

Research Article

Extension of the leafhopper genus *Multinervis* (Hemiptera, Cicadellidae, Megophthalminae, Agalliini) from Northern to Central Vietnam, with the description of one new species

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Abstract

A new species of the genus *Multinervis* Li & Li, 2013, *Multinervis phongdienensis* **sp. nov.**, is described from two locations in Central Vietnam, Thua Thien-Hue Province: Bach Ma National Park and Phong Dien District. It represents a second species for the genus and for the fauna of Vietnam, where the type species, *M. guangxiensis* Li & Li, 2013, has also previously been recorded from Northern Vietnam. Illustrations, a differential diagnosis to distinguish the two *Multinervis* species and a distribution map are provided. *Multinervis phongdienensis* **sp. nov.** is characterized by the absence of forewing crossveins in the claval region, the reduced subgenital plates being almost entirely fused, the absence of a subgenital angular projection and the unusual lateral flanges of the male connective.

Key words: Auchenorrhyncha, biodiversity, leafhopper, Membracoidea, plant pathogen, plant virus, taxonomy

Introduction

The tribe Agalliini Kirkaldy, 1901 is one of four recognised in the subfamily Megophthalminae Kirkaldy, 1906, along with Evansiolini Linnavuori & DeLong, 1977, Adelungiini Baker, 1915, and Megophthalmini Kirkaldy, 1906 (Dietrich 2005). This speciose tribe comprises 57 genera and more than 641 species (Dmitriev et al. 2022 onwards) worldwide. Currently, only 4 species in 3 genera of this tribe are known from Vietnam: *Austroagallia nitobei* (Matsumura, 1912), *Multinervis guanxiensis* Li & Li, 2013, *Sangeeta fyanensis* Viraktamath, 2011 and *S. linnavuorii* Viraktamath, 2011 (Viraktamath 2011; Dietrich et al. 2020). As a comparison, 48 species in 16 genera are recorded from this tribe in China, mostly from the southern regions (Viraktamath 2012; Li and Li 2013; Zhang 2014; Li et al. 2015). Species of this tribe feed mostly on grasses and broad-leaved herbs and may also feed on leguminous crops (Viraktamath 2011; Viraktamath et al. 2012; Li and Li 2013), with a number of species reported as disease vectors of plant viruses, phytoplasmas and Rickettsia-like organisms



Academic editor: Ilia Gjonov Received: 6 September 2024 Accepted: 11 January 2025 Published: 26 March 2025

ZooBank: https://zoobank.org/ BCF14C2F-523B-4525-9C8F-0BB73AF3929A

Citation: Semeraro L, Constant J, Pham T-H (2025) Extension of the leafhopper genus *Multinervis* (Hemiptera, Cicadellidae, Megophthalminae, Agalliini) from Northern to Central Vietnam, with the description of one new species. ZooKeys 1233: 1–14. https://doi. org/10.3897/zookeys.1233.136519

Copyright: [©] Linda Semeraro et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). (RLO) (Nielson 1979; Viraktamath 2011; Viraktamath et al. 2012; Wilson and Turner 2021). This includes species of *Aceratagallia* Kirkaldy, 1907 (*A. curva-ta* Oman, 1933, *A. longula* van Duzee, 1894 and *A. sanguinolenta* (Provancher, 1872)) which transmit Potato yellow dwarf virus (PYD), *Agallia constricta* van Duzee, 1894, which is a vector of PYD and phytoplasmas (Nielson 1968; Trivellone 2019), *Agalliopsis novella* (Say, 1830) which is a vector of clover club leaf RLO (Nielson 1968) and *Austroagallia torrida* Evans, 1935, known to be a vector of rugose leaf curl virus (Grylls 1954; Nielson 1968; Fletcher et al. 2017).

The currently monotypic genus *Multinervis* Li & Li, 2013, is represented by the species *M. guangxiensis* Li & Li, 2013, which was described from Guangxi, China (Li and Li 2013). More recently the species was reported for the first time from Vietnam, Cuc Phuong National Park, Ninh Binh Province by Dietrich et al. (2020).

Following a two-week field expedition to Central Vietnam to collect insects in Bach Ma National Park and Phong Dien District in 2023, a second species of this genus was discovered and is described and illustrated in this paper, with a distribution map of the two known species.

Material and methods

The specimens were collected by sweeping the vegetation with an entomological net and aspirating into a jar. The specimens were euthanized with ethyl acetate and later card-mounted for study. Following the methods outlined in Fletcher (2009), the abdomen of the male specimens was removed to examine the genitalia. The abdomens were first macerated in a 10% solution of potassium hydroxide (KOH), soaked at room temperature overnight (for at least 12 hours) and then thoroughly rinsed in 70% ethanol before transferring to glycerol in genitalia vials.

Specimens were examined and photographed under a Leica EZ4W stereomicroscope with an integrated camera. The photographs were stacked with CombineZP software and optimized with Adobe Photoshop CS3. Photographs of the habitat were taken with an Olympus Tough TG-6 camera.

The distribution map was produced with SimpleMappr (Shorthouse 2010).

Terminology of the general leafhopper morphology, wing venation and leg chaetotaxy follows Dietrich et al. (2022); for the male genitalia, it follows Dietrich et al. (2022) in part, and Blocker and Triplehorn (1985).

The following acronyms are used:

- CCRR Centre for Conservation of Vietnam Natural Resources and Rescue of Animals and Plants, Phong Dien, Thưa Thien-Hue Province, Vietnam
 GUGC Guizhou University, Guiyang Guizhou, China
 RBINS Royal Belgian Institute of Natural Sciences, Brussels, Belgium
- VNMN Vietnam National Museum of Nature, Hanoi, Vietnam

Taxonomy

Class Insecta Linnaeus, 1758 Order Hemiptera Linnaeus, 1758 Suborder Auchenorrhyncha Duméril, 1806 Infra-order Cicadomorpha Evans, 1946

Superfamily Membracoidea Rafinesque, 1815 Family Cicadellidae Latreille, 1825 Subfamily Megophthalminae Kirkaldy, 1906 Tribe Agalliini Kirkaldy, 1901

Genus Multinervis Li & Li, 2013

Multinervis Li & Li, 2013: 296 [description of the new genus *Multinervis* based on the type species, *M. guangxiensis*]; Dietrich et al. 2020: 265 [first record of the genus and species for Vietnam].

Type species. Multinervis guangxiensis Li & Li, 2013.

Diagnosis. The genus is recognised by a combination of characters including, a robust body; coloration generally brown; forewing, somewhat coriaceous, brown with contrasting pale yellow veins; crown very narrowly visible in dorsal view; face with striations along upper margin, dorsad of ocelli; ocelli in slight depressions, almost equidistant from each other as to compound eyes; granulose texture of head, pronotum, mesonotum and forewings; pronotum only weakly pitted, in lateral view slightly convex, anterior margin may be depressed, concave in lateral view; forewing bearing numerous accessory crossveins (veins appearing reticulated), with or without crossveins in claval region (between anal veins); forefemur with pale yellow and black/ dark brown bands; hindfemur with macrosetal formula 2+1; hindtibia with 7 macrosetae on AD margin. In male genitalia structures, the subgenital plates are fused at base; in lateral view, style apophyses are distinctly spiralled. Connective elongate, broader anteriorly than posteriorly (longer than broad), in ventral view, approximately racquet-shaped.

Differential diagnosis. According to Li and Li (2013), Multinervis is most similar to three other genera of Agalliini - Dryodurgades Zachvatkin, 1946, Paulagallia Viraktamath, 2011 and Sangeeta Viraktamath, 2011. However, Multinervis differs from Dryodurgades in having a shorter aedeagal shaft without apical and subapical processes, an elongate connective, longer than wide and subgenital plates fused together in basal portion (in Dryodurgades, the aedeagal shaft is longer with branched apical and often subapical processes, the connective is broader than long and the subgenital plates are not fused basally). Multinervis differs from Paulagallia in having pronotum only weakly pitted, pygofer dorsal margin not deeply excavated, only slightly concave, without short stout setae apically, subgenital plates with no macrosetae, aedeagus without lateral ridges or apical processes, and with dorsal apodeme about half as long as aedeagal shaft, and connective elongate (in Paulagallia, the pronotum is coarsely pitted, the male pygofer has a deep, angular dorsal marginal excavation around midlength, apex of pygofer bears short stout setae, subgenital plates bear macrosetae, the aedeagal shaft may have lateral ridges and small tooth-like processes apically, the aedeagal dorsal apodeme is almost as long as the shaft and the connective is about as long as wide). Based on Viraktamath (2011), a further characteristic by which Multinervis can be distinguished from the two above-mentioned genera includes the ocelli being approximately equally distant from each other as they are to the compound eyes (in Dryodurgades and Paulagallia the ocelli are closer to compound eyes than to each other). Li and

Li (2013) considered another genus, *Sangeeta* Viraktamath, 2011, to be close to *Multinervis* based on the striations of the face, but the latter differs in that it has multiple accessory forewing crossveins (while *Sangeeta* does not have additional crossveins). In *Multinervis* the face width across the eyes is greater than the face length (in *Sangeeta* the face is longer than wide), the number of AD macrosetae on the hindtibia is 7 (*Sangeeta* has 6±1 macrosetae), the dorsal margin of the pygofer is slightly concave (while in *Sangeeta* the pygofer has a distinct almost right-angled excavation) and the pygofer apex and subgenital plates are without macrosetae (in *Sangeeta* the pygofer apex has some stout setae and subgenital plates have a row of macrosetae).

Distribution. (Fig. 6) China (southern) and Vietnam (Northern and Central). **Hosts.** Unknown.

Species list. (type locality indicated by *):

- Multinervis guangxiensis Li & Li, 2013 [CHINA, Guangxi Province* VIETNAM, Ninh Binh Province]. Holotype: GUGC.
- Multinervis phongdienensis sp. nov. [VIETNAM, Thưa Thiên-Hué Province*]. Holotype: VNMN.

Multinervis phongdienensis sp. nov.

https://zoobank.org/8062B470-7E73-497C-AC0D-AA72EC3F0685 Figs 1-6

Type material. *Holotype* ♂ (Figs 1, 3), VIETNAM • Thưa Thiên-Hué Province, Bach Ma National Park; low altitude; 16°13'14"N, 107°53'10"E; 17 May 2023; alt. 100–200m; J. Constant and L. Semeraro leg.; I.G. 34.640; VNMN. *Paratypes*, VIETNAM • 1♀; same data as in holotype; RBINS • 1♀; same data as in holotype; VNMN • 1♂; Thưa Thiên-Hué Province, Phong Dien District; 16°30'27"N, 107°16'05"E; 23 May 2023; alt. 350–400m; J. Constant and L. Semeraro, leg.; I.G. 34.640; RBINS.

Diagnosis. Body robust. Colouration chestnut brown contrasted with pale yellow veins and markings on face. Forewing with veins loosely reticulated mainly on apical half, crossveins in claval area absent; anal vein A1 strongly curved from base. Subgenital plates relatively short, only just reaching posterior margin of pygofer (see Fig. 3A), subgenital plates fused along almost entire length. Aedeagal shaft, narrow in lateral view. Connective elongate, racquet-shaped, broader anteriorly than posteriorly, and in caudal view, posterior shaft with lateral membranous triangular flanges. Female seventh sternite much wider than long.

Differential diagnosis. *Multinervis phongdienensis* sp. nov. can be differentiated from *M. guangxiensis* in body length, being slightly smaller (4.5 mm versus 4.8–5.2 mm in *M. guangxiensis*); the general body shape being more squat (around 2.4× longer than wide at widest point versus 2.75×); the frontoclypeus mostly patchy chestnut brown medially (face generally paler and with a large pale yellow patch medially in *M. guangxiensis*); forewing venation is less reticulated (fewer crossveins), crossveins between anal veins are absent and there are fewer crossveins between discal and costal cells (versus crossveins present between the anal veins and relatively densely reticulated venation between discal and costal cells) anal vein A1 is strongly curved from near the base, not parallel with anal vein Pcu (versus very slightly curved, more or less parallel with Pcu); in the male genitalia, the subgenitial plates appear shorter and only



Figure 1. *Multinervis phongdienensis* sp. nov., holotype ♂ (VNMN) A dorsal habitus B ventral habitus C face, perpendicular view D lateral habitus.

just reaching posterior margin of the pygofer (Fig. 3A) (versus subgenital plates exceeding the posterior margin of the pygofer); in lateral view, the subgenital plate has a slightly rounded projection basodorsally, digitate projection absent (versus distinct digitate projection near base); in ventral view, the subgenital plates appear short and fused for most of their length (versus subgenital plates more elongate, only fused along basal 1/3); the aedeagus is similar in shape to *M. guangxiensis* but is narrower in lateral view; female seventh sternite posterior margin mostly transverse to very weakly broadly concave (versus posterior margin with a deep medial u-shaped notch).

Description. *Measurements and ratios*. Body length, 3 holotype, 4.5 mm; paratypes 3, 4.5 mm; 2 9, 4.5mm. Proportion of body length 2.4× the width (measured across widest part of body). Head across eyes slightly wider than pronotum. Pronotum 2.5× as broad as long. Scutellum 1.25× length of pronotum along midline.

General body colouration. Chestnut brown with contrasting pale-yellow markings and tegminal venation. Colouration of males and females mostly identical.

Head (Figs 1A–D, 2A–D) Head very short, visible in dorsal view, crown slightly produced dorsad with upturned lip-like margin, slightly emarginate posterior to compound eyes; crown, brown with diffuse paler brown patches; face, striated across dorsal margin, slightly rugose around ocelli, each ocellus in slight depression; colour of face mostly chestnut brown with distinctive pale yellow pattern – brown and yellow bands across dorsal area of face, brown band across ocelli, ventral to ocelli, pair of pale yellow bands tapered to a point mesally; frontoclypeus and anteclypeus chestnut brown with patches of pale yellow; lora pale yellow, brown along sutural margins of anteclypeus and lora; genae brown with pale yellow longitudinal band medially.

Thorax (Figs 1A, 2A) Pronotum with fine, shallow pitting posteriorly, depressed along anterior margin, posterior margin transverse or only weakly concave; pronotal colour generally brown – darker brown band across anterior margin, pair of diffuse pale-yellow stripes posterior to depression, medial disc chestnut brown, posterior margin bordered with very fine pale yellow outline. Proepisternum not visible. Mesonotum with paired shallow oval depressions medially, scutellar suture transverse, recurved laterally; mesonotum and scutellum brown anteriorly, pale brown/ yellow medially, pale yellow apically, scutellar suture brown.

Forewings (Figs 1A, D, 2A, D) Generally opaque chestnut brown, surface granular, apical margin outlined dark brown, from apex of claval suture to costal margin; veins pale yellow; basal half of costal cells with few crossveins, clavus and brachial cells without complete crossveins, female specimens with additional pale yellow flecks stemming from anal margin; anal vein A1 strongly curved at base, not parallel with vein Pcu; apical half of forewing, with numerous crossveins, appearing loosely reticulate.

Legs (Figs 1B-D, 2B-D) Pale brown/ yellow; tibiae pale brown/ pale yellow; fore femora striped with black and pale yellow bands; mid femora mostly pale yellow with black band preapically; hind femora pale yellow with longitudinal black markings dorsally, black band apically. Hind femoral macrosetae 2+1; number of macrosetae on hind tibia AD row = 7.

Abdomen In males and females, tergites generally brownish, paler brown medially, darker brown in patches, laterally pale yellowish; sternites pale brown to pale yellowish.



Figure 2. *Multinervis phongdienensis* sp. nov., paratype ♀ (VNMN) A dorsal habitus B ventral habitus C face, perpendicular view D lateral habitus.

Terminalia \circlearrowleft (Fig. 3) Pygofer, broad basally, dorsal margin weakly concave, posterior margin subquadrate, ventral margin broadly convex; short fine setae on posterior 1/8 of pygofer. Anal tube short, not reaching posterior margin of pygofer. Subgenital plates, only just reaching posterior pygofer margin; in lateral view, plates with laterobasal membranous lobe directed dorsad; in ventral view, plates fused along almost entire length; plates tapering apically, produced as triangular points, or slightly rounded at apex, posterior margin medially forming distinct v-shaped notch. Aedeagus symmetrical; more than half height of pygofer, positioned almost horizontally, apex directed anterodorsad, reaching near base of anal tube; gonopore positioned apically on ventral margin; in lateral view, shaft narrow,



Figure 3. Multinervis phongdienensis sp. nov., holotype ♂ (VNMN) A pygofer, subgenital plate and pregenital sternite, left lateral view B pregenital sternite and subgenital plates, ventral view C anal tube, dorsal view D aedeagus, left lateral view E aedeagus, caudal view F connective, ventral view G connective, left lateral view H left style, dorsal view I left style, lateral view.

slightly sinuate along posterior margin; dorsal apodeme less than half length of shaft; well-developed preatrium; in caudal view, aedeagus broad, lateral margins slightly convex, narrowing slightly towards apex. Connective, elongate, longer than wide, sclerotised portion wider anteriorly than posteriorly; posterior portion articulated with preatrium of aedeagus; in lateral view, oriented obliquely within pygofer, sinuous in profile; in ventral view, anterior portion, approximately racquet-shaped, arms divergent and then convergent distally; sclerotised arms separated throughout, medially membranous, anterior portion of arms enclosed by membranous tissue; elongate posterior stem of connective with membranous lateral triangular flanges. Style basal part elongate; apex of style, reaching near posterior pygofer margin; apophyses spiraled, with fine preapical setae visible from dorsal aspect.

Terminalia \bigcirc (Fig. 4) Seventh sternite pale brownish/ yellow, wider than long (length medially around 1/5 its width); transverse, posterior margin straight to broadly, weakly concave, lateral posterior angles slightly rounded. Pygofer pale



Figure 4. *Multinervis phongdienensis* sp. nov., paratype \bigcirc (VNMN) **A** abdominal tergites and pygofer, left lateral view **B** abdominal tergites and pygofer, dorsal view **C** abdominal sternites, pygofer and ovipositor, ventral view **D** second valvifer and third valvula, lateral view **E** first valvula, lateral view **F** second valvulae, lateral view.

yellow with some diffuse brown patches dorsally, surface slightly granulate; ovipositor pale yellow; apex of ovipositor slightly but distinctly exceeding length of pygofer; valvulae similar in form to generic description (Li and Li 2013); first valvulae with reticulated area along dorsoapical portion of shaft; second valvulae with small tooth-like process posterior to medial dorsal hyaline area, small teeth along dorsoapical margin.



Figure 5. *Multinervis phongdienensis* sp. nov., habitat **A** Bach Ma National Park, low altitude, 15 May 2023 **B** Phong Dien District, 23 May 2023.



Figure 6. Distribution map of Multinervis guangxiensis Li & Li, 2013 and M. phongdienensis sp. nov.

Etymology. The species epithet is derived from the collection locality, Phong Dien District which includes the site of CCRR, one of the two locations in Thưa Thiên-Hué Province, in which this species was found.

Biology and habitat. (Fig. 5) The biology of this species is not known. Specimens were collected in subtropical evergreen rainforest in a region bordering the Northern and Southern Vietnam lowland rainforests and Southern Annamites montane forests. In Bach Ma, specimens were only collected by sweeping the vegetation at low altitude (100–200 m) along a track. No specimens were collected at the higher cooler climate altitudes (towards the summit). However, in Phong Dien one specimen was collected at 350–400 m.

Host. Unknown.

Distribution. (Fig. 6) Vietnam • Thưa Thiên-Hué Province, Bach Ma National Park and Phong Dien District.

Comments. The holotype of the type species of *Multinervis* (*M. guangxiensis*) was not examined in this study, but specimens were compared to the description of the genus and species and the photographs and illustrations as presented in Li and Li (2013: figs 1–24).

Discussion

Following the description of this new species, there are now five species of Agalliini recorded from Vietnam and the total number of *Multinervis* species has increased to two, with both species recorded from Vietnam.

Multinervis was recorded for the first time in Vietnam by Dietrich et al. (2020). The specimen of *M. guangxiensis* photographed in Dietrich et al. (2020: fig. 21X) appears to have more densely reticulated veins on the forewing compared to the original description of this species in Li and Li (2013, figs 1–3). This may indicate intraspecific crossvein variability within *Multinervis*. In *M. phongdienensis* sp. nov., however, the crossvein pattern varies only slightly between specimens and is generally consistent between all four specimens examined. The male genitalia structures remain the most reliable feature for species determination.

