022; Wang et al. 2022). Thus, we suggest *L. pallidulus* group be treated as an independent species group.

Within the *L. pallidulus* group, the species can be distinguished by their claws (Table 1) and the following key is designed around this feature combined with other morphological characters.

Table 1. The characteristics of tarsal claws of *L. pallidulus* group.

Species	Male fore and mid-legs		Female fore and mid-legs	
	anterior claws	posterior claws	anterior claws	posterior claws
Lycocerus pallidulus	▨		▨	▨
L. guerryi	▨		▨	▨
L. guerryi atroapicipennis	▨		▨	▨
L. centrochinensis			▨	▨
L. genaemaculatus	▨	▨	▨	▨
L. hubeiensis	▨	▨	▨	▨
L. jelineki	▨		▨	
L. bilineatus	▨		▨	
L. zdeneki	▨		▨	▨
L. kubani	▨	▨	▨	▨
L. curvatus	▨		▨	
L. pictipennis	▨	▨	▨	▨
L. putzi	▨	▨	▨	▨
L. laterophysus sp. nov.			▨	
L. flavipennis sp. nov.	▨	▨	▨	▨
L. putzimimus sp. nov.	▨		Unknown	Unknown
L. maoershanensis sp. nov.	▨		▨	▨
L. chongqingensis sp. nov.			▨	▨
L. bispermathecus sp. nov.			▨	▨

Note: the gray denotes that the claw has a digitiform tooth at base; the white indicates the claw is simple.

Key to the species of *L. pallidulus* group

1 Elytra bicolored, mixed black with light yellow **2**
– Elytra uniformly light yellow **9**
2 Elytra with black median longitudinal bands **3**
– Elytra with black apices **4**
3 Pronotum uniformly orange; aedeagus: ventral process of each paramere stout and nearly truncated apically in lateral view (Fig. 6C) .... ***L. bilineatus* (Wittmer, 1995)**
– Pronotum yellow, dark brown in middle; aedeagus: ventral process of each paramere slender and nearly rounded apically in lateral view (Fig. 11C) .... ***L. curvatus* (Wittmer, 1995)**
4 Elytra black at apical 2/3, with a long triangular area pale yellow along suture **5**
– Elytra black at most at apical 1/5 .... **6**
5 Pronotum with a small dark brown rounded marking; aedeagus: ventral process of each paramere moderately shorter than dorsal plate, laterophyse furcate at apex (Fig. 9F) .... ***L. putzimimus* sp. nov.**
– Pronotum uniformly yellow; aedeagus: ventral process of each paramere much shorter than dorsal plate, laterophyse acute at apex (Fig. 9C) .... ***L. putzi* Švihla, 2011**
6 All claws simple in male; spermatheca with 2 spiral tubes (Fig. 15B) .... ***L. bispermathecus* sp. nov.**
– Fore and mid- anterior and or posterior claws legs each with a digitiform tooth at base in male; spermatheca with 1 spiral tube **7**

7 Fore and mid- anterior and posterior claws legs each with a digitiform tooth at base in male; aedeagus: ventral process of each paramere nearly straight in ventral view (Figs 4D, 5A), dorsal plate slightly longer or nearly as long as ventral process (Figs 4F, 5C) **8**

– Fore and mid-anterior claws each with a digitiform tooth at base in male; aedeagus: ventral process of each paramere distinctly bent inwards in ventral view (Fig. 3A), dorsal plate distinctly longer than ventral process (Fig. 3C)...***L. guerryi atroapicipennis*** **(Pic, 1914)**

8 Aedeagus: ventral process of each paramere obviously longer than laterophyse (Fig. 4F), the distance between ventral processes wider than that between lateral margins of dorsal plates (Fig. 4E) ....***L. hubeiensis*** **(Švihla, 2004)**

– Aedeagus: ventral process of each paramere nearly as long as laterophyse (Fig. 5C); the distance between ventral processes narrower than that between lateral margins of dorsal plates (Fig. 5B) .... ***L. kubani*** **(Švihla, 2004)**

9 Head bicolored, mixed yellow or orange with black.................................**10**

– Head uniformly yellow or orange..**12**

10 Vertex yellow, each side with a black marking around eye; aedeagus: the distance between ventral processes distinctly wider than that between lateral margins of dorsal plates (Fig. 4B) ....***L. genaemaculatus*** **(Wittmer, 1951)**

– Vertex unlike above, never with markings around eyes; aedeagus: the distance between ventral processes narrower than that between lateral margins of dorsal plates (Figs 8B, 9B) **11**

11 Head and pronotum yellow, vertex with a triangular dark brown marking in middle, pronotum with two irregular black markings near middle of anterior and posterior margins (Fig. 19A); aedeagus: ventral process of each paramere gradually thinned apically, as long as dorsal plate (Fig. 9C), inner apical angle of dorsal plate acute (Fig. 9B), laterophyse narrow and directing inwards in ventral view (Fig. 9A) ....***L. maoershanensis*** **sp. nov.**