The diagnostic characters of *Multinervis* are slightly redefined in this study to capture the additional range of character states, as found in the new species. For example, the striations on the face above the ocelli are described in Li and Li (2013) as being conspicuous in this genus but are only faintly visible along the apical margin of the face of *M. phongdienensis* sp. nov. In the male genitalia, the subgenital plates are originally described as being elongate and only fused in the basal 1/3, while in the new species, the subgenital plates are relatively short and fused for almost the entire length of the plate. The dorsally directed digitate process on the basal half of the subgenital plate was described as a character of *Multinervis*, but it is absent in the new species, possibly replaced by a membranous lobe.

Multinervis species have an elongate connective, which is approximately racquet-shaped. While this elongate connective feature is shared with a few other Agalliini genera, such as *Brasa* Oman, 1936 and *Humpatagallia* Linnavuori & Viraktamath, 1973 (Linnavuori and Viraktamath 1973; Nielson and Freytag 1998), it differs from many other genera in the tribe, which have a relatively wide and short connective. The connective shape and the presence of tegminal crossveins represent relatively rare features in the Old World Agalliini (Viraktamath 2011). Additionally, the connective of *M. phongdienensis* sp. nov. is particularly unusual in having membranous flanges on the lateral margins of the posterior stem. The flanges do not appear to be present in *M. guangxiensis* based on the descriptions and illustrations in Li and Li (2013). It is considered a peculiar diagnostic feature of *M. phongdienensis* sp. nov.

The new species described in this study only represents one of many species of Cicadellidae to be described from the 2023 expedition to Bach Ma National Park and Phong Dien District (including the Phong Dien Nature Reserve Nature Reserve and CCRR). It is complimentary to the work by Constant et al. (2023, 2024) and Constant and Pham (2024) in which one new genus and 11 new species of planthoppers (Nogodinidae, Tropiduchidae and Issidae, respectively) were described from specimens collected during the same expedition, indicating a rich biodiversity of Auchenorrhyncha in the Thưa Thien-Hue Province region.

Acknowledgements

We would like to thank Thanh Trung Vu and Thi Thu Hoai Nguyen (VNMN), Trần Nam Hải (Hanoi National University of Education), Le Canh Viet Cuong (Mientrung Institute for Scientific Research, Hue, Vietnam), our guides in Bach Ma National Park, Lâm Bá Vũ Nguyễn, and Quang Thanh Nguyễn for all of their involvement and kind assistance during the collecting trips; the authorities of Bach Ma National Park and of the CCRR in Phong Dien; Frederik Hendrickx and Thierry Backeljau (RBINS) for their support to our projects; and the two reviewers whose thoughtful comments improved this work.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The present study was funded by the National Foundation for Science and Technology Development (NAFOSTED 106.05-2021.22).

Author contributions

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

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Data availability

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

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Short Communication

Extracting specimen label data rapidly with a smartphone—a great help for simple digitization in taxonomy and collection management

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Abstract

We provide short tutorials in how to read out specimen label data from type- as well as handwritten labels in a rapid and easy way with a mobile phone. We apply them in general, but test them in particular for insect specimen labels, which are generally quite small. We provide alterative procedure instructions for Android and Apple based environments, as well as protocols for single and bulk scans. We expect that this way of data capture will be of great help for a simple digitization in taxonomy and collection management, independent from large industrial digitization pipelines. By omitting the step of taking/maintaining images of the labels, this approach is more rapid, cheaper, and environmentally more sustainable because no storage with carbon footprint is required for label images. We see the biggest advantage of this protocol in the use of readily available commercial devices, which are easy to handle, as they are used on a daily basis and can be replaced at relatively low cost when they come into (informatic) age, which is also a matter of cyber security.

Key words: Artificial intelligence, citizen science, collection digitization, data science, label transcription, labels, taxonomic impediment, taxonomic revisions

Introduction

Currently, there are immense efforts underway to digitize natural history collections on a large scale, including the associated information and metadata (e.g., Smith and Blagoderov 2012; Hardisty et al. 2020a, b; Belot et al. 2023; Groom et al. 2023; Ong et al. 2023). In these endeavors, among other things, the automatic capture of label data plays a central role (e.g., Beaman et al. 2006; Heidorn and Wei 2008; Lafferty and Landrum 2009; Granzow-de la Cerda and Beach 2010; Haston et al. 2012; Agarwal et al. 2018; Alzuru et al. 2019, 2020; Alzuru 2020; Owen et al. 2020; Belot et al. 2023; Zhang 2023; Takano et al. 2024). However, many of these very promising activities have been for a long time exclusive to large companies, museums or institutions with specialized technical infrastructure, and specially trained staff (e.g., Blagoderov et al. 2012) for the highly customized implementations used (e.g., https://picturae.com/).



Academic editor: Pavel Stoev Received: 1 November 2024 Accepted: 17 January 2025 Published: 26 March 2025

ZooBank: https://zoobank. org/70B1D529-3A50-414A-8DAE-D76BFE019BC1

Citation: Ahrens D, Haas A, Pacheco TL, Grobe P (2025) Extracting specimen label data rapidly with a smartphone—a great help for simple digitization in taxonomy and collection management. ZooKeys 1233: 15–30. https://doi.org/10.3897/ zookeys.1233.140726

Copyright: © Dirk Ahrens et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Most of the current digitization initiatives aim at a one-go retro-digitization of large collections (Engledow et al. 2018; Hardisty et al. 2020a, b; Helminger et al. 2020; De Smedt et al. 2024). However, this approach comes with limitations: 1) collections are continuously growing and developing (see also Balke et al. 2013); and 2) the scientific community produces a large amount of high-quality biodiversity data independently of the collection institutions with their ongoing research on the specimens, in which amateur scientists are also largely involved (Löbl et al. 2023). The latter is achieved by the often-remote study of the collection material, far away from collections and large digitization pipelines. Especially in insects, taxonomic specialists are rare, and specimens are often loaned by shipment overseas to obtain best identifications from world-leading specialists. In this, working processes are quite different from those of vertebrates or plants in often leading in new methodologies, such as large-scale digitization. However, these data often do not end up in big data repositories, also due to the lack of time and stimulus, as well as the work-overload of taxonomists.

Therefore, more flexible solutions are needed that allow for more efficient data processing and speed up biodiversity/species discovery and help to overcome taxonomic impediment. This would be perfectly in line with the idea of integrating specimen databases and revisionary systematics (Schuh 2012). Advantages of a revision-based digitization (see also Meier and Dikow 2004), i.e., that biodiversity data come from taxonomic revisionary studies rather than from uncritical retro-digitizing of museum specimen data, are the following (based on Meier and Dikow (2004) and Schuh (2012)): 1) the data are provided in association with the most accurate identifications, 2) the data have the most complete taxonomic and geographic coverage, 3) the data satisfy these points in a cost-effective way, and 4) the occurrence data and images are citable and acknowledgeable. This last point enables errors to be retracted and corrected).

Recently, we found that mobile devices, which nowadays are used by almost everyone, can be of assistance in speeding up data collection and digitization, including biodiversity discovery. By simple experimentation, we discovered that mobile phones can be used in association with cloud-like environments (such as Google Workspace or Apple iCloud). Because we think that these workflows will be useful to a larger audience, we prepared this short paper on how to rapidly and easily read out specimen label data using a smartphone.

Most digitization approaches capture digital metadata (e.g., labels) with the intermediate step of digital images (Nelson et al. 2012). This comes with difficulties and the considerable cost of image processing and storage (Tann and Flemons 2008; Hardisty et al. 2020b). It is more sustainable to skip this step, with the greatest benefit to cost, if data can be read and spell-checked at the same moment without the burden of intermediate images, which are scientifically and practically unnecessary for non-type specimens.

Material and methods

Resources needed

A mobile or smartphone; a recent model with macrophotography capacity.
 A stable internet connection.

- A computer connected with internet and logged into a Google account (via Google Chrome Browser) or AppleID account.
- 4) A database or text file to insert the specimen data.
- 5) "Google Lens" or "Google Translate" to be installed on the mobile phone.

For our testing here, we used a Motorola Edge 30 Neo (system: Android ver. 14), a Motorola G5g Plus (system: Android ver. 10/11), a Motorola G82 G5g (system: Android ver. 13), and an iPhone 15 Pro Max (system: iOS ver. 17.7).

We explored data extraction from labels with different approaches and alternative label conditions (Fig. 1). Some of the tutorials for each of these approaches can be found to be more suitable for different technical situations of the user than others. We describe subsequentially each of these approaches in simplified step by step tutorials. Tutorials are accompanied by screen shots and examples of retrieved data.

With any computer operational system and an Android mobile device:

Variant 1

- 1) Open the "Google Translate" or "Google Lens" apps in your mobile phone.
- 2) Focus on the label to be scanned and zoom virtually by using the touch screen so that the label fills the screen as much as possible. There is no need to be perfectly focused, but all letters should be recognizable.
- 3) Scan by clicking on the circle with the magnifier lens on it.
- 4) Mark the label text (Fig. 1) via cursor selection using the touch screen of the mobile phone.
- 5) Select "Copy to Computer".
- 6) Confirm the selected device (computer with which you are logged into your Google account and via Google Chrome Browser) by choosing "Select".¹
- 7) On the computer: simply paste from clipboard into your target document (verbatim label citation).
- 8) Finally, you may proofread the scan (while having still your specimen in front of you) and manually correct misspellings or readings.
- 9) Finished.

Alternatively, in step 5 you may choose "Copy" and then paste this copied content into an open Google Docs document on the mobile device. This document can be accessed on via the same Google account on the synchronized computer. This step is sometimes necessary if the internet connection is too slow (see results below). This also works outside of the Google Cloud environment but is a little more complex: Files can be shared between Android, Windows or Mac devices using the "*KDE Connect*" app (https://kdeconnect.kde.org). The latter app works also on Linux. All devices must be in the same WIFI network. After installing the "*KDE Connect*" app, the text can be transferred to the computer.

¹ This function works exclusively with the Google Chrome web browser.



Figure 1. Exemplary specimens used for experimental real-time label scans **A** printed labels scanned on pin **B** printed labels scanned separately **C** partly handwritten labels scanned on pin **D** partly handwritten labels scanned separately.

Variant 2 (bulk scans)

- 1) Open the "Google Keep Notes and Lists" app on your mobile phone.
- 2) Click on "+" and then on "image" icon.
- 3) Click on "Take photo" to capture the image, focus on the label you want to scan. Click on "photo" button, and then on the "checkmark" icon to save it.
- 4) Click on the image, then on the three dots in the upper right corner, and then on "grab image text". The text will appear as a note and can be manually corrected for spellings or readings errors. A title for the note can be added. This function seems to work only on newer Android systems; here we used successfully Android ver. 13 and ver. 14. With an older Android ver. 10 or 11 smartphone, this option did not work.
- 5) Repeat steps 2–4 for each label you want to scan. They will be saved as separate notes.
- 6) Select all notes, click on the three dots in the upper right corner, and then on "copy to Google Docs" (This step can be alternatively done already

on the computer via the respective google account; see Fig. 5). A single Google Docs document containing all images and texts will be generated. This step can be done on your mobile or on a computer logged into your Google account.

7) On your computer: open your Google Docs file, and the final corrections can be made and downloaded.

With an "Apple-only" environment

Requirements: Make sure you have a recent iPhone or iPad model with macro photography capabilities and the most recent operating system (iOS 15 and later). You will also need a Mac computer and an Apple iCloud account (at least the free version). An internet connection of the phone (e.g., via WLAN) is not necessary for data collection, if you collect your data from the specimen labels first on your phone (bulk scans) and go back to your Mac computer later.

a) Using "Notes" app:

- 1) Open the "*Notes*" app on your iPhone and set up a new note for your current project.
- In your note, tap the camera symbol at the bottom and choose "scan text" from the pop-up menu. A camera window opens in the bottom part of your note.
- 3) Aim your camera at the text block you want to scan. Yellow brackets will show you which text block the software sees as target. Once the desired target text is within the brackets press the insert button at the bottom of the camera window. The targeted text will be read and automatically transferred to your note.
- 4) Briefly check the result in your note.
- 5) Go to the next line in your note and scan the next target text in the same way, thus accumulating information from multiple specimen labels or multiple specimens as you like.
- 6) Once finished with the data collecting, return to your desktop Mac computer. If the phone had telephone connection with your provider while you took the scans or on your way back to your desktop computer, the "Notes" app should automatically synchronize with your Apple Account in the background so that when you open the "Notes" app on your desktop computer, you should find all the scanned data there.
- 7) Continue to copy and paste the information accumulated in your "*Notes*" app to the document or database of your choice.

b) Using the Shortcuts app:

The "Shortcuts" app of iOS can be used to program an automated process from taking the photo, extracting the text and filling a table in Apple's spreadsheet app "Numbers". Make sure that your "Shortcuts" and "Numbers" apps are synchronized for all of your devices via your iCloud drive. We assembled a "Short-cuts" algorithm as a proof of concept. Fig. 4 shows the algorithm.

Without internet connection, using Bluetooth with a Windows PC and an Android 14 mobile phone

1) Download the app ("Google keeps - Notes and Lists") on the mobile phone

2) Open Bluetooth options in the computer

3) Pair the computer and mobile phone

4) Click on receive files via Bluetooth

5) Open the app and click on the picture icon

6) Click on "take photo" and take the photo

7) Click on the captured picture

8) Click on the three dots in the upper right corner

9) Click on "grab image text" and select the extracted text²

10) Click on the three dots in the lower right corner and click on "send"

11) Click on "send via other apps" and choose the Bluetooth symbol

12) Choose a folder to save the html file in the computer

13) Copy the text from the html-file into a text editor for final spelling corrections

We expect this approach to work in a similar way also in Linux and Apple environments.

Results

In Table 1 we summarize the major characteristics of data capture using these methods. We show directly pasted content and the amount of real-time spelling corrections needed. In type-printed labels the corrections were minimal, but handwritten labels needed more correcting, depending on the size and style of the handwriting. In these cases, scanning the labels separately from the pin without distortion helped a lot (Fig. 1A, B). In printed labels, direction (Fig. 2C) and distortion of labels did not matter much (Fig. 2D). We were able to scan up to three labels (from the distorted side view) still mounted on a pin and without rotating the labels on, or removing them from, the pin (Fig. 2D).

Low image resolution was not a problem, and we could zoom-in digitally, so the labels almost filled the screen of the phone. However, during our initial testing, we found that much smaller images were also successful in capturing data (Fig. 1A-D).

Processing time per specimen was fast, and we estimated that full data capture, including spelling corrections, was 3–10 seconds per specimen. Processing time was often a little longer for badly handwritten labels, when an insect pin or other labels covered parts of the label text, or when the internet connection was slow. The total time gain per label was larger with labels containing much information or with multiple labels. For example, in the labels shown in Fig. 2B, typing the data by hand into the computer required 60 seconds, including spell check, but scanning the label using Approach 1 took 8 seconds (including a manual spell check). For the data of Fig. 2C, manual typing and spell-check required 121 seconds, while Approach 1 took 10 seconds (including manual spell check).

² This function works only on newer Android systems; here we used successfully Android v. 13 and v. 14. With an older Android v. 10 or 11 smartphone, this option unfortunately did not work.



Figure 2. Steps of scanning (exemplified by a screenshot from mobile phone) of real-time data collection, and examples of labels **A** step 1: marking of the text to be captured via touch screen of the mobile phone (example - printed labels scanned on pin) **B** step 2: select from menu bar (at the right side under three dots) "Copy to computer" (example - printed labels scanned separately). As to be seen, different labels at different levels on the pin can be scanned simultaneously and do not need to be removed from the pin **C** Screenshot showing the capture of multidirectional printed labels scanned on the pinned specimen in "*Google Lens*" **D** Screenshot showing the capture of multiple distorted, printed labels scanned on the pinned specimen in "*Google Lens*" **E** Screenshot showing the initial capture of a printed label scanned separately from the specimen in "*Google Keep*" **F** Screenshot showing the extracted data resulting from **E**.

Table 1. Summary of label configuration/ view (with reference to Figs 1, 2) and the obtained resulting text in the final database. Text corrected by real-time manual corrections are indicated in **bold**.

Label configuration/ view	Text as pasted from computer's clipboard	Verbatim finalized data (after manual correction)
Fig. 1A (labels scanned on pin, distorted)	Belivr vista Peretra、インタ	"Bolivia Buenavista Pereira XI.48 / Museum Frey Tutzing/
	Museum Frey	Ex Coll. Frey, Basel, Switzer land " (CF).
	Tutzing	
	Ex Coll. Frey, Basel, Switzer	
Fig. 1B (labels scanned separately, not distorted)	Bolivia Buengvista Pereira X198	"Bolivia Buenavista Pereira XI.48 / Ex Coll. Frey, Basel,
	Ex Coll. Frey, Basel,	Switzerland/ Museum Frey Tutzing" (CF).
	Switzerland	
	Museum Frey Tutzing	
Fig. 1C (partly handwritten labels scanned on pin, distorted)	North IRAQ, KURDISTAN Duhok, Akre, Bjeel 2.V.2018,	"North IRAQ, KURDISTAN Duhok, Akre, Bjeel 2.V.2018,
	leg.1.H.Mudhafar	leg.1.H.Mudhafar/ Maladera insanbilis (Brsk.) de t . D. Abrens 2023"
	Maladera	
	del. D. Ahrens 2023	
Fig. 1D (partly handwritten labels scanned separately, not distorted)	Maladus dusanabilis (Boy)	Malad era in sanabilis (B rsk) det. D. Ahrens 2023
	det. D. Ahrens 2023	
Fig. 2C	Tucuman:	"Tucuman: Argentina. H.E.Box. B.M.1930-238./ Est. Expt.
	Argentina. H.E.Box. B.M.1930-238.	Agric. No 2486/ TUCUMAN XI-I 191/ A H Rosenfeld Collector/ Astaena argentina Moser/ Ex Coll Frey Basel
	Est. Expt	Switzerland / Museum Frey Tutzing"
	Agric. No 2486	
	TUCUMAN 101/	
	AHRosenfeld Collector	
	Astaena argentina Moser	
	Ex Coll. Frey, Basel, Switzerland	
	Museum Frey Tutzing	
Fig. 2D	Argentiniel w.Wittmer	"Argentinien W. Wittmer/ L. Cabral Coral Salta 1160 m
	L. Cabral Coral	3.XII.1985/ Ex Coll. NHM Basel, Switzerland" (NHMB)
	Salta 1160 m	
	3.XII.1985	
	Ex Coll.NHM	
	Basel, Switzerland	
Fig. 3A	四川∶峨嵋山 _{⋧∼}	"四川:峨嵋山 1957. VII. 31 中國科學院"
	19573131	
	中國科學院	

We refrained from more extensive comparisons in the time needed between manual typing and data scanning, because the former depends much on the typing skills of the person doing the data entry. The comparative numbers from a few test replicates given here, refer to a scientist without proficiency in typing (performed by D.A.).

In some instances, in Approach 1, we had to use the deviation via a Google Docs document due to bad internet connection, when the copy process failed due to slow data transfer. This was then usually two "clicks" (or seconds) slower, but not a major delay compared to the amount of time required for manual typing.

The iPhone workflow test with was done with a larger label (Fig. 3B). In the workflow 2a) above, using the Notes app on the Apple iPhone, the image recognition tried to identify and focus blocks of text within the label, but did not to capture the label as a whole. To capture multiple bits of information the process had to be reiterated accordingly. Once the data has been collected in

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Figure 3. Other exemplary specimens used for experimental label scans **A** for Chinese language labels (printed) **B** printed Herpetology collection label that was scanned in the test of the Apple "*Shortcuts*" app algorithm. Note the incomplete text in the third text line and the cut off text "image 0355" below (compare to the corresponding data entries in C) **C** Screenshot of the automatically scanned collection label as transferred into cells of the spreadsheet app "*Numbers*". Although the text scan was very reliable, incomplete text will need editing: the somewhat cut off text "image 0355" of the label was interpreted as "Tmaee 0355". The time stamp in the first column corresponds to the file name of the respective photo saved as backup in the Shortcuts directory.

Notes, further copy-paste editing is necessary to transfer the data to a database. Workflow 2b), using "*Shortcuts*" app automation (Fig. 4) scans the whole label and also stores the data with a timestamp directly into a spreadsheet app. Furthermore, the photos are stored in the user's Apple iCloud account (as backups for potential later reference), but this step is optional in the algorithm. The result of the scanning is shown in Fig. 3B, C. Note that incomplete text in the original caused interpretation problems (truncated third line and partially hidden bottom part of "image 0355"). In addition, the algorithm tends to place

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Ask Where To Save:			Next Action Suggestions		
Overwrite If File Exists: 🗹			☑ If☑ Choose from Menu		
Extract text from LABEL			Set Variable		
Text			Text		
Text from Image			 Bet Contents of URL Ø URL 		
0			Add to Variable		
Open Test	Show More		Get Dictionary Value		
⊟ List			Match Text Comment		
Current Date			Get Item from List		
			 Show Alert Replace Text 		
+ - 2 items			Stop This Shortcut		
Add 🔳 List to the Bottom of Test in A in Test			 Count Open URLs 		
			Choose from List		
			Split Text		

Figure 4. iOS "*Shortcuts*" app algorithm. From top to bottom: The first step will open the iPhone's Camera app and lets you photograph the label. The photo ("LABEL") is then resized (optional, to reduce space) and saved in the background to the "*Shortcuts*" directory in your iCloud account with the current date (and time) as file name. Then the text is extracted from the photo and stored to a text container. The next step opens the spreadsheet "Test" in app "*Numbers*"; an empty target spreadsheet file (here: "Test") must be prepared beforehand and waiting in the "*Shortcuts*" folder of your iCloud account. Current Date and Text items are then collected in the "List". The List items are finally entered into different columns in the spreadsheet file "Test" and a sheet with the name "A".

each recognized line of text in a separate cell. If several lines belong to the same block of information, editing of the cells was necessary. The scan of the label and filling of the cells in the spreadsheet took less than 10 seconds. The algorithm analyzed the label as lines of text and allocated one cell per line in the spreadsheet. This means that the locality information in our example was split up into two cells in our test. Depending on which further tasks the user wants to accomplish copy-paste processing of such splits is necessary.

The approach using a Bluetooth connection between the mobile phone and the computer appeared to be slightly longer (by the increased number of "device



Figure 5. Screenshot of bulk-scanned labels via "*Google Keep*", inspected afterwards directly from the computer interface, during the step of copying to of the label text to a Google document (interface here in Portuguese).

clicks") than the direct approach (with the internet connection). Yet it saved a substantial amount of time for extracting the label data compared with manually typing. The use of Bluetooth may be necessary in situations where a good internet connection is unavailable, such as in collections. However, unfortunately the "grab image text" function of "*Google Keeps*" did work only with a newer smartphone (Android 13 or 14), not on an older device with Android 10 or 11.

Bulk approaches are available under the Google and Apple environment (Fig. 4) with the "*Google Keeps*" and "*Notes*" applications, respectively. In both, images are temporarily stored in the mobile devices, which can be later saved or discarded. While bulk approaches save time with data transfer, they have the disadvantage that potentially incomplete scans are only discovered when the specimens no longer at hand.

Discussion

While new technologies, including artificial intelligence, are entering in our daily life, their use and application in biodiversity research is yet rather limited, although there have been developments in Al-powered label recognition (Johaadien and Torma 2023; Weaver et al. 2023; Takano et al. 2024). Herein, we addressed the scanning of data from labels using a smartphone using different operating systems. To our knowledge, this has not previously been explored and applied to insect collection specimens in particular. There are solutions for large-scale mass digitization of collections (Blagoderov et al. 2012; Tegelberg et al. 2014; Engledow et al. 2018; Belot et al. 2023). All these solutions require manual separation of specimens and labels to separately photograph them. Initial trials with robotic technology (e.g. Dupont and Price 2019) are promising, but such methods can only be used by larger institutions with the budget to do so. With partly omitting the so far obligatory step of taking and permanently storing images of the labels, our direct approach to data capture is more rapid and environmentally more sustainable. In some of our procedures, data extraction happens without delay in the background, and there still is the option to retain the images if wanted. For a simple extraction of distributional data for taxonomic revisions or faunistic studies, we see no scientific necessity for long-term storage of images of specimen labels. Moreover, spell checking of the scanned and extracted data can be done when the specimen is at hand, and the data is finalized almost immediately.

However, depending on the individual needs and working conditions, the user has the choice on the individual workflow. It is possible to scan 50 labels in a row (i.e., bulk workflow) before transferring the data to the computer. Then in some critical cases, having a backup photo is good for quality assessment and spell checking.

Another great advantage is that these protocols use commercial devices that are simple to handle and which cost little to replace when they come into (informatic) age which is also a matter of cybersecurity. Unfortunately, in biosystematics, specialized devices are often overpriced, technologically obsolete, or require often expensive updates and service. Since biodiversity research in invertebrates, and especially entomology, is done in part by amateur scientists (and even professionals may lack funds for their "descriptive research"), funding may be lacking or limited.

Our results revealed that some functions may not be available on older smartphones running earlier operating systems. Here, the "grab image text" function did not work on Android 11 or older. Similarly, the other approaches might not work with even older devices, even if they work with the same app. However, we could not explore these limitations in detail, because we had only a limited selection of smartphones at hand.

The increasingly high reliability of text recognition and the rapid data transfer may make the use of machine readable barcode labels and QR codes superfluous in collection management, since connected data can be easily inferred from numerical voucher numbers on labels. Considering that optical character recognition (OCR) software, even when coupled with very advanced AI-technologies, possibly might give more errors than reading machine-readable codes (barcodes, 2D-codes), more rigorous tests are needed to check and compare the accuracy of a smartphone-based workflow compared to standard barcode and QR readers in this field.

Our solutions and tutorial proposed here are well suited for fast, secure recording of collection objects, e.g. when visiting a collection or when selecting individual objects. We are aware that habits, skills, and specific workflows influence the way we integrate such devices and text recognition capabilities. We are convinced that they will make a significant contribution and help to alleviate the taxonomic impediment (e.g., de Carvalho et al. 2005, 2007; Engel et al. 2021), as the workload for taxonomists capturing data of the material they study will be reduced by at least tenfold.

Finally, we note that there might be even more options and possibilities to scan labels with mobile devices. These options might evolve as quickly as mobile phones and artificial intelligence technology improve. Nevertheless, we expect that our paper will be an inspiration to others to continue exploring options on how to successfully apply this technology in their workflows and to share what they have learnt.

Acknowledgements

We thank the numerous colleagues who provided insightful discussions and encouraged us to pursue this topic. Furthermore, we thank Ilia Gjonov, Salza Palpurina, and Pavel Stoev for their helpful comments on the former version of our manuscript, as well as our colleague Christina Blume (LIB Bonn) for helping with some of the test runs of the apps.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

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Data availability

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

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Research Article

Biological resources of Eunotidae, Herbertiidae, Pteromalidae and Eulophidae (Hymenoptera, Chalcidoidea) from the Altun Mountain National Nature Reserve, China, with description of ten new species

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Academic editor: Jonah M. Ulmer Received: 3 November 2024 Accepted: 4 March 2025 Published: 26 March 2025

ZooBank: https://zoobank. org/614FD808-9FC8-4B90-A388-CFFA304E0425

Citation: Kang N, Hu H-Y (2025) Biological resources of Eunotidae, Herbertiidae, Pteromalidae and Eulophidae (Hymenoptera, Chalcidoidea) from the Altun Mountain National Nature Reserve, China, with description of ten new species. ZooKeys 1233: 31–54. https://doi. org/10.3897/zookeys.1233.140823

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Abstract

In this study, we explore the biological resources of Eunotidae, Herbertiidae, Pteromalidae and Eulophidae in the Altun Mountain Nature Reserve, Xinjiang, China. Sixty-one species are listed and we described ten new species, including *Eunotus caeruleus* Kang & Hu, **sp. nov.** and *Eunotus argenteus* Kang & Hu, **sp. nov.** of Eunotidae, *Herbertia altunensis* Kang & Hu, **sp. nov.** of Herbertiidae, *Thinodytes splendens* Kang & Hu, **sp. nov.**, *Erdoesina maculata* Kang & Hu, **sp. nov.** of Pteromalidae and two new species *Diaulinopsis altunensis* Kang & Hu, **sp. nov.** and *Hyssopus altunensis* Kang & Hu, **sp. nov.** of Eulophidae. Detailed illustrations of all new species are included to support identification and further study.

Key words: Alpine, biological control, checklist, Eulophidae, Eunotidae, Herbertiidae, new species, population density, Pteromalidae, species abundance

Introduction

Pteromalidae and Eulophidae are among the largest families within the superfamily Chalcidoidea (Insecta, Hymenoptera), notable for their vast diversity and global distribution. Currently, over 4000 species across 33 subfamilies and 640 genera of Pteromalidae have been documented worldwide, while Eulophidae encompasses more than 6000 species belonging to 328 genera across 5 subfamilies. In China alone, 388 species of 51 genera in Eulophidae, 110 genera and over 450 species in Pteromalidae have been recorded (Noyes 2019). Through years of research on morphological combining molecular phylogeny, the taxonomic status of the families Pteromalidae and Eulophidae has been changed (Burks et al. 2022; Cruaud et al. 2022). The two subfamilies Eunotinae Ashmead, 1904 and Herbertiinae Bouček, 1988 of Pteromalidae have been elevated to the family level of Eunotidae and Herbertiidae accordingly. At present, 23 genera and 17 species have been recorded in Eunotidae, of which 8 species have been recorded in China, and 3 genera and 10 species of Herbertiidae have been recorded worldwide, with only one species recorded in China (Bouček 1988). These families primarily consist of species which parasitize primarily hosts in the orders Lepidoptera, Coleoptera, Diptera, Hemiptera, as well as other arthropods (Cruaud et al. 2022). Their role in the biological control of agricultural pests highlights their ecological importance and biogeographical relevance.

Although Pteromalidae, Eunotidae, Herbertiidae and Eulophidae are widely distributed and commonly found in diverse habitats, they tend to show reduced species diversity and larger body sizes in alpine environments (Kang et al. 2023). To date, there are few records of these families from such regions (Table 1), with most collections concentrated in the Himalaya Mountains.

The Altun Mountain National Nature Reserve, situated in the southeastern Xinjiang Autonomous Region of northwest China, lies at the northern edge of the Tibetan Plateau and the southern boundary of the Tarim Basin. This reserve is distinguished by its unique extreme environmental conditions, including low temperatures, extremely arid, strong winds and high ultraviolet radiation. It features a variety of habitats, such as expansive sandy and gravel deserts, wetlands, and alpine steppes, with an average elevation exceeding 4500 m. These harsh conditions promote the development of unique species.

In this study, we enhance our understanding of Pteromalidae, Eunotidae, Herbertiidae and Eulophidae within the Altun Mountain Nature Reserve by listing 61 species, including 10 ten new species described and illustrated. This work marks a crucial advancement in addressing the taxonomic and ecological complexities of these families in an alpine region that has been historically overlooked in entomological studies.

Material and methods

All the examined specimens were collected by net sweeping, yellow pan traps, and Malaise traps in July from 2019 to 2021; yellow pans were left from 2 to 24 h at each site, and alcohol in Malaise traps was changed every 10 (±5) days to 1 month. The specimens were sorted and preserved in absolute ethanol immediately, stored at -20 °C in the lab. Selected specimens of both sexes were first air dried, then critical point dried (CPD, K850, Quorum), point-mounted or slide-mounted with labels, and then examined under a Nikon SMZ745T stereomicroscope using the available keys (Bouček 1988; Trjapitzin, 1989; Bouček and Rasplus 1991; Huang and Xiao 2005). Habitus photographs were taken with a Nikon D7000 digital camera connected to a Nikon SMZ25 stereomicroscope, at least 35 images were used for stacking to achieve high-quality images, and plates were compiled using Adobe Illustrator software. All specimens were deposited in the Insect Collection of the College of Life Science and Technology, Xinjiang University, Urumqi, Xinjiang, China (ICXU) (Fig. 1).

The taxonomic terminology and abbreviations follow relevant professional monographs (Graham 1969; Bouček 1988; Bouček and Rasplus 1991; Gibson et al. 1997; Huang and Xiao 2005). The following abbreviations are used in the text:

- **F1–6** Funicle segment from the first to the sixth;
- **POL** Distance between the posterior ocelli;
- **OOL** Distance between the eye margin and the adjacent posterior ocellus;
- OCL Distance between the posterior ocellus and the occipital margin;
- **Gt**₁₋₇ Gastral tergite segment from the first to the seventh.

 Table 1. Records of high altitude Pteromalidae and Eulophidae.

Family	Group	Alpine area	Reference
Pteromalidae	Tridymus sp.	Canadian Arctic	(Kevan 1973)
	Asaphes suspensus, Dibrachys cavus, Pachyneuron nelson, Schizonotus latus et al.	Ladakh region of the Trans- Himalaya	(Sureshan 2012)
	Herbertia indica, Pteromalus puparum	Kashmir Himalaya	(Bhat et al. 2017)
	Asaphes suspensus, Ammeia pulchella, Halticopterina triannulata et al.	Karatau Ridge and adjacent area in Western Tien Shan	(Dzhanokmen 2017)
	Sphaeripalpus, Halticoptera, Lamprotatus, Thektogaster	Tibet Plateau	(Xiao and Huang 2004)
Eulophidae	Chrysocharis	Italian Alps, high altitude of India	(Khan 1984; Hannes and Hansson 1997)
	Diglyphus isaea	Himalaya Mountains,	(Sha et al. 2007)
	Neotrichoporoide, Tamarixia, Pronotalia	Georgia	(Japoshvili and Kostjukov 2016)
	Diglyphus, Cirrospilus, Hemiptarsenus	North-western Himalayas, India	(Kumar and Sharma 2016)



Figure 1. A–F Location and habitat of protected area sampling sites **A** geographical location of the Altun Mountain National Nature Reserve **B** alpine meadow, 4240 m **C** plateau desert, 4050 m (main vegetation is *Thermopsis alpina* (Pall.) Ledeb.) **D** plateau wetland, 3890 m (main vegetation is *Neotrinia splendens* (Trin.) M. Nobis, P. D. Gudkova & A. Nowak) **E** alpine wetland, 3450 m (main vegetation is *Myricaria* sp.) **F** gravel desert, 3790 m (main vegetation is *Oxytropis* sp.).

Results

Eunotidae

Eunotus caeruleus Kang & Hu, sp. nov. https://zoobank.org/75ACC7E5-C7F5-49A0-87EE-24A960A866E5 Fig. 2A-F

Type material. *Holotype*. • ♀, point-mounted, China, Xinjiang, Ruoqiang County, Altun Mountain Nature Reserve, 38°4'22.5288"N, 89°7'10.7472"E, Altitude: 3681.55 m, 13.VII.2020, Coll. Ning Kang by sweeping net. *Paratypes*. • 1 ♀,

1 \Diamond , card mounted, same data as holotype except 15.VII.2020; • 3 \bigcirc , 5 \Diamond \Diamond , card mounted, 16.VII.2021. Coll. Shun-Gang Luo, Ning Kang, Hong-Ying Hu (All deposited in ICXU).

Description. Female. Length 1.3 mm. Body dark blue (Fig. 2A), eyes dark red, antenna dark brown. Legs with all coxae same color as the body; femora and tibiae dark brown, except trochanter and their apices deep yellow, forewing transparent covered densely with setae (Fig. 2D).

Head, distinct inverted triangle, in frontal view 1.45× as broad as high (Fig. 2B), the inner margin of the compound eye curved outward, eye height 0.61× as long as interocular distance, 0.85× as long as scape. Clypeus margin straight, without tooth, mandible bidentate, dark yellow. Antenna located before the lower eye margin and the distance from median ocellus by 3.96× height of the distance from clypeus margin, scape not reaching anterior ocellus, pedicel short and square, 0.6× as long as F1; F1-F4 all longer than width, funicle length 1.5× as long as width, each funicle segment with two rounds of dark plate-shaped sensilla; clava shorter than the combined length of the last two funicle segments, 3.35× as long as broad; length of flagellum and pedicel combined longer than head width (0.9×). Head in dorsal view 3.18× as broad as long, POL 4.2× OOL.

Mesosoma not distinctly convex, covered with reticulated and dense engraving, notauli distinct and complete (Fig. 2C). Pronotum 0.7× as long as mesoscutum length, anteriorly not margined; scutellum 0.6× as long as mesoscutum, frenal line absent. Propodeum 0.35× as long as scutellum, reticulation irregular, the rear of both sides protruded, nucha short (Fig. 2E). Forewing 2.2× as long as broad, covered with dense setae, without speculum and marginal fringe, marginal vein 1.04× as long as postmarginal vein, postmarginal vein 1.15× as long as stigmal vein, the angle between stigmal vein and postmarginal vein 40° (Fig. 2D).

Gaster 1.6× as long as broad, shorter than the combined length of head and mesosoma, Gt_1 distinctly longer than other tergites, 0.56× as long as the gaster, smooth without distinct markings (Fig. 2E).

Male. Length 1.0 ± 0.2 mm, N = 5 (Fig. 2F), similar to female in body color and habitus, but differs as follows. The last two flagella significantly shorter than the first two funiculus, square and short. Gaster short and flat.

Host. Unknown.

Etymology. *"caeruleus"* means dark blue, signifying the dark blue body color of the female species.

Diagnosis. The new species is morphologically similar to *E. parvulus*, but distinctly different in several key traits: the body color of the latter is dark green and gaster dark brown, while the color of new species is dark blue; POL: OOL of the latter is 4.5 while the new species is 4.2; pedicel $2\times$ as long as width, longer than F1, and significantly longer than the new species; all funicle segments broader than long and transverse while for the new species they are obviously longer than width; clava $2\times$ as long as broad, obviously shorter than the new species; propodeum with complete median carina and costulae, while the new species is covered with irregular reticulation; and Gt₁ 0.86× as long as gaster, significantly longer than the Gt₁ of the new species.



Figure 2. A–F *Eunotus caeruleus* Kang & Hu, sp. nov., female holotype A habitus, lateral view B head, frontal view C mesoscutum, dorsal view D forewing E gaster, dorsal view F Male, body, lateral view. Scale bars: 500 μ m (A, D); 200 μ m (B, C, E, F).

Eunotus argenteus Kang & Hu, sp. nov.

https://zoobank.org/3F61ECBE-C822-46A3-8978-67B0EEE8002F Fig. 3A-G

Type material. *Holotype.* • ♀, card mounted, Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 36°58'10.8984"N, 90°14'44.916"E, Altitude: 4021.95 m, 16.VII.2021. Coll. Ning Kang. *Paratypes.* • ♀, 38°0'1.9512"N, 89°0'31.3164"E,



Figure 3. A–G *Eunotus argenteus* Kang & Hu, sp. nov., female holotype A habitus, lateral view B habitus, dorsal view C head, frontal view D head, dorsal view E mesoscutum, dorsal view F forewing G antenna. Scale bars: 500 µm (A, B); 200 µm (C–G).

Altitude: 3717.15 m, 19.VII.2020. Coll. Ning Kang, by sweeping net; • 2 \bigcirc , 38°0'17.5428"N, 88°53'20.5044"E, Altitude: 3771.58 m, 19.VII.2021. Coll. Ning Kang, by sweeping net (All deposited in ICXU).

Description. Female. Length 1.3 mm (Fig. 3A), body black green, gaster dark brown; ocelli silver white, eyes deep red; antenna light brown, pedicel and clava dark brown; legs brown, coxae same color with body, tarsi yellow, distal tibiae dark brown; forewing hyaline, venation dark brown.
Head 1.45× as broad as high (Fig. 3C), the inner margin of eyes curved laterally, the distance between eyes 1.43× as their height. Antennal insertion below the lower ocular line, distance from upper margin of torulus to lower margin of anterior ocellus 7.2× distance from lower margin of torulus to lower margin of clypeus; scape 1.12× as long as eye height, not reaching anterior ocellus, pedicel longer than the combined length of first two funicular segments; five funicular segments(Fig. 3G), the first two segments transverse, the last three segments gradually lengthened, clava clavate, length of flagellum and pedicel combined longer than head width (0.77×). Head in dorsal view, 2.75× as wide as long, POL 3.9× OOL (Fig. 3D); Head in lateral view, 0.66× as broad as height, malar sulcus not distinct, eye height 1.24× malar space.

Head 1.25× as broad as mesosoma. Mesosoma not distinctly convex, pronotum 0.35× as long as mesoscutum (Fig. 3B), mesoscutum 0.85× as long as scutellum, notauli complete and distinctly straight, scutellum 1.74× as broad as long, frenal line absent (Fig. 3E). Fore wing 2.25× as long as broad (Fig. 3F), covered with dense hair, marginal vein 1.48× postmarginal vein, postmarginal vein 0.94× stigmal vein, stigmal vein slightly capitate.

Gaster sessile, abdomen flattened, $1.35 \times$ as long as thorax, approximately as long as broad. Gt, $0.5 \times$ as long as the gaster.

Male. Unknown.

Hosts. Unknown.

Etymology. 'argenteus' means silver, as in the color of the compound eyes of this species.