– Vertex black, clypeus yellow, pronotum yellow, with a black wide median longitudinal band (Fig. 18A); aedeagus: ventral process of each paramere shorter than dorsal plate and expanded near base in lateral view (Fig. 8C), inner apical angle of dorsal plate rounded (Fig. 8B), laterophyse broad and directing outwards in ventral view (Fig. 8A) .... ***L. laterophysus*** **sp. nov.**

12 All claws simple in male **13**

– Fore and mid-anterior and posterior claws each with a digitiform tooth at base in male..**14**

13 Body larger, 8.0–10.0 mm in length; aedeagus: ventral process of each paramere nearly vertical in lateral view (Fig. 3F), dorsal plates of parameres with inner margins abruptly diverging near middle (Fig. 3E); abdominal sternite VIII strongly narrowed posteriorly, the portion between lateral and middle emarginations triangular at apices, which distinctly extending over apices of latero-apical angles (Fig. 16B) ....***L. centrochinensis*** **(Švihla, 2004)**

– Body smaller, 7.8–9.0 mm in length; aedeagus: ventral process of each paramere slightly bent ventrally in lateral view (Fig. 10C), dorsal plates of parameres with inner margins feebly protuberant near base (Fig. 10B); abdominal sternite VIII moderately narrowed posteriorly, the portion between lateral and middle emarginations rounded at apices, which slightly extending over apices of latero-apical angles (Fig. 17E)....................... .... ***L. chongqingensis*** **sp. nov.**

14 Fore and mid-anterior and posterior claws with a digitiform tooth at base in male..**15**

– Fore and mid-anterior claws with a digitiform tooth at base in male **16**

15 Aedeagus: basal piece very large, ~ 3× longer than dorsal plate of each paramere in lateral view (Fig. 8F), dorsal plate narrow (Fig. 8E), laterophyse slightly bent inwards in ventral view (Fig. 8D)................................ .... ***L. flavipennis* sp. nov.**

– Aedeagus: basal piece nearly as long as dorsal plate of each paramere in lateral view (Fig. 7F), dorsal plate wide (Fig. 7E), laterophyse obviously bent outwards in ventral view (Fig. 7D) .... ***L. pictipennis* (Wittmer, 1995)**

16 Fore and mid-anterior and posterior claws each with a digitiform tooth at base in female **17**

– Fore and mid-anterior claws each with a digitiform tooth at base in female.. ***L. jelineki* (Švihla, 2004)**

17 aedeagus: ventral process of each paramere obviously shorter than laterophyse in ventral view (Fig. 4D), laterophyse slender, nearly approaching to each other in ventral view (Fig. 4D), inner apical angle of dorsal plate emarginate at apex (Fig. 4E)................................ ***L. zdeneki* (Švihla, 2004)**

– aedeagus: ventral process of each paramere obviously longer than laterophyse in ventral view (Fig. 3A), laterophyse stout, nearly directing outward in ventral view (Fig. 3A), inner apical angle of dorsal plate subrounded at apex (Fig. 3B)...**18**

18 Female abdominal sternite VIII with the portions between middle and lateral emarginations wide and rounded at apices (Okushima, 2005: fig. 22d); aedeagus: ventral process of each paramere slightly bent inwards in ventral view (Okushima, 2005: fig. 22a), dorsal plate of each paramere narrow and obviously separate apically (Okushima, 2005: fig. 22c)........... .... ***L. pallidulus* (Wittmer, 1995)**

– Female abdominal sternite VIII with the portions between middle and lateral emarginations narrower and right-angled at apices (Fig. 16C); aedeagus: ventral process of each paramere distinctly bent inwards in ventral view (Fig. 3A), dorsal plate of each paramere slight wide and feebly separate apically (Fig. 3C) .... ***L. guerryi* (Pic, 1906)**

Conclusions

The *Lycocerus pallidulus* subgroup originally placed in *L. maculicollis* group is suggested as an independent species-group herein. This group is mainly distributed in the southern China and easily recognized by the middle-sized body and pale yellow or even transparent elytra, sometimes with black longitudinal bands or markings, as well as the genitalia of both sexes. In total 19 species are currently attributed to this group, including, *L. laterophysus* sp. nov., *L. flavipennis* sp. nov., *L. putzimimus* sp. nov., *L. maoershanensis* sp. nov., *L. chongqingensis* sp. nov. and *L. bispermathecus* sp. nov., discovered from China. These species can be distinguished from one another by the body coloration, structures of tarsal claws in both sexes, shapes of aedeagus and abdominal sternite VIII of female. The results of this study provide a better understanding about the morphological and specific diversities of *Lycocerus*, to improve the classification of this speciose genus.

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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: HL, YW. Data curation: YW. Formal analysis: YW. Funding acquisition: HL, YY. Investigation: YY. Methodology: YW. Project administration: YY. Resources: XY. Software: YW. Supervision: YY, HL, XY. Visualization: YW. Writing - original draft: YW. Writing - review and editing: YY.

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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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