Herbertiidae

Herbertia altunensis Kang & Hu, sp. nov.

https://zoobank.org/2B750C67-7087-4C99-BED6-9B4DCC4EAA44 Fig. 4A-G

Material examined. *Holotype.* • \bigcirc , China: Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 38°4'22.5288"N, 89°7'10.7472"E, Altitude: 3681.55 m, 13.VII.2020. Coll. Ning Kang, by sweeping net; *Paratypes.* • 2 \bigcirc \bigcirc , 13.VII.2020. same locality, Coll. Ning Kang; • 1 \bigcirc , 19.VII.2021. same locality, Coll. Ning Kang (All deposited in ICXU).

Description. Female. Length 1.5 mm (Fig. 4A); body dark aeneous, with green metallic reflection, antenna concolorous with body, clava dark brown, eyes and ocelli dark red, coxae and femurs concolorous with body, tibiae light brown, forewing hyaline, venation dark brown.

Head in frontal view 1.25× as wide as high (Fig. 4B), eyes separated by 1.24× their height. Antennal insertion below lower ocular line, distance from upper margin of torulus to lower margin of anterior ocellus 5.1× distance from lower margin of torulus to lower margin of clypeus, clypeus margin slightly concave. Antenna stubby (Fig. 4D), length of flagellum and pedicel combined longer than head width (0.68×), each funicular segment transverse, and width is significantly greater than length, gradually widen from base to end, each funicular segment with a round of short sensilla, clava clavate. Head in dorsal view 4.35× as broad as long, POL 2.3× OOL, without occipital margin (Fig. 4C). Head in lateral view, malar sulcus not distinct, malar space 0.44× eye height.



Figure 4. A–G *Herbertia altunensis* Kang & Hu, sp. nov., female holotype **A** habitus, dorsal view **B** head, frontal view **C** head, dorsal view **D** antenna **E** forewing **F** mesosoma, dorsal view **G** propodeum, dorsal view. Scale bars: 500 μm (**A**); 200 μm (**B–D**, **F**); 250 μm (**E**, **G**).

Mesosoma flattened, head and mesoscutum covered with distinct white setae and rough reticulation (Fig. 4F), notauli distinct and complete, pronotum 3.65× as long as broad, mesoscutum 2.95× as long as broad, notauli complete, scutulum 0.95× as long as broad, frenal line absent. Propodeum rectangular (Fig. 4G), plicae and median carina distinct and complete, white setae covered on both sides. Forewing hyaline (Fig. 4E), covered with dense setae, speculum absent, the distance from uncus to the postmarginal vein 0.63× the width of stigma, marginal vein 1.73× postmarginal vein, postmarginal vein longer than stigmal vein (2.8×). Gaster sessile, ovate, 2.45× as long as broad, Gt_1 covers 1/2 of gaster, ovipositor not exerted.

Male. Unknown.

Hosts. Unknown.

Etymology. 'altunensis' means the collection site.

Diagnosis. The new species is similar to *H.indica*, but noticeably different from *H. indica* in body color black with dark blue metallic reflection, head in frontal view as broad as height, pronotum 0.2× as long as broad, scutellum equal in length and width, and marginal vein: postmarginal vein: stigmal vein = 23:9.6:4.

Pteromalidae

Erdoesina maculata Kang & Hu, sp. nov.

https://zoobank.org/D94063EB-C71E-4780-93CC-A1FC9A3C31CF Fig. 5A-G

Material examined. *Holotype.* • \bigcirc , China, Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 37°48'14.2092"N, 89°54'41.2884"E, Altitude: 3449.93 m. 16.VII.2021. Coll. Ning Kang. by sweeping net. *Paratypes.* • 2 \bigcirc \bigcirc , 1 \bigcirc , 37°48'14.2092"N, 89°54'41.2884"E, Altitude: 3449.93 m. 12.VII.2021. (All deposited in ICXU).

Description. Female. Length 2.2 mm (Fig. 5A), body dark blue, eyes and ocelli dark red with purple metallic reflections, antennal scape light yellow, pedicel brown, flagellum dark brown; legs yellow except tibiae ends white, forewing hyaline with darkened area below the marginal and stigmal vein, venation brownish.

Head in frontal view 1.45× as broad as high (Fig. 5B), lower margin of clypeal curved, malar sulcus not distinct, mandible with four teeth. Antennal insertion on lower ocular line (Fig. 5C), antennal scape length 1.1× eye height, pedicel longer than F1, in lateral view 1.86× as long as broad, funicular segments slight-ly broader than length, clava longer than the combined length of last two funicles, length of flagellum and pedicel combined longer than head width (1.1×). Head in dorsal view (Fig. 5D), POL 1.54× OOL; Head in lateral view (Fig. 5F), malar sulcus absent, malar space 0.36× eye height.

Mesosoma slightly convex, pronotum short, mesoscutum 2.07× as broad as long, notaular lines shallow and incomplete, scutellum 1.48× as broad as long, frenal line not distinct (Fig. 5G). Propodeum smooth, median carina and plicae raised and complete; nucha short, rectangular smooth without reticulation. Forewing infuscate below the marginal vein and stigmal vein (Fig. 5E), speculum large and open below, basal cell bares except a few hairs apically, marginal vein 1.43× postmarginal vein, postmarginal vein 1.1× as long as the stigmal vein.

Gaster petiole short rectangular, slightly longer than thorax, the length of each segment uniform, the ovipositor does not protrude significantly from the end of the abdomen.

Male. Length 2 mm, body smaller, gaster flat, antennal flagellum with whorled finer setae.

Hosts. Unknown.

Etymology. "maculata" means dark spot, signifying the dark area of the female forewings.

Diagnosis. The genus includes two species (Noyes, 2019): *E. alboannulata* mainly found in Europe and Central Asia, which has distinctly enlarged femora, gaster rounded, forewing marginal vein 1.5× as long as the stigmal vein, and basal cell bare; and *E. boarmiae* which has the marginal vein 1.3× as long as the stigmal vein, ovipositor slightly protruding from the end of the abdomen, head in dorsal view POL 1.05× OOL, and the combined length of two annuli and F1 as long as scape. The morphology of the new species is significantly different from the above two species.

Homoporus flavus Kang & Hu, sp. nov.

https://zoobank.org/A118D174-FBD9-4503-AAA6-ED7D503CB84A Fig. 6A-G

Material examined. *Holotype.* • \bigcirc , China, Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 37°18'21.9924"N, 90°20'33.2592"E, Altitude: 3907.8 m, 7.VII.2019. Coll. Ning Kang, by sweeping net. *Paratypes.* • 1 \bigcirc 1 \bigcirc , 37°23'51.6516"N, 90°10'59.7216"E, Altitude: 3855.19 m, 7.VII.2019. Coll. Ning Kang, by sweeping. • 2 \bigcirc 1 \bigcirc , 37°23'49.056"N, 90°10'52.3092"E, Altitude: 3854.2 m, 2020.VII.18. • 1 \bigcirc 2 \bigcirc , 37°23'53.0664"N, 90°11'11.8644"E, Altitude: 3843.19 m, 2021.VII.14 (All deposited in ICXU).

Description. Female. Length 2.2 mm, body slender, head and thorax dark green, with metallic reflection, gaster dark brown (Fig. 6A); eyes and ocelli dark red, mandible yellowish-brown; antennal flagellum light yellow, scape dark green with metallic reflection, pedicel dark brown; coxae concolor with body, legs yellow except basal part of femurs dark brown; forewing hyaline, venation albescent.

Head in frontal view 1.3× as wide as high (Fig. 6B), covered with rough reticulate, clypeal margin straight, clypeus with longitudinal striation. Antennal insertion on lower ocular line, distance from upper margin of torulus to lower margin of anterior ocellus 1.6× distance from lower margin of torulus to lower margin of clypeus. Antennal scape not reaching vertex, two discoid anelli, F1 shortest and square, clava 2.55× as long as wide, and with tubercles at apical, length of flagellum and pedicel combined longer than head width (0.86×). Head in lateral view, malar sulcus slender not distinct, eyes height 1.62× malar space. Head in dorsal view (Fig. 6C), 2.45× as wide as height, POL 1.84× OOL.

Mesosoma flattened (Fig. 6D), pronotum without carina, significantly narrower than the mesoscutum, mesoscutum 1.22× as wide as long, notauli incomplete, scutulum 1.32× as wide as long, frenal line not distinct, propodeum with finer reticulate in the middle area, median carina and plica not distinct, nucha short. Forewing hyaline (Fig. 6E) marginal vein 1.28× postmarginal vein, postmarginal vein longer than stigmal vein (1.38×), basal cell bare, speculum small.

Gaster sessile, flattened, 2.43× as long as wide, slightly longer than the combined length of head and thorax, ovipositor does not protrude.

Male. Length 1.7 \pm 0.1 mm (Fig. 6F), body slender, head and thorax dark blue, gaster dark brown, forewing hyaline, venation light brown, antennal pedicel dark brown, anellus light yellow, flagellum brown, each funicular segments with whorled light seta (Fig. 6G).

Hosts. Unknown.

Etymology. 'flavus' means golden yellow, which is the distinctive color of its antennae.





Diagnosis. The new species is similar to *H. sinensis*, but differs in having POL 1-1.11× OOL, each funicular segment longer than broad, marginal vein 2.3× stigmal vein, and mesosoma 2× as long as broad.

Stenomalina viridis Kang & Hu, sp. nov. https://zoobank.org/D2946720-B2EC-4E63-8785-327CEBA78DFF Fig. 7A-F

Material examined. *Holotype*. • ♀, China, Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 36°58'10.8984"N, 90°14'44.916"E, Altitude: 4021.95 m, 21.VII.2020.



Figures 6. A–**G** *Homoporus flavus* Kang & Hu, sp. nov., female, holotype **A** habitus, dorsal view **B** head, frontal view **C** head, dorsal view **D** mesoscutum, dorsal view **E** forewing **F** male, habitus **G** head, frontal view. Scale bars: 500 μm (**A**); 200 μm (**B**–**G**).

Coll. Ning Kang, by sweeping net. *Paratypes.* • 4 $\bigcirc 2$ \Diamond , 36°56'25.8576"N, 90°16'48.2376"E, Altitude: 4052.62 m, 21.VII.2020. Coll. Ning Kang, by sweeping. • 2 $\bigcirc 1 \Diamond$, 36°58'10.8984"N, 90°14'44.916"E, Altitude: 4021.95 m, 16.VII.2020. Coll. Ning Kang, by sweeping. • 50 \bigcirc , 36°56'32.91"N, 90°16'40.4508"E, Altitude: 4047.73 m, 16.VII.2021. Coll. Ning Kang, by sweeping.

Description. Female. Body length 2.3 mm (Fig. 7A), body green with metallic luster, eyes and ocelli dark red, scape and pedicel concolor with body, flagellum

dark brown, femur dark brown except light yellow at both ends, forewing hyaline, venation brown.

Head in frontal view 1.4× as wide as high (Fig. 7B), clypeal suture obvious, clypeus with longitudinal striation, clypeal margin protruded with three teeth, middle tooth sharp and long, side teeth shorter. Antennal insertion above lower ocular line, distance from upper margin of torulus to lower margin of anterior ocellus 1.55× distance from lower margin of torulus to lower margin of clypeus, scape length 0.65× eye height, not reaching anterior ocellus, pedicel pyriform (Fig. 7E), 1.65× as long as broad, as long as F1, two discoid anelli, funicular segments square, and gradually widen towards the end, calva 2.25× as long as broad, each funicular segment with sensilla, length of flagellum and pedicel combined longer than head width (1.08×). Head in lateral view, POL 1.63× OOL.

Mesosoma slender (Fig. 7C), pronotum anteriorly not margined, notauli incomplete, mesoscutum 1.49× as long as broad, covered with rough reticulation, scutellum slightly convex, 1.02× as long as broad, frenal line strongly impressed, reticulation of frenum similar to the scutellum, propodeum length 0.46× as long as scutellum, median carina slim, plicae not distinct. Forewing costal cell with few setae apically, basal cell bare, speculum large and opened below, marginal vein 1.55× postmarginal vein, postmarginal vein 0.65× stigmal vein, stigma oblong with long uncus (Fig. 7F).

Gaster sessile and oval, slightly longer than thorax, $Gt_1 0.35 \times ength$ of gaster. **Male.** Body length 2.1 ± 0.1 mm (Fig. 7F), flagellum with whorled setae. **Hosts.** Unknown.

Etymology. *'viridis'* means emerald green, emphasizing its distinctive body color.

Diagnosis. The new species is similar to *S. muscarum* (L.), but can be differentiated by the pronotum anteriorly margined, forewing marginal vein 2.2–2.5× stigmal vein, and male flagellum black with metallic luster.

Thinodytes splendens Kang & Hu, sp. nov.

https://zoobank.org/F62377E7-873A-4D38-AFA4-94DA6AF318AB Fig. 8A-G

Material examined. *Holotype*. • \bigcirc , China, Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 37°18'21.9924"N, 90°20'33.2592"E, Altitude: 3907.8 m, 7.VII.2019. Coll. Ning Kang, by sweeping net. *Paratypes.* • 1 \bigcirc 1 \bigcirc , 37°23'51.6516"N, 90°10'59.7216"E, Altitude: 3855.19 m, 7.VII.2019. Coll. Ning Kang, by sweeping net.

Description. Female. Length 2.2 mm (Fig. 8A), body dark green with metallic reflection; eyes dark red, mandible dark brown; flagellum dark brown, scape and pedicel concolor with body; tibia brown, tarsus dark brown; forewing hyaline, venation brown.

Head in frontal view 1.53× as long as wide (Fig. 8B), eyes height 2.75× malar space, clypeus smooth, anterior margin of clypeus with three asymmetrical teeth, mandible four teeth. Antennae inserted above level of ventral edge of eyes, formula 11263, scape 0.95× as long as eye height, length of flagellum and pedicel combined longer than head width (1.2×), anterior four funicular segments 1.63× as long as broad, the last two funicular segments 1.15× as





long as broad, clava with micropilosity area at the ventral of last two segments (Fig. 8E), and slightly shorter than last two funicular segments. Head in dorsal view (Fig. 8D), POL 1.45× OOL. Head in lateral view (Fig. 8C), malar sulcus not distinct, malar space 0.36× eyes height.

Pronotum covered with large reticulates (Fig. 8F), mesoscutum convex, 2.05× as broad as long, notauli complete but shallow, scutellum 1.33 as broad as long, frenal line not distinct, reticulations on scutellum smaller than mesoscutum, frenum smooth without reticulates. Propodeum as long



Figures 8. A–G *Thinodytes splendens* Kang & Hu, sp. nov., female holotype A habitus, lateral view B head, frontal view C head, lateral view D head, dorsal view E antenna F mesoscutum G forewing. Scale bars: 500 μm (A, G, E); 200 μm (B–D).

as scutellum, median carina and plica not distinct, each side of the upper margin with elliptical depression, callus covered with white dense setae. Forewing with 2–3 rows hairs on the end of costal cell (Fig. 8G), 4 rows of setae in the basal cell, speculum oval and closed below, marginal vein as long as postmarginal vein, 2× as long as stigmal vein.

Gaster petiole slightly longer than propodeum, 1.93× as long as wide, abdomen oval-shaped and significantly shorter than the thorax, 0.63× as long as thorax. Gt₁ 0.48× as long as gaster.

Male. Length 1.8 \pm 0.1 mm, gaster flat and short, flagellum covered with dense setae.

Hosts. Unknown.

Etymology. '*splendens*' means luminous, emphasizing its distinctive brightgreen body color among the species in the genus.

Diagnosis. By comparing the morphology with the seven species of this genus that have been described in the world (Heydon 1995), it was found that the new species is most similar to *T. cyzicu*, but the body color of the new species is obviously bright green, while the body color of the latter species is blue-black, the head in frontal view is 1.25× as long as wide, the propodeum has a complete median carina and plica, and the spiracular sulcus wide and deep.

Eulophidae

Diaulinopsis altunensis Kang & Hu, sp. nov.

https://zoobank.org/70983ACC-A8BD-40F3-9F4F-7DBF74904647 Fig. 9A-G

Material examined. *Holotype*. • ♀, China, Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 38°5'37.572"N, 89°16'17.1372"E, Altitude: 3471.91 m, 15.VII.2020. Coll. Ning Kang, by sweeping net. *Paratypes*. • 1 ♀ 2 ♂, 37°49'28.8588"N, 89°9'9.5328"E, Altitude: 4237.16 m, 12.VII.2020. Coll. Ning Kang, by sweeping; • 1 ♂, 37°48'14.2092"N, 89°54'41.2884"E, Altitude: 3449.93 m, 12.VII.2020, by net sweeping; • 1 ♂, 37°39'47.6172"N, 88°45'35.1864"E, H: 4007.46 m, 13.VII.2020, • 2 ♀, 37°58'30.1512"N, 88°58'25.158"E, Altitude: 3849.56 m, 14.VII.2020; • 4 ♀ 2 ♂, 38°5'37.572"N, 89°16'17.1372"E, Altitude: 3471.91 m, 9–15.VII.2020, by Malaisetrap; • 6 ♂, 36°56'32.91"N, 90°16'40.4508"E, H: 4047.73 m, 16.VII.2020, by net sweeping.

Description. Female. Length 1.4 mm, body metallic green (Fig. 9A), antenna dark brown except scape complete brown with metallic tints, anterior of eyes, toruli and part of frontal suture with some yellowish marks, eyes and ocellus red, mandibles yellow and maxillary palp white without expansion; legs yellow-ish-white, coxae with body color, basal 3/4 of femur dark brown with metallic tints, terminal tarsus brown. Gaster dark brown with metallic green tints.

Head with finely reticulate, in frontal view 1.05× as wide as high (Fig. 9B), POL: OOL = 3.65. Frontal sulcus obtuse "V" shaped and yellow marked, malar sulcus straight, 0.4× as eye height; clypeus truncate, without oral fossa. Antenna inserted below the level of lower margin of eyes, with two discoid anelli (Fig. 9D); scape 4.5× as long as wide, 0.8× as long as eye height; clava 2.4× as long as wide and 1.4× as long as F1 + F2, F1 1.3× as wide as the width of pedicel in lateral view; funiculars quadrate, each with a single row of sensilla and irregularly distributed.

Mesosoma, pronotum conoid (Fig. 9C), 0.2× as long as wide, mesoscutum 0.8× as long as wide, with complete and deep notauli; scutellum 1.0× as long as wide and 1.0× as long as mesoscutum, with lateral grooves; ratio distance between grooves/distance between one groove and lateral margin of scutellum 2.3, meshes of reticulation more elongate than mesoscutum; dorsellum 0.2× as long as scutellum, propodeum 0.2× as long as scutellum, smooth with fine reticulation, propodeal callus with four pale setae. Submarginal vein of forewing with six setae and without speculum (Fig. 9E), marginal vein: postmarginal vein: stigmal vein = 3:1.7:1.



Figures 9. A–G *Diaulinopsis altunensis* Kang & Hu, sp. nov., female holotype **A** habitus, lateral view **B** head, frontal view **C** mesoscutum **D** head, lateral view **E** forewing. Male **F** habitus, lateral view **G** head, lateral view. Scale bars: 250 μm (**A**, **C**, **D–G**); 100 μm (**B**).

Metasoma, gaster sessile, dark brown with metallic tints throughout, about $1.7-1.8 \times$ as long as wide.

Male. Length $1.7 \pm 0.1 \text{ mm}$ (Fig. 9F), body slighter, antennal scape obviously enlarged (Fig. 9G).

Hosts. Unknown. There are many Agromyzidae (Diptera) flies and *Oxytropis*, *Carex* plants (gravel desert) in their habits.

Etymology. 'altunensis' means the collection site.

Diagnosis. The new species is similar to *D. albimaxillia*, but can be distinguished by the significantly enlarged white maxillary palp and gaster with a large spot in the anterior 1/2 of the latter. Among species of the genera, only this species has the scape and gaster dark brown with metallic green tints throughout.

Hyssopus altunensis Kang & Hu, sp. nov.

https://zoobank.org/CD4F004E-9024-47EE-B5DB-12F67F6E3A65 Fig. 10A-I

Material examined. *Holotype*. • ♀, China, Xinjiang, Ruoqiang, Altun Mountain Nature Reserve, 37°18'21.9924"N, 90°20'33.2592"E, Altitude: 3907.8 m, 7.VII.2019. Coll. Ning Kang, by sweeping net. *Paratypes*. • 1 ♀ 1 ♂, 37°23'51.6516"N, 90°10'59.7216"E, Altitude: 3855.19 m, 7.VII.2019. Coll. Ning Kang, by sweeping.

Description. Female. Length 2.4 mm, body and thorax dark green, with metallic reflection, gaster dark brown (Fig.10A), antennae brown, eyes red; coxae yellow-ish-brown, distal tarsus dark brown; forewing hyaline, tegula brown, venation brown.

Head in frontal view square (Fig.10B), 1.25× as wide as high, scape 1.85× as long as eyes, clypeus margin 3.25× malar space, clypeus with long white hair; antennal insertion close to clypeus margin, below the lower ocular line; pedicel longer than each funicular, 2.34×, anelli short and transverse, F1 longer, F2-F4 0.88× as long as wide, each funicular with 2 row of sensilla, clava clavate, with a strong constriction between its second and third segments, 1.95× as long as wide. Head in lateral view (Fig.10G), eyes 1.55× as height as wide, malar sulcus straight, malar space 1.65× as long as eyes height. Head in dorsal view, 4.25× as wide as height, POL 2.15× OOL.

Mesoscutum not distinctly convex (Fig.10C), smooth with fine reticulation, pronotum large and hemisphere, covered with irregular setae, 1.3× as long as mesoscutum; mesoscutum smooth with 2 pairs of setae, 2.85× as wide as long, without setae; scutulum 1.25× as long as mesoscutum, sublateral groove connected terminally, frenum smooth without reticulation, significantly shorter than propodeum; propodeum with complete median carina (Fig.10F), without plica and costulae, nucha short. Forewing hyaline (Fig. 10D), 2.26× as long as wide, marginal cell with 2–3 rows of setae, speculum not distinct.

Gaster 1.95× as long as wide (Fig.10E), full oval shape, ovipositor sheath slightly protruding the end of the abdomen.

Male. Length 1.8 \pm 0.1 mm (*N* = 5) (Fig. 10H), antennal scape significantly expands, 1.5–1.6× as long as wide (Fig.10I); F1 with at least 2 rows of sensilla, F2-4 with 1 row of irregular setae; each coxa concolor with body, femur dark brown; gaster flattened and shorter than thorax.

Hosts. Unknown.

Diagnosis. Comparing the morphological characters of the genus *Hyssopus* (Schauff, 1985), the new species is similar to *Hyssopus geniculatus* (Hartig, 1838), but the latter has a body black, eyes black, ocellus brown, each coxa black, antennal insertion on lower ocular line, and the flagellum is widened gradually towards the end. The new species differs from *Hyssopus flavgasterus* by the body dark green, with blue luster, eyes dark gray, and antennal insertion below the lower ocular line.



Figures 10. A–I *Hyssopus altunensis* Kang & Hu, sp. nov., female holotype A habitus, lateral view B head, frontal view C mesoscutum D forewing. Male E habitus, dorsal view F forewing G head, mesoscutum, lateral view H male, habitus, lateral view I antenna. Scale bars: 500 μm (A, D, H); 200 μm (B, C, E–G, I).

Geographical distribution of Eunotidae, Herbertiidae, Pteromalidae and Eulophidae in the Altun Mountain National Nature Reserve

The Altun Mountain region exhibits pronounced topographic and vegetative contrasts between its eastern and western sectors. The eastern foothills are

characterized by gentle terrain with verdant vegetation, while the western alpine zone demonstrates rugged topography and harsher habitat conditions. We systematically examined the east-west disparity in the four families' species diversity and community structure.

The distribution of the four families across various habitats within the Altun Mountain Nature Reserve is extensive but notably uneven. Species abundance and population density are significantly higher in the eastern part of the reserve compared to the western part, while both population numbers and species richness are lower. The eastern sector of the nature reserve exhibits higher vegetation richness and coverage compared to the western region, with a marginally elevated mean annual temperature that collectively is more favorable for species survival. Species of Eunotidae and Herbertiidae are mainly distributed in the western part of the reserve, having a typical psammophytic desert ecosystem characterized by arenaceous soil substrates (sand content > 85%), where xerophytic leguminous shrubs constitute 22-35% of the total vegetation cover, demonstrating significant adaptation to arid edaphic conditions. Among Pteromalidae, the subfamilies Miscogasterinae and Pteromalinae are widely distributed with substantial populations, while Eulophidae, Entedontinae and Eulophinae exhibit a similar distribution pattern. At the genus level, Selderma, Pachyneuron, Callicarolynia, Halticoptera, Neochrysocharis, Entedon, Diaulinopsis are notably prevalent throughout the reserve. The distributions of Halticoptera trinflata Huang, 1991 and Callicarolynia yixiekea Kang & Hu, 2022 were found to be clustered, with H. trinflata showing a strong association with Thermopsis alpina (Pall.) Ledeb. Additionally, we observed that sites with higher coverage of flowering vegetation had significantly greater species abundance.

Acknowledgments

We thank the Bazhou Altun National Nature Reserve Administration for admission to scientific investigation. Sincerely thank Zeng-Qian Huang, Shu-Han Guo and Shun-Gang Luo for their help during the field collecting and sample sorting. In addition, many thanks to the driver and guide Li Youyi's careful navigation and route planning during the scientific investigation in Altun Mountain National Nature Reserve.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the National Natural Science Foundation of China (Grant Number 31860612) and the Autonomous Region Graduate Scientific Research Innovation Project (Grant Number XJ2019G025).

Author contributions

Conceptualization: NK. Data curation: NK. Funding acquisition: HYH. Investigation: HYH, NK. Methodology: NK. Writing - original draft: NK. Writing - review and editing: NK.

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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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Appendix 1

Checklist of Eunotidae, Herbertiidae, Pteromalidae and Eulophidae in Altun Mountain National Nature Reserve

The checklist is the result of a three-year investigation in the Altun Mountain National Nature Reserve. The following 61 species of the four families were found:

Eunotidae Ashmead, 1904

- 1. Eunotus caeruleus Kang & Hu, sp. nov.
- 2. Eunotus argenteus Kang & Hu, sp. nov.

Herbertiidae Bouček, 1988

3. Herbertia altunensis Kang & Hu, sp. nov.

Pteromalidae Dalman, 1820 Asaphinae Ashmead, 1904

4. Asaphes petiolatus (Zetterstedt, 1838)5. Asaphes vulgaris Walker, 1834

Miscogasterinae Walker, 1833 Miscogastrini:

- 6. Seladerma saurus Walker, 1844
- 7. Xestomnaster mazares (Walker, 1844)

Sphegigastrini:

- 8. Ammeia pulchella Delucchi, 1962
- 9. Sphaeripalpus vulgaris Huang, 1990
- 10. Callicarolynia yixiekea Kang & Hu, 2023
- 11. Cyrtogaster vulgaris Walker, 1833
- 12. Sphegigaster intersita (Graham, 1969)
- 13. Halticoptera trinflata Huang, 1991
- 14. Halticoptera moczari (Erdös, 1954)
- 15. Halticoptera circula Walker, 1833
- 16. Halticoptera atherigona (Huang, 1990)
- 17. Merismus splendens Graham, 1969
- 18. Thinodytes splendens Kang & Hu, sp. nov.

Pireninae Haliday, 1844

19. Gastrancistrus hamillus Graham, 1969

Pachyneurinae Rohdendorf, 1962

- 20. Pachyneuron aphidis (Bouché, 1834)
- 21. Pachyneuron solitarium (Hartig, 1838)
- 22. Pachyneuron korlense Xiao, Jiao & Huang, 2009
- 23. Pachyneuron grande Thomson, 1878
- 24. Pachyneuron planiscuta Thomson, 1878

Pteromalinae Dalman, 1820 Pteromalini:

- 25. Chlorocytus splendensis Li & Hu, 2017
- 26. Chlorocytus polichna (Walker, 1848)
- 27. Caenocrepis arenicola (Thomson, 1878)
- 28. Cyclogastrella qinghaiensis Jiao & Xiao, 2015

- 29. Dibrachys (Dibrachys) microgastri (Bouché, 1834)
- 30. Erdoesina maculata Kang & Hu, sp. nov.
- 31. Homoporus flavus Kang & Hu, sp. nov.
- 32. Mesopolobus semenis Askew, 1997
- 33. Mesopolobus bruchophagi (Gahan, 1917)
- 34. Nasonia vitripennis (Walker, 1836)
- 35. Pteromalus microps (Graham, 1969)
- 36. Pteromalus sequester Walker, 1835
- 37. Stenomalina viridis Kang & Hu, sp. nov.
- 38. Trichomalopsis brevis Kang & Hu, sp. nov.
- 39. Trichomalopsis closterae Kamijo, 1983
- 40. Urolepis maritima (Walker, 1834)

Eulophidae Westwood, 1829: Entedoninae Förster, 1856

- 41. Chrysocharis mediana Förster, 1861
- 42. Entedon cyanellus Dalman, 1820
- 43. Neochrysocharis aratus (Walker, 1838)
- 44. Neochrysocharis formosus (Westwood, 1833)
- 45. Neochrysocharis iris Erdös, 1954

Eulophinae Westwood, 1829

- 46. Cirrospilus vittatus Walker, 1838
- 47. Diaulinopsis altunensis Kang & Hu, sp. nov.
- 48. Diglyphus isaea (Walker, 1838)
- 49. Diglyphus begini (Ashmead, 1904)
- 50. Elachertus parvispecularis Zhu & Huang, 2001
- 51. Elachertus lateralis (Spinola, 1808)
- 52. Hyssopus altunensis Kang & Hu, sp. nov.

Tetrastichinae Förster, 1856

- 53. Aprostocetus (A.) eurystoma Graham, 1961
- 54. Aprostocetus (A.) emesa (Walker, 1839)
- 55. Aprostocetus (Chrysotetrastichus) celtidis (Walker, 1839)
- 56. Aprostocetus (A.) caudatus Westwood, 1833
- 57. Baryscapus fossarum Graham, 1991
- 58. Baryscapus gradwelli Graham, 1991
- 59. Holcotetrastichus rhosaces (Walker, 1839)
- 60. Kolopterna kohatensis Graham, 1987
- 61. Oomyzus sokolowskii (Kurdjumov, 1912)



Research Article

New insights into the mitogenomic phylogeny and evolutionary history of Murinae (Rodentia, Muridae) with the description of a new tribe

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Academic editor: Raquel López-Antoñanzas Received: 31 October 2024 Accepted: 17 February 2025 Published: 27 March 2025

ZooBank: https://zoobank. org/739E8E73-362C-4ECF-A942-A172CA959C04

Citation: Liu S, Zhao S, Wang J, Fu C, Wang X, Liu S, Chen S (2025) New insights into the mitogenomic phylogeny and evolutionary history of Murinae (Rodentia, Muridae) with the description of a new tribe. ZooKeys 1233: 55–74. https://doi.org/10.3897/ zookeys.1233.140676

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Abstract

Murinae is the largest known subfamily of Muridae and includes 15 tribes and 3 genera (incertae sedis). Although the phylogeny of Murinae has been studied, its phylogenetic relationships have not been completely elucidated. We used phylogenetic framework and molecular dating methodologies with the vast majority of available mitochondrial genomes to disentangle the phylogenetic relationships and evolutionary history of Murinae. Sixteen tribes were identified within the Murinae subfamily. Among these, fifteen tribes were found to be consistent with those currently recognized. Hapalomyini (Clade A) was located at the base of the Murinae clade with strong nodal support contrary to previous studies, which showed that Phloeomyini diverged first. The Clade B consisted of Micromyini, Rattini, and the genus Vernaya. Vernaya cannot be accommodated in any existing tribe. The origin of Murinae dates back to 17.22 Ma. The split between Micromyini and Vernayini was dated to 11.69 Ma during the Miocene, indicating that they were both early branches of Murinae. Combined with the differences between Vernaya and its sister tribes (Micromyini and Rattini) in morphology, skull and teeth, we validated a new tribe, Vernayini tribe nov. We believe that it is necessary to combine morphological and molecular perspectives (especially from a genome-wide perspective) to determine the phylogenetic position of tribes with an uncertain taxonomic position in Murinae.

Key words: Divergence analysis, evolutionary history, mitochondrial genome, nocturnal rodents, phylogenetic analysis, taxonomy, tribal classification, *Vernaya*

Introduction

Murinae comprises 135 genera and 656 species and is the largest subfamily of Muridae (Wilson and Mittermeier 2018). Muridae exhibit a greater degree of variation in their diversification rates compared to any other rodent group (Fabre et al. 2012). In addition, owing to the medical importance and genomic benefits of Murinae species, sequencing, phylogeny, and divergence analyses are particularly important. The phylogenetic relationships of some subfamilies have been explained from various aspects; however, those of Murinae have not been fully elucidated (Steppan et al. 2005). To fill this void in knowledge, Steppan et al. (2005) clarified the phylogeny of Murinae, which consisted of seven distinct geographic lineages. They achieved this by employing three nuclear genes and one mitochondrial fragment and inferred that Murinae originated in Southeast Asia. Musser and Carleton (2005) divided Murinae into 29 divisions. Lecompte et al. (2008) proposed describing the phylogenetic relationships of Murinae at the tribe level and pointed out that Phloeomyini is basal to Murinae. Fabre et al. (2013) deduced the phylogenetic relationships of Murinae and reconstructed the biogeographic history, also showing that Phloeomyini is located at the base of Murinae; they found that Micromys belongs to Murini, rather than a separate tribe, and that Murinae has nine tribes. Meschersky et al. (2016) determined that Chiropodomys is neither a member of the Micromys division nor is it closely related to Hapalomys. Missoup et al. (2016) clarified the phylogenetic relationships among Arvicanthini species. In the same year, Pagès et al. (2016) used molecular analysis and morphological comparison methods to determine the taxonomical status of the Micromys and Pithecheir divisions in Murinae. According to recent research, Murinae is divided into 15 tribes, and 3 genera with an undetermined status (Wilson and Mittermeier 2018). This suggests that many genera have uncertain taxonomic status in Murinae, which are named Murinae incertae sedis (Pagès et al. 2016).

Vernaya belongs to the subfamily Murinae and is regarded as a monotypic genus containing only one species, *Vernaya fulva*. Zhao et al. (2023) performed molecular and morphological analyses on this taxon, and determined that *Vernaya* is not a monotypic genus. Instead, it contains four species: *Vernaya fulva* (Allen, 1927), *Vernaya foramena* Wang, Hu & Chen, 1980, *Vernaya nushanensis* Zhao, Liu, Jiang, Liu & Chen, 2023, and *Vernaya meiguites* Zhao, Li, Wang, Jiang, Liu & Chen, 2023. Thus, the phylogeny of the genus in Murinae remains unknown.

Mitochondrial genomes have been used in many studies to explore the phylogeny of species (Rasmussen and Arnason 1999; Kuang et al. 2019; Song et al. 2022; Huo et al. 2023). However, based on their genomes, no studies have investigated the phylogeny of Murinae. To determine the phylogenetic relationships of Murinae and the taxonomic status of *Vernaya* in Murinae, we conducted whole mitochondrial genome sequencing for one individual of each of the four species of the genus, constructed a phylogenetic tree of Murinae, and performed a divergence time calculation to determine the evolutionary history.

Material and methods

Specimen collection, DNA extraction and sequencing, assembly and annotation

In the present study, we sequenced one individual of each of the four species in *Vernaya*. The four specimens were collected using snap traps from Yunnan and Sichuan, China (we sequenced the mitochondrial genomes (mtGenomes) of the first four species in Table 1). All individuals in this table were used for morphological comparative analysis later.

The implementation of trapping across all locations included a broad utilization of various trap models, such as Victor snap traps, Museum Special traps, and Sherman live traps. The process of capturing the specimens was executed

Field ID	Museum number	Locality
csd4405	SCNU02747	Lanping, Yunnan
csd3540	SAF201518	Pingwu, Sichuan
csd3561	SAF19287	Yunlong, Yunnan
csd3537	SAF201653	Meigu, Sichuan
csd1525	SAF19383	Habahe, Xinjiang
XZ021	SCNU00173	Yadong, Xizang
	Field ID csd4405 csd3540 csd3561 csd3537 csd1525 XZ021	Field ID Museum number csd4405 SCNU02747 csd3540 SAF201518 csd3561 SAF19287 csd3537 SAF201653 csd1525 SAF19383 XZ021 SCNU00173

Table 1. Collection information of specifie	Table 1
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with the aid of cage-style traps. Once captured, the small mammals were transported to the lab, where they underwent bloodletting under the influence of isoflurane anesthesia, administered on a heated mat to ensure their comfort and to reduce any potential suffering. Fresh liver or muscle was obtained and preserved in anhydrous ethanol in the field, and stored in a -80 °C freezer upon return to the laboratory. Tissues and specimens were stored at the Sichuan Academy of Forestry Sciences (SAF) and Sichuan Normal University (SCNU). All fieldwork complied with legal regulations in China, and sampling was carried out following local legislation. This study was approved by and conducted according to the guidelines of the Animal Ethics Committee of Sichuan Normal University.

We used the animal tissue DNA extraction kit of Chengdu Foregene Company Limited to extract DNA following the manufacturer's instructions, and then sent it to Novogene (Beijing, China) for high-throughput sequencing using the Illumina NovoSeq 6000. The original data were spliced using MitoZ in the Linux system with reference to the whole-genome sequence of *Vernaya* species (Meng et al. 2019). The Mitos database (http://mitos.bioinf.uni-leipzig. de/index.py) was used to preliminary annotate the mtGenomes and to detect the sequences of protein-coding genes, rRNA, and tRNA (Bernt et al. 2013). The sequences were annotated using CGView (https://proksee.ca/) to obtain a structural map of the mitochondrial genome (Grant et al. 2023).

Phylogenetic analysis of Murinae

In the present study, based on the mtGenomes of four species of *Vernaya*, the taxonomic status of the genus was explored. According to the latest classification relationship of Murinae, we downloaded the mtGenome sequences of some Murinae species from NCBI (https://www.ncbi.nlm.nih.gov/), based on the classification of the tribes of Murinae (except for three tribes) (Wilson and Mittermeier 2018). For species without available mtGenome sequences, *Cyt b* gene sequences were obtained from NCBI (Table 2).

We chose *Meriones tamariscinus* Pallas, 1773 and *Meriones meridianus* Pallas, 1773 as outgroups and downloaded their complete mitogenomes from the NCBI. The sequences of Murinae species obtained from NCBI and the sequences of four individuals of *Vernaya* were imported into MEGA 5 software. The gene sequences were compared; inconsistent or uncertain sequences were manually corrected and removed, and the aligned FASTA file was exported. We applied Bayesian inference (BI) and maximum-likelihood (ML) methods to infer the phylogenetic relationships. BI analyses were performed using BEAST v. 1.7 (Drummond et al. 2012). First, jModelTest v. 2.1.7 was used to calculate the optimal

Tribes	Species	Complete mitochondrial sequence	Cyt b gene
Outgroups	Meriones tamariscinus	NC034314	
	Meriones meridianus	NC027684	
Vernayini	Vernaya foramena	OR085220	
	Vernaya fulva	OR085222	
	Vernaya meiguites	OR085219	
	Vernaya nushanensis	OR085221	
Malacomyini	Malacomys edwardsi	MN964121	
Rattini	Bandicota indica	KT029807	
	Niviventer andersoni	NC060500	
	Rattus rattus	NC012374	
	Chiromyscus langbianis	NC084241	
	Leopoldamys sabanus	MT259591	
	Leopoldamys neilli	JX573334	
	Maxomys surifer	NC036732	
	Margaretamys parvus		MN273044
Micromyini	Micromys minutus	NC027932	AB201996
	Micromys erythrotis	MW389539	
Praomyini	Stenocephalemys albipes	NC051514	
	Heimyscus fumosus	NC049120	
	Hylomyscus denniae	MN845743	
	Mastomys coucha	NC036018	
	Praomys rostratus	NC049115	
Millardiini	Millardia meltada	MN807616	
Chiropodomyini	Chiropodomys gliroides	NC049121	KJ772301
	Chiropodomys gliroides	MN964124	
Hydromyini	Baiyankamys habbema		MN273033
	Melomys burtoni	NC049118	
	Xeromys myoides		EU349790
	Leggadina lakedownensis	NC014696	
	Pseudomys chapmani	NC014698	
Vandeleurini	Vandeleuria oleracea		KY754177
Apodemini	Apodemus draco	HQ333255	
	Apodemus latronum	HQ333256	
	Tokudaia osimensis	LC778283	
	Tokudaia osimensis		AB033703
Pithecheirini	Pithecheir parvus		MG189672
Otomyini	Otomys sungae		JF795993
	Otomys zinki	10050011	JF795989
	Otomys typus	NC053811	
	Otomys irroratus	MK166028	
	Parotomys brantsii	Noossass	KY/54096
Arvicanthini	Golunda ellioti	NC053815	MN807614
	Golunda ellioti	1010075	KY986802
	Desmomys harringtoni	MN807595	MT084863
	Arvicanthis nairobae		MK239825

Table 2. Accession numbers of mtGenomes and Cyt b sequences of the species of Murinae.

Tribes	Species	Complete mitochondrial sequence	Cyt b gene
Arvicanthini	Arvicanthis somalicus	NC053801	
	Dephomys defua	NC053808	MF992073
	Hybomys lunaris		MF680490
	Hybomys trivirgatus	NC053810	
	Rhabdomys pumilio		AF533116
	Thallomys paedulcus		KU724036
Hapalomyini	Hapalomys delacouri	MZ159976	MG189666
Phloeomyini	Phloeomys cumingi		MH330620
	Batomys granti		EU349738
	Crateromys schadenbergi		MH330619
Murini	Mus spretus	OR840825	
	Mus musculus	LC644162	KF839627

model, GTR+G (Posada and Manousiouthakis 2008). BEAUti was used to set the following parameters: model calculation involved selecting an inhomogeneous model gamma, an alternative model GTR, a relaxed molecular clock, and the Yule process; the search chain was run for 100 million generations and sampled every 5000 generations; and the remaining parameters were set to default. We then used BEAST v. 1.7 to construct a phylogenetic tree. We employed Tracer v. 1.6 to verify that the effective sample sizes (ESSs) exceeded 200; therefore, the result tended to be reliable (Rambaut and Drummond 2013). Burn-in was discarded via TreeAnnotator v. 1.6.1. ML analyses were conducted using W-IQ-TREE (http://iqtree.cibiv.univie.ac.at) as described by Trifinopoulos et al. (2016). The analyses used the rapid bootstrapping algorithm with 1000 replicates. The final BI tree and ML tree were decorated and embellished using FigTree v. 1.4.3, and the posterior probability and bootstrap values of each branch were displayed (Rambaut 2016). We also evaluated the phylogeny of Murinae using BI and ML methods based on the complete mitochondrial sequences and removed three tribes containing only Cyt b sequences to locate the Vernaya genus. The sequences used are shown in Suppl. material 1: table S1. The process of all phylogenetic tree building and annotation steps followed the above protocol.

Estimating dates of divergence

We estimated the divergence time by using two types of sequences. For species that do not have mtGenome sequences, we utilized *Cyt b* sequences. For other species, we employed mtGenome sequences from some species belonging to 15 tribes within Murinae. Additionally, we used mtGenome sequences from one individual of each of the four selected species of *Vernaya*. Sequence selection was the same as that for the phylogenetic tree. Data were analyzed using BEAST v. 1.7. Divergence times were estimated using five fossil-based calibration intervals as described by Pagès et al. (2016) and Aghová et al. (2018). We used the following constraints: (1) The stem Apodemini fossils (11 Ma min.) from the Early Vallesian were used to constrain the split between Apodemini/ Millardiini (Most Recent Common Ancestor (MRCA) of *Apodemus/Tokudaia*) and Praomyini/Murini (MRCA of *Mus/Praomys/Mastomys* Clade) (upper 95%: 8.91–21.8 Ma) (Suárez and Mein 1998; Vangengeim et al. 2006); (2) The first

fossil record of *Mus (Mus auctor)* was used to represent the minimum divergence at 5.7 Ma (upper 95%: 4.66–11.07 Ma) between different *Mus* lineages (*Mus musculus/Mus pahari/Mus setulosus*) (Jacobs et al. 1990; Jacobs and Downs 1994; Lundrigan et al. 2002); (3) We used the African crown Arvicanthini lineage from the Late Miocene (median age 6 Ma; from the Tortonian) and a soft maximum prior extending to the Serravalian (upper 95%: 3.91–16.81 Ma) as a constraint of the MRCA of Arvicanthini (Winkler 2002); (4) We set a minimum constraint for the MRCA of Hydromyini, using the first Australian fossil evidence dated at 3.4 Ma (upper 95%: 1.3–14.21 Ma); and (5) The divergence time of Murinae (15.9 Ma, upper 95%:14.06–18.15 Ma) was used as calibration point in the present analyses (Aghová et al. 2018).

All fossil dating age constraints are considered lognormal distributions (Tedford et al. 1992; Ho 2007; Rowe et al. 2008; López-Antoñanzas et al. 2024). The best-fitting substitution models for each partition were selected using the jModelTest results. A general time-reversible model was used as the substitution model. The Yule process of speciation was selected as the tree prior and combined with a relaxed lognormal molecular clock model. Each analysis was performed for 100 million generations, with samples collected every 5000 generations. The posterior distribution and ESS for parameters greater than 200 were calculated using Tracer v. 1.6. TreeAnnotator v. 1.6.1, which was set to the top 10% of the generation, was used to determine the necessary burn-in portion. We then viewed and identified the divergence tree in FigTree v. 1.4.3.

Abbreviations

mtGenome: mitochondrial genome; **MRCA**: The most recent common ancestor; **QTP**: Qinghai-Tibetan Plateau; **Ma**: Megaannus; V. foramena: Vernaya foramena; V. f. foramena: Vernaya foramena foramena; V. fulva; Vernaya fulva; V. meiguites: Vernaya meiguites; V. nushanensis: Vernaya nushanensis; **SCNU**: Sichuan Normal University; SAF: Sichuan Academy of Forestry Sciences.

Results

Characteristics of the mtGenomes in Vernaya

The structure of mtGenomes exhibit a striking resemblance to that found in typical vertebrates and other rodents. It comprises 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and 1 control region in *V. fulva* (Fig. 1a), *V. foramena* (Fig. 1b), and *V. nushanensis* (Fig. 1d), and two control regions (two D-loop) in *V. meiguites* (Fig. 1c), which is not common in mammals, including rodents. The size of the mitochondrial genome is 16,334 bp in *V. meiguites* to 16,351 bp in *V. foramena*. While ND6 and eight tRNA genes reside on the light strand, the remaining mtGenome genes (such as PCGs, rRNAs, and other tRNAs) along with the control region, are located on the heavy strand (Fig. 1a–d). All *Vernaya* mtGenomes were AT-rich, with AT content ranging from 65.38% (*V. fulva* and *V. foramena*) to 65.78% (*V. meiguites*), suggesting strand heterogeneity in the nucleotide composition.



Figure 1. MtGenomes structure map of the species of Vernaya a Vernaya fulva (SCNU02747) b Vernaya foramena (SAF201518) c Vernaya meiguites (SAF201613) d Vernaya nushanensis (SAF19287)).

Phylogenetics and divergence in Murinae based on mtGenomes

We constructed a BI phylogenetic tree and a ML tree based on the mtGenomes of four species of *Vernaya* and the mtGenome sequences of the other Murinae species, except for those of three tribes (Fig. 2 for BI tree and Fig. 3 for ML tree). The accession numbers of the sequences are listed in Table 2.

Three clades and sixteen tribes were retrieved from Murinae in our study, and fifteen of them correspond to currently recognized tribes (Figs 2, 3).

In both phylogenetic trees, the positions of Clade A, Clade B, and Clade C within Murinae were roughly the same. Hapalomyini (Clade A), located at the base of Murinae, was the first to differentiate and was strongly supported (PP = 1.00, BP = 100), with the assumptions proposed in previous research (Badenhorst et al. 2012; Meschersky et al. 2016; Pagès et al. 2016). Clade B consisted of Micromyini, Rattini, and *Vernaya* (PP = 0.95), all of which were grouped together and were well supported (PP = 1.00, BP = 100). The *Vernaya* genus cannot be accommodated in any existing tribe. Interestingly, it was sister to



Figure 2. Phylogenetic and molecular dating results for Murinae and close-relative lineages. The tree is a chronogram (uncorrelated log-normal molecular clock) based on a BEAST MCMC analysis of the mtGenome sequences (except for three tribes). We propose elevating the genus *Vernaya* to tribe Vernayini. Clocks indicate the fossil calibration points used for molecular dating (referring to Pagès et al. (2016) and Aghová et al. (2018)). Red stars indicate calibration points. The values at the nodes are posterior probabilities (PP) obtained by Bayesian analysis. t1-t11 represent the divergence time of some important nodes. NCBI accession numbers for each species are shown on the branch; different colors of the branch and taxa represent different tribes. Numbers represent: I. Quaternary; II. Pliocene; III. Miocene; IV. Oligocene.





Micromyini, and combined to form a sister group to Rattini (Figs 2, 3, Suppl. material 1: fig. S1). In both trees, the remaining 12 tribes constituted a large branch (Clade C). However, there were numerous disparities in this branch between the BI tree and ML tree. For example, our ML tree grouped Chiropodomyini together with Hydromyini (BP = 99), while our BI tree placed Pithecheirini as sister taxa to the Chiropodomyini tribe but with weak support (PP = 0.57). Since the location of *Vernaya* within Murinae is the same in both trees, we shall mainly discuss the BI tree.

In the BI tree, Clade C was composed of four smaller clades. One of these smaller clades was made up of Phloeomyini, Pithecheirini, Chiropodomyini, and Hydromyini. Phloeomyini was positioned at the base of this particular clade. But in some previous studies, Phloeomyini was located at the base of Murinae (Steppan et al. 2005; Lecompte et al. 2008; Fabre et al. 2013; Schenk et al. 2013; Missoup et al. 2016). However, Chiropodomyini was sister to Pithecheirini, which was different from a previous study (Pagès et al. 2016). Malacomyini was the first to diverge from the large clade that comprised Malacomyini, Millardiini, Praomyini, Apodemini, Murini, Vandeleurini, Otomyini, and Arvicanthini. The clade consisting of Millardiini, Praomyini, Apodemini, and Murini was a sister to the clade consisting of Vandeleurini, Otomyini, and Arvicanthini. Within the clade consisting of Millardiini, Praomyini, Apodemini, and Murini, Murini was sister to Apodemini, and they formed a sister group with Praomyini, and then formed a sister group with Millardiini. However, in previous studies, Murini was sister to Praomyini and the clade combining the two tribes was sister to Apodemini (Steppan et al. 2005; Lecompte et al. 2008; Fabre et al. 2013; Schenk et al. 2013; Pagès et al. 2016). In the clade consisting of Vandeleurini, Otomyini and Arvicanthini, Vandeleurini diverged first, followed by Otomyini and Arvicanthini. Otomyini and Arvicanthini were sister groups, which was similar to previous studies (Fabre et al. 2013; Schenk et al. 2013; Missoup et al. 2016; Pagès et al. 2016).

The estimated divergence time is shown in the BI topology in Fig. 2. The results showed that the divergence between Gerbillinae and Murinae is estimated to have occurred during the Oligocene (23.75 Ma, 95% CI = 15.71-35.10). The MRCA of Murinae can be traced back to the Miocene (17.22 Ma, 95% CI = 13.47-25.55), which was also the time when Hapalomyini first diverged from Murinae. Cladogenesis between Clade B and Clade C was dated to 14.17 Ma (95% CI = 11.16-20.57 Ma). The divergence between the clade consisting of Phloeomyini, Pithecheirini, Chiropodomyini, and Hydromyini and the large clade consisting of the other tribes in Clade C was dated to 13.49 Ma (95% CI = 10.63-19.57 Ma). In Clade B, the divergence between Rattini and the other two groups (Micromyini and Vernaya) was estimated to have occurred around 13.37 Ma (95% CI = 10.19-19.73 Ma). The MRCA of this tribe was calculated to have occurred in the Miocene, approximately 10.98 Ma (95% CI = 7.40-17.43 Ma). The split between Micromyini and Vernaya dates to 11.69 Ma (95% CI = 8.29-18.49 Ma) during the Miocene. This chronology, along with the MRCA chronology of Micromyini, indicates an early branch of both Vernaya and Micromyini of Murinae.

Discussion

The higher-level classification of Murinae remains controversial. Fabre et al. (2015) summarized the progress of rodent taxonomy, but retained the "division" arrangement of the rodent subfamily instead of the tribe arrangement. Pagès et

al. (2016) re-examined the *Micromys* and *Pithecheir* divisions using molecular and morphological evidence to demonstrate the multilineage status. The current classification of Murinae includes 15 tribes (Wilson and Mittermeier 2018). Previous studies have obtained different phylogenetic trees of Murinae based on different gene segments; however, the locations of some genera or species in Murinae have not been resolved. Therefore, the taxonomic classification of some tribes with an uncertain taxonomic status should be elucidated (Pagès et al. 2016). Our research results showed that we need to further sample, sequence, analyze and study the tribes in Clade C.

The teeth and outline of the tribes confirm our molecular results and a review of a new tribe. Through the construction of phylogenetic trees, we confirmed the monophyly of *Vernaya* within Murinae, with strong nodal support (Fig. 2, PP = 1.00; Fig. 3, BP = 100; Suppl. material 1: fig. S1, PP = 1.00; Suppl. material 1: fig. S2, BP = 100); it is designated as an independent tribe in Murinae. In both the ML and BI trees (Figs 2, 3), *Vernaya* formed a clade (Clade B) with Micromyini and Rattini, suggesting close phylogenetic relationships among these groups. The morphology, skull, and teeth are signature features of Murinae (Carleton and Musser 1984). Numerous studies have erected novel taxa based on the disparities in these characteristics (Pu et al. 2022; Chen et al. 2024). Specimens of *Vernaya* and its sister groups were examined. *Vernaya* had a larger head-to-body length ratio than *Micromys* and *Rattus* (Fig. 4A–C).

Moreover, there were longitudinal depressions in the interorbital region of the skull in Vernaya (Smith and Xie 2008). However, this was absent in Micromys and Rattus (Fig. 5A). We examined the molars of three genera: Vernaya, Micromys, and Rattus. Revising the molar traits of the species in light of our novel molecular classification resulted in an overall consistent picture (Fig. 5B, C). The dental morphology of Vernaya displays characteristics that fall within the broad spectrum of morphological diversity displayed by Micromyini and Rattini. In Micromyini, the presence of an obvious odontoid on the first transverse ridge of M¹ is a characteristic shared by both Vernayini and Micromyini. In Rattini, the separation of the paracone and metacone on the second and third transverse ridges of M¹ in Vernayini is similar to the separation of the paracone and metacone seen in Rattini (Fig. 5B, C). In addition, Table 3 also shows some similarities between the Vernava genus and the other two tribes. However, M³ of Vernaya has two tabular transverse ridges that are not found in Micromyini or Rattini. Additionally, some dissimilarities were observed between the upper and lower molars (Fig. 5C).

A character comparison between M^1 and m_1 of *Vernaya* and its sister taxon is presented in Table 3 and Fig. 5B, C. The dental morphology of *Vernaya* strongly differs from that of Rattini and Micromyini in its posteroloph of the third transverse ridge of M^1 ((5) in Fig. 5B, C), and its tma and the first transverse ridge of m_1 are connected and protruding ((6) in Fig. 5B, C).

This level of dental dissimilarity supports the phylogenetic position of these tribes. Our molecular phylogenetic analyses indicated that *Vernaya* cannot be accommodated in any of the existing tribes. This is supported by the morphological data, which provide strong support for *Vernaya* from related taxa (*Micromys* and *Rattus*). Accordingly, a new tribe is warranted and is described below as tribe Vernayini, following the naming of tribes by Lecompte et al. (2008).



Figure 4. Skin specimens from three different genera in dorsal (**A**), lateral (**B**), and ventral (**C**) view. a is a skin specimen of *Micromys minutus* (SAF19383); b is a skin specimen of *Vernaya fulva* (SCNU02747); c is a skin specimen of *Rattus tanezumi* (SCNU00173).



Figure 5. Comparison of skull and teeth morphology. Based on the results of phylogenetic trees, the skull and molar morphology of species in *Vernaya* were compared with that of the species in the genera closely related in the phylogenetic tree (*Micromys* and *Rattus*) **A** comparison of skull morphology. Arrows indicate the presence or absence of longitudinal depressions in the interorbital region of the skull **B** comparison of morphology of the first molar. The 6 images are hand-drawn pictures of the molars M¹ (upper 3 images) and m₁ (lower 3 images). The numbers in the figure correspond to those in Table 3 **C** comparison of the morphology of the molars. The 6 images are physical images of the upper molars (upper 3 images) and lower molars (lower 3 images). The numbers in the figure correspond to those in the Table 3. *Micromys minutus* (SAF19383); *Vernaya fulva* (SCNU02747); *Rattus tanezumi* (SCNU00173). **Table 3.** Dental characters of genus *Vernaya* compared with its sister taxa. The morphological comparison in light of the molecular phylogeny obtained in this study. Refer to Pagès et al. (2016) for the naming of each part.

	Corresponding number in Fig. 5B, C		Micromys minutus	Vernaya fulva	Rattus tanezumi
M^1	1	First transverse ridge of M ¹	Obvious odontoid	Obvious odontoid	Unobvious odontoid
	2	t7	Present	Present	Absent
	3	Second and third transverse ridge of M ¹	Paracone- metacone junction	Paracone-metacone separation	Paracone-metacone separation
	4	t9	Degeneration	Large and no degradation	Large and no degradation
	5	Third transverse ridge of M ¹	Posteroloph absent	Posteroloph present	Posteroloph absent
m ₁	6	tma and the first transverse ridge of m ₁	Separated	Connected and protruding	Connected but not prominent
	7	Second transverse ridge of m ₁	3 posteroloph	2 posteroloph	2 posteroloph

Systematics

Order: RODENTIA Bowdich, 1821 Suborder: Myomorpha Brandt, 1855 Superfamily: Muroidea Illiger, 1811 Family: Muridae Illiger, 1811 Subfamily: Murinae Illiger, 1811

Vernayini Liu, Zhao, Liu & Chen, tribe nov. https://zoobank.org/89A47D54-870B-45CE-B5C5-EA463CC63019

Etymology. The tribal name is formed by adding to the stem of the name of the type genus *Vernaya*, the suffix ini; thus, the name of the tribe becomes Vernay + ini = Vernayini.

Type genus. Vernaya Anthony, 1941.

Genera included. The single genus *Vernaya* comprises four species: *Vernaya fulva* (Allen, 1927), *Vernaya foramena* Wang, Hu & Chen, 1980, *Vernaya mei- guites* Zhao, Li, Wang, Jiang, Liu & Chen, 2023 and *Vernaya nushanensis* Zhao, Liu, Jiang, Liu & Chen, 2023.

Diagnosis. Vernavini is a tribe of small, arboreal, nocturnal rodents within the subfamily Murinae, which are well-adapted for climbing. These rodents are characterized by their medium-sized bodies, long, soft fur, and a unique combination of morphological features that distinguish them from other rodent groups. The hindfoot is less than 25 mm in length, and the total skull length is under 35 mm. Notably, the tail is particularly long, approximately twice the length of the head and body combined, and it is covered with fine scales, remaining hairless. Both the fifth digit of the forefoot and the first digit of the hindfoot are equipped with flattened nails, with the thumb capable of opposing the other fingers. The first digit of the hindfoot is semi-opposed and has a flattened nail instead of a claw, and both the fifth finger and the fifth toe have one claw each. The skull of Vernayini rodents features a longitudinal depression in the interorbital region, which also frequently contains two unossified sockets. The molar morphology of this tribe is guite distinctive, featuring an incisive foramen that extends backward to the front of the first molar. The third molar, M³, is notable for its two tabular transverse ridges. Furthermore, the posterior lobe of the third transverse ridge on the first molar, M¹, is

significantly different from what is seen in Rattini and Micromyini. Members of this tribe are skilled climbers, frequently active on large trees and plants like bananas. Their activity peaks during the morning, and their diet includes plant fruits, seeds, and insects (Pan et al. 2007; Smith and Xie 2008; Wei et al. 2022).

Distribution. Vernayini is primarily found in the mountainous regions of southwestern China, extending into northern Myanmar. The four species within this tribe have distinct distribution patterns (Zhao et al. 2023), as follows:

Vernaya fulva: found in western Yunnan Province, China, west of the Lancang River, and extends into northern Myanmar.

Vernaya foramena: distributed in the Qinling Mountains of northern Sichuan, southern Gansu, southwestern Shaanxi, central Sichuan, the Qionglai Mountain District in western Sichuan, and northeastern Chongqing.

Vernaya meiguites: distributed in the Meigu Dafengding National Nature Reserve, Mabian Dafengding National Nature Reserve, and adjacent areas, as well as the Gongga Mountains in the middle of the Hengduan Mountains.

Vernaya nushanensis: found in Xuemeng Mountain, Lushui, Caojian, Dali, Yunnan Province, China, likely with the Lancang River (upper Mekong River in China) as its eastern boundary.

The divergence of arboreal animals may be related to climatic and geological movements. Both Vernaya and Micromys are climbing species. Micromys is found in various regions of the world, including Myanmar, India, Vietnam, and Russia. In China, its distribution extends to Sichuan, Chongqing, Yunnan, and the southern part of Shaanxi (Wei et al. 2022). This distribution overlaps with the distribution of Vernaya species. According to the divergence time results, we noticed that the divergence of Micromys and Vernaya occurred approximately 12 Ma ago. At this time, what caused the two genera to diverge caught our attention. During the 14–12 Ma, the Qinghai-Tibetan Plateau (QTP) is thought to have experienced a rapid uplift (Coleman and Hodges 1995; Dettman et al. 2003). Moreover, the uplift of the Sanjiang area in the southern section of the Hengduan Mountains is believed to have occurred at 25-17 Ma and 13-8 Ma (Zhong and Ding 1996). These events have created complex land conditions and diversified climate evolution (Wu et al. 2001), leading to the differentiation and evolution of specific species. Therefore, based on these findings, we speculate that the two climbing genera, Micromys and Vernaya may have been closely related in the early stage; later, due to the uplift of the QTP and the change in climate, the habitats of the two have changed and occupied different ecological niches, which led to their divergence. Moreover, should Vernaya and Micromys be subject to distinct selective pressures within their mitochondrial genomes, the discovery of adaptive sites that fuel speciation is a thrilling prospect that beckons us to delve deeper into this evolutionary enigma.

Conclusions

We sequenced the mitochondrial genomes of four species of *Vernaya* and found that there were 2 D-loops in *V. meiguites*. There are relatively few relevant data and studies, and more research is needed to reveal the specific mechanisms and biological significance behind this phenomenon. In addition, we determined the phylogenetic relationships of Murinae based on the vast majority of its mtGenome data for the first time. We revealed many new details

concerning the overall phylogenetic structure of Murinae and described its evolutionary history. We also propose a new tribe namely Vernayini Liu, Zhao, Liu and Chen, trib. nov. We believe that it is necessary to combine morphological and molecular data (especially from a genome-wide perspective) to determine the phylogenetic position in Murinae of some tribes (Vandeleurini, Pithecheirini, Phloeomyini, etc.) with an uncertain position, and the taxonomic status of Murinae *incertae sedis* (*Nilopegamys* and *Hadromys*). Further sampling and research are necessary to analyze the origin, evolution and extinction of various tribes of Murinae and the adaptation mechanisms involved.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

All animal experiments for this project were approved by the Ethics Committee of Sichuan Normal University. No human subjects were used in this study.

Funding

The work was supported by the National Natural Science Foundation of China (32370496) to Liu Shaoying and the National Natural Science Foundation of China (32070424) to Chen Shunde, and the Natural Science Foundation of Sichuan Province (2025ZNSFSC0277) to Chen Shunde.

Author contributions

SYL and SDC conceived and designed the research. SPZ performed the experiments and analyzed the data. SL analyzed the data and wrote the paper. SYL, SDC, SPZ, JW, CKF and XMW revised the manuscript. The author(s) read and approved the final manuscript.

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Data availability

The sequence and annotation of the mtGenomes of *Vernaya fulva*, *Vernaya foramena*, *Vernaya nushanensis*, and *Vernaya meiguites* were submitted to the NCBI. The accession numbers respectively in GenBank are OR085222 (https://www.ncbi.nlm.nih.gov/nuccore/OR085222), OR085220 (https://www.ncbi.nlm.nih.gov/nuccore/OR085220), OR085221 (https://www.ncbi.nlm.nih.gov/nuccore/OR085221), OR085219 (https://www.ncbi.nlm.nih.gov/nuccore/OR085219).

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Supplementary material 1

Bayesian and Maximum likelihood trees for Murinae and close-relative lineages based on the mtGenome and the accession numbers of mitochondrial sequences for the trees

Authors: Shuang Liu, Songping Zhao, Jing Wang, Changkun Fu, Xuming Wang, Shaoying Liu, Shunde Chen

Data type: docx

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Link: https://doi.org/10.3897/zookeys.1233.140676.suppl1



Research Article

A new species of *Elpidium* Müller, 1880 (Crustacea, Ostracoda) from Hispaniola, with an updated key to the species of the genus, and its molecular phylogenetic positioning within the Cytheroidea

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Abstract

The ostracod genus Elpidium, a specialist of phytotelma habitats, has received increased attention during the past decade, with a proliferation of described species, rising from seven to nineteen. These recent studies emphasize the high diversity and endemicity of the genus, and its wide distribution in the Neotropics. Yet many regions are still to be inspected for the presence of *Elpidium*. In this work, a new species collected from Hispaniola is described, for which only undetermined previous records existed, despite several species being known from the neighboring islands of Cuba, Jamaica, and Puerto Rico. Elpidium alarconi sp. nov. belongs to the group with a basal expansion on the hemipenis distal lobe, which contains six other species (E. chacoense, E. cordiforme, E. higutiae, E. maricaoense, E. merendonense and E. picinguabaense) and can be distinguished from them by the different shape of the basal expansion (appearing long and digitiform) and by a thin, pointed and L-shaped lower ramus. An updated key is constructed to allow identification of the 20 species of Elpidium described to date, based on shell morphology and soft parts anatomy. The first sequence of the molecular marker 18S for a described species of Elpidium is also presented, and shows its close affinities to members of the genera Gomphodella, Metacypris, and Cytheridella, all in the same clade, separated from the branch where Limnocythere is positioned in the phylogenetic tree. These molecular results, together with strong morphological differences, support the promotion of the subfamily Timiriaseviinae to the family rank, independent from the Limnocytheridae, to which it formerly belonged.

Key words: Dominican Republic, Limnocytheridae, Neotropical aquatic biodiversity, phytotelmata, Timiriaseviidae

Introduction

The Ostracoda are a diverse group of crustaceans present in a wide variety of habitats, from deep oceans to mountain springs (Smith and Horne 2002; Mesquita-Joanes et al. 2012). Planktonic ostracods are diverse in marine environments (Angel 1993) but most ostracod species, either marine or nonmarine, are mainly benthic, hypogean or nekto-benthic. However, there are entire groups of species with a very specialized way of life; the Entocytheridae is a



Academic editor: Simone Nunes Brandão Received: 1 May 2024 Accepted: 2 January 2025 Published: 28 March 2025

ZooBank: https://zoobank. org/13478F15-8F2A-4CD0-ABC7-6A3DE7A24AC0

Citation: Mesquita-Joanes F, Gálvez Á, Palero F, Rueda J (2025) A new species of *Elpidium* Müller, 1880 (Crustacea, Ostracoda) from Hispaniola, with an updated key to the species of the genus, and its molecular phylogenetic positioning within the Cytheroidea. ZooKeys 1233: 75–106. https://doi.org/10.3897/ zookeys.1233.126611

Copyright: © Francesc Mesquita-Joanes et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). speciose family of ostracods exclusively living as symbionts of other crustaceans (Hart and Hart 1974; Mestre et al. 2014); the Terrestricytheridae have the ability to live in humid soils, devoid of a permanent layer of free water (Horne et al. 2004); and members of the genus *Elpidium* are known to dwell in phytotelmata, i.e., very small water bodies accumulating in between plant structures (Jocque et al. 2013).

The most common environments among phytotelmata are tree holes, bromeliads and pitcher plants, and all of them can host relatively simple communities of organisms in tightly organized food webs (Maguire 1971; Kitching 2000). Among the metazoans inhabiting phytotelmata, mosquitoes are possibly one of the most common and more intensely studied groups (Frank 1983). Crustaceans, although less studied than insects, can also be diverse and common, including Copepoda, Decapoda, Ostracoda and Anomopoda (Jocque et al. 2013). Except *Elpidium* ostracods, however, most other species of crustaceans are usually not exclusive from phytotelmata, but rather live in a wide variety of aquatic habitats. Among ostracods living in plant containers, we can also find species that live in other habitats, such as those belonging to the Candonidae, Cyprididae or Darwinulidae (Jocque et al. 2013), but the cytheroid genus *Elpidium* stands out as almost exclusively living in bromeliad phytotelmata (but see Acosta-Mercado et al. 2012), and whose species usually have restricted geographic distributions.

The genus *Elpidium* was established by Müller (1880) to accommodate globular ostracods with a flat ventral surface, which he found very frequently in Brazilian bromeliads: "Ella ali vive em abundancia e quasi que não ha Bromelia sem a sua colonia de Cytherideos; é provavel que, com as Bromelias, ella se estenda por todo o Brazil" (Müller 1881: 27) ["It lives there in abundance, and practically there is no Bromelia without its own colony of cytheroids; probably, as happens with bromeliads, it must be spread all over Brazil"]. No further species of *Elpidium* were described until the middle of the next century (Tressler 1941, 1956). However, during the past few decades, there has been a renewed interest in the genus, and at present we know of 19 described species of Elpidium (Pereira et al. 2023; Díaz et al. 2024), distributed in Brazil, Argentina, Honduras, Guatemala, US, Jamaica, Puerto Rico and Cuba, plus some undetermined species from Dominican Republic, Mexico and Costa Rica (Picado 1913; Tressler 1956; Pinto and Purper 1970; Danielopol 1975; Colin and Danielopol 1980; Acosta-Mercado et al. 2012; Pérez et al. 2012; Pinto and Jocqué 2013; Danielopol et al. 2014; Pereira et al. 2019, 2022; Mercado-Salas et al. 2021). The last published key for the identification of *Elpidium* species appeared eleven years ago (Pinto and Jocqué 2013), when only seven species were known to science. With the present survey, we describe for the first time a species of Elpidium for the island of Hispaniola and provide an updated identification key including all species described hitherto. In addition, we discuss the biogeography of the genus, and use molecular methods, for the first time in a described species of Elpidium, together with morphological data, to evaluate its phylogenetic relationships with other ostracod taxa, supporting the validity of the Timiriaseviidae as a distinct family, separated from Limnocytheridae s.s., as suggested by previous authors (Tanaka et al. 2021).

Materials and methods

Study area and sampling and laboratory methods

Samples were collected from two localities in the municipality of Jarabacoa (Dominican Republic), in the island of Hispaniola. Jarabacoa is located in La Vega province, in a valley of the Cordillera Central (central ranges) with a mean altitude of 530 m a.s.l. The area is characterized by a siliceous substrate, and wet tropical climate, with an average annual temperature of 20 °C and 1723 mm of mean annual precipitation (Climate-Data.org 2023). The Cordillera Central is included in a single biogeographical area, the Central-Eastern district, which has one of the highest richness of plant genera and endemic species of Hispaniola (Cano-Ortiz et al. 2017).

Ostracod samples were collected in the frame of a wider survey and various projects on the aquatic invertebrate biodiversity of Hispaniola, which sampled varied habitats, focusing particularly on potential predators of mosquito larvae (Rodríguez Sosa et al. 2019; Olmo et al. 2024). Invertebrate samples were collected by suction from the water stored in between the base of bromeliad leaves, using either a plastic Pasteur pipette, or a 60 mL syringe coupled to a 40 cm flexible hose following Júnior et al. (2017). Most of the Bromeliaceae plants were located in private gardens or nearby, and were tentatively determined as belonging to the genus *Neoregelia*. In the laboratory, the samples were filtered through a 350 µm mesh size filter and fixed in 70% ethanol.

The dissection of ostracod specimens for optical microscopy inspection was done following the protocol described in Namiotko et al. (2011). Soft parts were embedded in HydroMatrix[®] for the preparation of permanent slides. Shells were stored dry in micropaleontological slides. Drawings were done using a camera lucida on a Leica microscope. Some pictures were taken using a Nikon[®] Eclipse E800 epifluorescence microscope, either with white light or with UV light (340–380 nm) plus a blue filter (435–485 nm). Some individuals were critical-point dried *in toto* or without the valves. These individuals, plus separated valves of other individuals, were coated with a thin layer of Au-Pd for SEM observation in a Hitachi S-4800 or a SCIOS-2 at the University of València.

Taxonomy, chaetotaxy, descriptions, and abbreviations

In this work we follow Mesquita-Joanes et al. (2024) in accepting the suggestion of Tanaka et al. (2021) to raise the subfamily Timiriaseviinae to the family rank, and provide a diagnosis of the family. This diagnosis is established after the differences indicated by Martens (1995) and Danielopol et al. (2018) between Timiriaseviinae and Limnocytherinae. However, most ostracodologists have traditionally considered the Timiriaseviinae as a subfamily within the Limnocytheridae, ever since the review by Colin and Danielopol (1978) (e.g., Savatenalinton et al. 2008; Karanovic and Humphreys 2014; Danielopol et al. 2018; Meisch et al. 2019).

The selection of critical characters to build the identification key was based on those used by Pinto and Jocqué (2013), plus those stressed by Danielopol et al. (2014), supported in some cases by some of the characters included in the phylogenetic tree of Pereira et al. (2022).

Ср	carapace;	Md-palp	mandibular palp;
CL	carapace length;	Мх	maxillula;
н	height of valves;	T1	first thoracopod;
L	length of valves;	T2	second thoracopod;
LV	left valve;	Т3	third thoracopod;
RV	right valve;	CR	caudal ramus;
W	width of shell;	Нр	hemipenis;
A1	antennula;	DL	distal lobe;
A2	antenna;	CoP	copulatory process;
Md	mandibula;	LR	lower ramus.

Abbreviations used in the text and figures include the following:

Chaetotaxy nomenclature follows mainly Broodbakker and Danielopol (1982), Martens (1987), Meisch (2000), and Pereira et al. (2023). We follow mostly Sames (2011a, 2011b) and Danielopol et al. (2014) for carapace traits terminology. However, terms used for the description of the general shape in dorsal or ventral view of the carapace follow those commonly used for leaves summarized by Hickey (1973), by applying those terms for the tip of leaves to the shape of the anterior part of the carapace, and those for the base of leaves to the shape of the posterior part of the carapace. Note that these terms differ in some cases from those used in the ostracod literature, and in *Elpidium* descriptions in particular, but are more widely used in general in Biology for morphological descriptions.

Molecular methods

Ethanol-fixed ostracods were individually transferred to 1.5 mL microtubes using a thin brush. Single specimens from the type locality (e.g., P459=MUVHN-ZY0040) were digested at 55 °C overnight using 180 µL T1 buffer and 20 µL proteinase K, and DNA was extracted with the Nucleospin DNA extraction kit (Macherey-Nagel[™]) following the manufacturer's instructions. The large ribosomal subunit (18S) gene region was amplified using primers 18S_5F 5'-GCG AAA GCA TTT GCC AAG AA-3' and 18S_9R 5'-GAT CCT TCC GCA GGT TCA CCT AC-3' (Carranza et al. 1996). Amplifications were carried out using ~ 10 ng of genomic DNA in a reaction containing 1 U of Tag polymerase (Amersham), 1× buffer (Amersham), 0.2 mM of each primer and 0.12 mM dNTPs. The polymerase chain reaction (PCR) thermal profile included an initial denaturation step at 94 °C for 4 min, followed by 30 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 20 min. Sequences were obtained using the Big-Dye Ready Reaction kit v. 3.1 (Applied Biosystems) on an ABI Prism 3770 automated sequencer at the MACROGEN sequencing facilities. Chromatograms for each DNA sequence were checked with BioEdit v. 7.2.5 (Hall 1999) and sequence alignment was conducted with Muscle v. 3.6 (Edgar 2004). Model selection was carried out for the sequence alignment using the Bayesian Information Criterion (BIC) as implemented in ModelTest-NG v. 0.1.7 (Darriba et al. 2020). Maximum likelihood phylogenetic reconstruction was then completed with the corresponding DNA substitution model with ultrafast bootstrap (1000 replicates) as implemented in IQ-TREE v. 2.0 (Minh et al. 2020).

Repository

The holotype, allotype, and paratypes with codes MUVHNZY0021-0042 are deposited in the Natural History Museum of the University of Valencia (**MUVHN**, Burjassot, Spain).

Results

Taxonomic account

Class Ostracoda Latreille, 1802 Subclass Podocopa G.O. Sars, 1866 Order Podocopida G.O. Sars, 1866 Suborder Cytherocopina Baird, 1850 Superfamily Cytheroidea Baird, 1850

Family Timiriaseviidae Mandelstam, 1960

Diagnosis. [Based on the list of characteristic traits of the subfamily Timiriaseviinae by Martens (1995) and Danielopol et al. (2018) and on the types of hinge and sieve pores respectively by Danielopol et al. (2014) and Danielopol et al. (2018)]. Cytheroid Ostracoda with globular shells, particularly in the females, which are larger than males and have a brood pouch (i.e., shell sexual dimorphism apparent). Hinge lophodont, adont, or protodont (Danielopol et al. 2014). Sieve pores absent or type B if present (Danielopol et al. 2018). Terminal segment of the antennula usually short, not longer than the previous segment. Fused part of the antennula Ya aesthetasc with adjacent seta short or not distinguishable, less than one third the length of the aesthetasc. Ventral seta on the second antennular segment situated in a medial or proximal position, not in the distal margin, or absent. Maxillular palp not subdivided in two segments, and with a reduced number of setae. Distal lobe of hemipenis moveable, not fused to the rest of the hemipenis.

Genus Elpidium F. Müller, 1880

Type species (by original designation): *Elpidium bromeliarum* F. Müller, 1880. **Type locality.** Itajaí, Santa Catarina state, Brazil.

Other species included. *E. alarconi* sp. nov.; *E. chacoense* Díaz et al., 2024; *E. cordiforme* Pereira et al., 2023; *E. eriocaularum* Pereira et al., 2023; *E. heberti* Pereira et al., 2019; *E. higutiae* Pereira et al., 2023; *E. inaequivalve* Danielopol, 1981; *E. laesslei* (Tressler, 1956); *E. litoreum* Pereira et al., 2022; *E. littlei* Pereira et al., 2019; *E. maricaoense* (Tressler, 1941); *E. martensi* Danielopol et al., 2014; *E. merendonense* Pinto & Jocqué, 2013; *E. oxumae* Pereira et al., 2023; *E. picinguabaense* Pereira et al., 2023; *E. pintoi* Danielopol, 1981; *E. purium* Pereira et al., 2023; *E. purperae* Danielopol, 1981; *E. wolfi* Pereira et al., 2019.

Diagnosis. [Modified after Danielopol et al. (2014) and Pereira et al. (2022, 2023)]. Timiriaseviidae of intermediate size (0.6–1.1 mm) with sexually dimorphic carapace, broad and ventrally flat. Females relatively wider than males, due to the presence of a brood pouch, and usually also larger. Valves symmetric or

asymmetric in dorsal view, carapace surface of most species with subtle ornamentation of minute and shallow pits (except E. laesslei, which is strongly ornamented). At the mouth part, a funnel structure is internally built in the carapace between both valves. Four apparent adductor muscle scars arranged subvertically (at ~ 15-30° oblique from the vertical axis towards the anterior part from top to bottom). Hinge protodont, with a bar on the smaller valve, which may have prototeeth anteriorly and posteriorly, and a groove in the larger one. A1 apparently six-segmented in most species: with five clearly separated segments, but in most species the fourth segment appears as partially subdivided (4a + 4b). A1 with a dorsal apical expansion in the first segment. A2 sexually dimorphic; three terminal claws in the last segment, one of which is pectinated only in males. Last segment of A2 distally with a small hyaline formation. Mx with two spatulate claws and three normal setae in each of the second and third endites. Hp strongly sclerotized, CR reduced to a pair of setae. Distal lobe very apparent and varied in shape, usually subtriangular or subrectangular, but in some species with a small (pointed or digitiform) expansion in its internal border, always with a basal seta. CoP curved (hook-like, curled, U-shaped or L-shaped), with a tip either subdivided or not in ejaculatory glans and duct. Lower ramus varied in shape. Upper ramus absent. Female abdomen rounded, with a dorsal spine-like seta, sclerotized genital lobes, and three setae on each CR lobe.

Elpidium alarconi sp. nov.

https://zoobank.org/D973DE83-200E-4BEF-AE4D-05174A5FD155 Figs 1-7

Type locality. Rancho Baiguate (La Joya Sector, Jarabacoa, República Dominicana) 19°6'49"N, 70°37'8"W, 530 m a.s.l., sampled on 7/2/2019 and 12/4/2021 by J. Rueda and P. Alarcón. Tank bromeliads growing at the base of several tree trunks in a secondary natural forest, with a wide cover, and presence of domestic animals (horses, dogs) in the vicinity, near the Baiguate River.

Type material. *Holotype* • 1 adult ♂; soft parts dissected and stored on a permanent microscopic slide, valves dry in a micropaleontological slide (MUVHN-ZY0021). Allotype • 1 adult 2; soft parts dissected and stored on a permanent microscopic slide, valves dry in a micropaleontological slide (MUVHNZY0022). *Paratypes* • 10 adult ♂♂ and 17 adult ♀♀. Six of the males (MUVHNZY0023 - MUVHNZY0026, MUVHNZY0035, MUVHNZY0036) dissected and stored as the holotype, valves coated and used for SEM; one male (MUVHNZY0027) used in toto for SEM, after applying critical-point drying (CPD), and stored in a micropaleontological slide; another male (MUVHNZY0039) with valves untreated and bodies (CPD and coated) in a micropaleontological slide. Seven females (MUVHNZY0028-0033, MUVHNZY0037) dissected and stored as the holotype, valves coated and used for SEM; another female (MUVHNZY0038) with valves untreated and bodies (CPD and coated) in a micropaleontological slide. Two adult males and six females stored in toto in ethanol 96% (MUVHN-ZY0034). Soft body remains of three adult females used for DNA extraction stored in ethanol (codes MUVHNZY0040-0042).

Diagnosis. Elpidium species of intermediate size (~ 700–800 μ m), with a dark-colored carapace. Females slightly longer and wider than males, and with



Figure 1. *Elpidium alarconi* sp. nov. A Cp dorsal view (MUVHNZY0026) **B** Cp ventral view (MUVHNZY0025) **C** LV, external view (MUVHNZY0035) **D** RV external view (MUVHNZY0035) **E** LV internal view (MUVHNZY0036) **F** RV internal view (MUVHNZY0036) **G** LV subventral view (MUVHNZY0036) **H** RV subventral view (MUVHNZY0036) **I** LV internal view (MUVHNZY0036), detail of posterior part, and zoom on lateral row of pores (inset) **J** RV internal view (MUVHNZY0036), detail of posterior part, and zoom on lateral row of pores (inset) **K** Cp detail anterior part, right external view (MUVHNZY0035) **L** Detail adductor muscle scars, RV internal view (MUVHNZY0036). Scale bars: 400 μm (**A**–**H**); 100 μm (**I**, **J** general); 20 μm (**I**, **J** inset); 50 μm (**K**, **L**).

a truncate posterior margin in dorsal view; males with a barely obtuse posterior margin. Valves (quasi-)symmetric in dorsal view. Surface of valves covered with minute and shallow pits. LV embracing RV along all free margins. Hinge protodont, with a strongly built bar in the RV, including one (proto-)tooth at each extreme of the bar. LV with a hinge groove. A1 apparently six-segmented (i.e., segments 4a and 4b partially separated). DL of male Hp with a long digital expansion, CoP L-shaped, with tip not subdivided, and LR very slender (thinner than CoP), L-shaped and with a pointed tip.

Description. Male. Adult shell large (L > 0.7 mm), according to size groups established for limnocytherids s.l. by Gidó et al. (2007), but of intermediate size compared to other Elpidium species. Cp subovate in dorsal and ventral view (Fig. 1A, B). Maximum width slightly displaced to posterior part, at ~ 45% of total length. Cp in dorsal view: anteriorly pointed, barely acute; posteriorly bluntly pointed, obtuse, with more rounded outline than anterior margin. Valves almost symmetrical in dorsal view; LV slightly longer and embracing RV along all free margins (Fig. 1B). Valves elongate in lateral view (Fig. 1C, D), posterior margin broadly rounded, anterior margin infracurvate, i.e., narrowly rounded towards anteroventral region. Maximum length at ~ 33% of maximum height. Ventral margin slightly convex in lateral view, flat in ventral (Fig. 1B) and frontal (Fig. 5A) views. Surface of valves smoothly punctate, overall covered with minute foveolae and sparsely with normal (type-A2) pores, many of which hold a sensory seta (Fig. 1A, C, D, I-K). These foveolae more conspicuous, denser, and deeper near anterior margin of valves, in a narrow beak-like zone (Fig. 1K). This zone partially corresponds internally to the area of the inner lamella between outer margin and selvage (Fig. 1E, F). Calcified inner lamella wider anteriorly (~ 12% of valve L) than posteriorly (6% of valve L). Selvage strongly built in the RV (Fig. 1F, H), anteriorly positioned approximately half way between anterior margin and inner margin of calcified inner lamella. Hinge protodont, sensu Danielopol et al. (2014). RV dorsally with a hinge bar (Fig. 1H), showing anterior and posterior prototeeth. LV with a hinge groove (Fig. 1G), anteriorly with enlarged socket. Both valves antero-ventrally with selvage protruding towards external margin, building the typical funnel-like structure of Elpidium ostracods at mouth position. Four large adductor muscle scars (Fig. 1F, L) aligned in a slightly oblique row (leaning 30° from vertical axis towards anterior part, from top to bottom), located just in front of central area of valves. Three of these scars elongate, bottom one subovate. Another smaller, rounded scar situated in front of top one of the four central muscle scars. Both valves postero-ventrally with a row of submarginal (type-A2) pores and setae located in the peripheral part of the marginal infold (Fig. 1I, J). Carapace colored dark brown.

A1 (Figs 2A, 3A–D). Apparently six-segmented, i.e., with clear separation between segments 4a and 4b under standard microscope, but this separation weaker than other segments (Fig. 3A). Separation not observed under UV-light in a fluorescence microscope, compared to other segmentation (Fig. 3B). This separation observed only in the internal part of fourth segment under SEM, but not in the external part (Figs 3C, D, 5E). First segment trapezoidal, strongly built, dorsally with a subapical subtriangular expansion, partially covered with pseudochaetae. Second segment elongate, more than thrice longer than wide, dorsally covered with pseudochaetae along its margin, ventrally with a long plumose seta, attached slightly behind middle of segment, and reaching midlength of fourth segment. Third segment rectangular, with a seta at its dorso-apical margin, this seta slightly longer than next segment. Segment 4a rectangular, ~ 2× longer than wide, dorsally with two small apical setae (not attaining the middle of next segment) and ventrally one seta as long as next segment. Fifth segment (segment 4b) dorsally with three apical setae of varied length; longest one attaining one third of Ya aesthetasc, second longer one as long as last segment, smallest one ~ 1/2 the length of last segment. Ventrally with a long apical seta, surpassing the middle of Ya aesthetasc. Last (sixth) segment with three



Figure 2. *Elpidium alarconi* sp. nov. *A* (MUVHNZY0021) **A** A1, top arrow points to subtriangular expansion on first segment; bottom arrow points to the partial separation between segments 4a and 4b **B** A2, hy: hyaline formation **C** Md palp (top) and coxa (bottom) **D** Mx. Scale bars: 100 μm.

apical setae and Ya aesthetasc. One seta as long as Ya, another slightly longer than last two segments, another one slightly longer than last three segments. A2 (Fig. 2B). Protopod two-segmented. First segment short and ring-shaped, second segment elongate and smoothly curved, > 2.5× longer than wide. Exopod with a very small seta and a spinneret seta, not surpassing tip of claws. First



Figure 3. *Elpidium alarconi* sp. nov. details of A1 and A2 **A** A1, arrow points to the partial separation between segments 4a and 4b. Picture in white transmitted light. \checkmark (MUVHNZY0035) **B** A1, arrow points to the partial separation between fourth and fifth segment (4a and 4b). Picture using UV light and blue filter in a fluorescence microscope. \checkmark (MUVHNZY0035) **C** SEM image of right A1, external view; arrow points to the lack of separation between fourth and fifth segment (4a and 4b). \checkmark (MUVHNZY0039) **D** SEM image of right A1, internal view; arrow points to the separation between fourth and fifth segment (4a and 4b) (Same individual as in C:MUVHNZY0039) **E** SEM image of left A2, internal view; arrow points to a ventroapical minute seta on the penultimate segment. Q(MUVHNZY0038) **F** SEM image of left A2, external view; one arrow points to a ventroapical minute seta on the last segment, and another to the (crumpled) hyaline formation (hy). Q (MUVHNZY0038). Scale bars: 50 µm (**A–D**); 30 µm (**E, F**)

segment of endopod subquadrate, ventrally with an apical long seta, ~ 2/3 of the length of next segment. Second endopodal segment elongate, ~ 5× longer than wide. Ventrally with one small seta and Y aesthetasc, situated slightly in front of mid-length of segment. This small seta slightly shorter than aesthetasc. Another large and thick seta attached to ventro-apical margin, together with a minute seta (Fig. 5F, as in the female: Fig. 3E). Dorsally with two subapical short setae, one ~ 1/2 the length of the other. Last segment subquadrate, with three claws, shortest and ventral one pectinated with a row of strong teeth (Fig. 5F). A very small hyaline formation located ventro-apically, at the base of pectinated claw, but together with a minute seta (as in the female: Fig. 3F).

Md (Fig. 2C). Coxa slender, with curved posterior half and straight anterior one. Distally with eight teeth, progressively smaller from anterior (dorsal) to posterior (ventral) ones, most of them bicuspidate and/or with adjacent interdental spines and setae (X-setae). Dorsally with large serrate seta, not reaching the base of dorsal teeth. Ventrally with one small plumose seta, slightly longer than ventralmost small tooth. Md-palp four-segmented and curved. First segment (basis) with two ventral plumose setae, one ~ 2/3 the length of the other. Dorsally with exopod (respiratory plate) with three broad rays and a small reflected ray. Second segment (first endopodal segment) with two ventro-apical plumose setae, one of them half the length of the other. Third segment subquadrate, ventrally holding an apical long smooth seta, dorsally with three long apical smooth and thin setae, together with a thicker plumose seta, all of similar length. Last segment small and subquadrate, with three terminal thin setae of similar length, one of these claw-like, the other two smooth.

Mx (Fig. 2D). Elongate, subrectangular protopod. Exopod (respiratory plate) with 16 distal unequal rays and a proximal reflexed ray. Endopod with three endites and a palp. First endite with three subequal setae. Second and third endites each with two spoon-shaped (spatulate), claw-like setae, and three smooth, thin setae. Palp unsegmented, distally with two long plumose setae, longer than tip of endite setae, plus a minute subapical dorsal seta.

T1 (Figs 4A, 5H). Four-segmented. First segment the longest. Ventrally with a large seta, situated well behind mid-length of segment. Dorsally with proximal long seta, slightly surpassing distal margin of segment. Dorso-apically with two subequal knee-setae. Second segment elongate, 6× longer than wide, ventrally with strong apical seta, as long as next segment. Third segment without setae. Fourth segment with apical claw bearing a minute seta at its swollen base, and as long as third segment.

T2 (Figs 4B, 5H). Larger than T1 and four-segmented. First segment strong, bearing ventrally a subproximal long setae, attaining distal edge of segment. Dorsally with one medial long seta, surpassing distal margin of segment, and an apical knee-seta, ~ 1/2 the length of previous seta. Second segment slender and long, ventrally with apical strong seta, almost as long as next segment. Third segment without setae. Fourth segment similar to previous one but slightly shorter and with an apical claw. This claw as long as third segment, and with a proximal minute seta.

T3 (Figs 4C, 5H). Larger than T2 and four-segmented. First segment ventrally with a proximal large seta, 2/3 as long as segment. Dorsally with a thin medial seta, attaining distal edge of segment, and a small distal knee-seta, ~ 1/2 the length of previous seta. Second segment long, > 8× longer than wide, and with an apical strong seta, ~ 2/3 the length of next segment. Third segment devoid of setae and 3× longer than wide. Last segment similar but slightly smaller than previous one, bearing a very long claw, longer than second segment, and with a minute seta at its base.

Hp (Figs 4D, 5A–D). Large sclerotized and muscular body with DL, distal seta, CoP and LR. DL with a long basal digital expansion. Width of DL, including digital expansion, longer than its length. This expansion flexible at its tip, so that in some slide preparations for optical microscopy, it can be distally folded. Distal seta shorter than digital expansion. DL with lateral margins almost parallel in its mid length, but converging in a subtriangular, pointed shape at its distal part (Figs 4D, 5B). CoP L-shaped, progressively narrowing towards the tip (Fig. 4D), without separation between distal glans and ejaculatory duct (Fig. 5C, D). LR slender, very thin, L-shaped and with a finely pointed tip (Figs 4D, 5C, D). Depending on the position of LR in slide preparations for optical microscopy, L-shape might not be seen clearly in one or both hemipenes. A slight difference



Figure 4. *Elpidium alarconi* sp. nov. \bigcirc (A–D) (MUVHNZY0021) and \bigcirc (E) (MUVHNZY0037) A T1 B T2 C T3 D Hp E A2; hy: hyaline formation. Scale bars: 100 µm.

between left and right LR shape observed in the development of the L-angle, somehow resembling a piolet with a small adze rather than an L (Fig. 5C, D). CR with one pair of intermediate-size, plumose setae and numerous pseudochaetae (Figs 4D, 5B).



Figure 5. *Elpidium alarconi* sp. nov. A **A**–**E** MUVHNZY0027 **F**–**H** MUVHNZY0039 **A** complete frontal view of individual with extended penis **B** detail of penis **C** detail of digital expansion of DL, CoP and LR (right hemipenis) **D** detail of digital expansion of DL, CoP and LR (left hemipenis) **E** detail of A1 showing separation between segments 4a and 4b in internal part of left A1 (leftmost arrow) and the lack of separation between them in the external part of right A1 (right arrow) **F** detail of right A2 distal part (internal view) **G** labrum, ventral view **H** left T1-T3. Scale bars: 400 μm (**A**); 100 μm (**B**, **G**, **H**); 30 μm (**C**, **D**, **F**); 40 μm (**E**).

Labrum (Fig. 5G) large, subquadrate in ventral view. Anteriorly and ventro-laterally with arrays of long pseudochaetae. Posteriorly, near the mouth entrance, with two submarginal pappose setae and a marginal row of short setulae forming an apparently serrated margin.



Figure 6. *Elpidium alarconi* sp. nov. ♀ **A** Cp dorsal view (MUVHNZY0028) **B** Cp ventral view (MUVHNZY0029) **C** LV, external view (MUVHNZY0037) **D** RV external view (MUVHNZY0037) **E** LV internal view (MUVHNZY0037) **F** RV internal view (MUVHNZY0037) **G** LV subventral view (MUVHNZY0037) **H** RV subventral view (MUVHNZY0037) **I** LV internal view (MUVHNZY0037), detail of posterior part, and zoom on lateral row of pores (inset) **J** RV internal view (MUVHNZY0037), detail of posterior part, and zoom on lateral row of pores (inset) **J** RV internal view (MUVHNZY0037), detail of posterior part, and zoom on lateral row of pores (inset) **J** RV internal view (MUVHNZY0037), detail of posterior part, and zoom on lateral row of pores (inset) **J** RV internal view (MUVHNZY0037), detail of posterior part, and zoom on lateral row of pores (inset) **J** RV internal view (MUVHNZY0037), detail of posterior part, and zoom on lateral row of pores (inset) **J** RV internal view (MUVHNZY0037).

Description. Female (only sexually dimorphic features described) (Figs 6, 7). Cp slightly longer, distinctly wider, and slightly more asymmetric than male, posteriorly not pointed but truncate or even slightly cordate in dorsal and ventral views (Fig. 6A, B). These Cp differences between male and female correspond to species group A, according to Danielopol et al. (2014). In lateral external view (Fig. 6C, D), female valves with a straight ventral margin and a less arched posterior margin than males. In internal view, more developed socket-like hinge structures posteriorly in the inner margin of both valves (Figs 6E–H, 7B, C), and posteroventrally wider distance between outer margin and external outline, due



Figure 7. *Elpidium alarconi* sp. nov. \bigcirc **A** Posterior part of abdomen (MUVHNZY0022) **B** LV internal view (MUVHNZY0033) **C** RV internal view (MUVHNZY0033). Scale bars: 100 μ m (**A**); 500 μ m (**B**, **C**).

to the wider development of valves in this area (Fig. 6E, F, I, J). Posterior part of female hinge bar also with stronger tooth, coupled to a tooth-like pointed inner margin in RV (Fig. 6F, H), not observed in male valves (Fig. 1H).

A2 (Figs 3E, F, 4E). None of the three claws in distal segment pectinated. Y aesthetasc smaller than in male, i.e., of similar length than adjacent seta.

Abdomen (Fig. 7). Centrally with a spine-like seta in dorsal position. Genital lobes semicircular, with internal trabecula and showing internal tubes. CR with two equal adjacent plumose setae in an apical position plus a separate stronger plumose seta, laterally situated, close to genital lobe.

Measurements. Male. L: 739 μm (671–778, *n* = 7); W: 559 μm (524–596, *n* = 5); H: 423 μm (418–430, *n* = 3). **Female.** L: 773 μm (711–836, *n* = 9); W: 645 (556–711, *n* = 5); H: 422 (373–476; *n* = 4).

Differential diagnosis. Other Elpidium species with similar Cp, i.e., with LV embracing RV, symmetric in dorsal view, not ornamented and with sexual dimorphism of group A, include E. bromeliarum, E. pintoi, E. littlei, E. litoreum, and E. purium, but none of these species have a digital expansion at the base of the DL, although E. littlei has some subdigitiform, elongate triangular expansion. The species E. maricaoense and E. merendonense have a similar digital expansion (although smaller than in E. alarconi sp. nov.), but their Cp are asymmetrical in dorsal view. The Brazilian species E. cordiforme has a similar digital expansion, but its Cp is strongly cordiform in dorsal view, and the CoP and LR of Hp are notably different. Another Brazilian species, E. picinguabaense and the Argentinian E. chacoense also have a digital expansion in the DL. However, this expansion is shorter than in the new species. In addition, the female Cp of E. picinguabaense is not posteriorly truncate, but narrowly rounded, and the LR of the Hp is distinctly larger than in E. alarconi sp. nov. The female Cp of E. chacoense is not truncate posteriorly in dorsal view, but barely pointed. Elpidium higutiae, also from Brazil, has a similar Cp shape to E. alarconi sp. nov., and it also has a digital expansion on the DL, but this expansion is shorter than in E. alarconi sp. nov. and its LR is larger and thicker than in the new species. In fact, the very thin L-like shape of the LR in E. alarconi sp. nov. is a unique trait that allows distinction from all other Elpidium species.

Ecology and distribution. Besides the type locality of Rancho Baiguate, it has also been found in Pinar Dorado Hotel (19°7'2"N, 70°37'58"W), 549 m a.s.l., sampled on 20 March 2018 by J. Rueda and P. Alarcón. This site is in the same municipality of Jarabacoa, but in the Pinar Dorado Sector. Tank bromeliads (possibly of the genus *Neoregelia*) growing at the base and the trunk of several trees in a relatively anthropized habitat composed of a law garden surrounded by pine trees, with a pool and a bar located nearby. In the type locality, the species was collected from the same type of bromeliads. Paratypes MUVHN-ZY0035, MUVHNZY0036, and MUVHNZY0037 were collected from this locality; other types were collected in the type locality.

Etymology. The species is named after Dr. Pedro María Alarcón-Elbal, who organized the sampling campaign in República Dominicana, obtained financial support and encouraged the senior author JR to study the invertebrates of the area.

Molecular phylogeny

We have obtained new 18S rDNA sequences for *Elpidium alarconi* sp. nov. and *Cyprideis torosa*, with GenBank accession numbers PP648174 and PP648175, respectively. The 18S rDNA sequence alignment had 739 bp in length and followed the GTR substitution model according to BIC model selection. The phylogenetic tree obtained (Fig. 8) placed *Elpidium alarconi* sp. nov. in the same clade as *Metacypris*, *Cytheridella*, and *Gomphodella*, all of them belonging to the Timiriaseviidae (formerly subfamily Timiriaseviinae). This clade becomes clearly separated from the genus *Limnocythere*, and therefore the Limnocytheridae s.s. Interestingly, the clade formed by the Timiriaseviidae genera, holds a more basal position within the Cytheroidea, splitting earlier than Limnocytheridae, but also than other families, including Xestoleberididae, Loxoconchidae, Cytheridae and Cytherideidae, among others.



Figure 8. Maximum Likelihood tree inferred using the GTR model on the 18S rDNA alignment data. Bootstrap support values (percentage of trees in which the associated taxa clustered together) larger than 70 are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Specimen *Elpidium*-P459 stored as paratype with code MUVHNZY0040 in the repository.

Identification key to species of Elpidium

1	RV ventrally overlapping LV
-	LV ventrally overlapping RV4
2	Female CL = 0.7-0.9 mm. Hp: DL lateral margins parallel or convergent
	from base to mid-length3
-	Female CL \geq 0.9 mm. Hp: DL lateral margins divergent from base to mid-
	length E. oxumae
3	Female Cp in dorsal view: greatest width at posterior half of CL. Hp: DL tip
	blunt, DL lateral margins parallel or slightly convergent from base to mid-
	length E. martensi
-	Female Cp in dorsal view: greatest width at mid-length of CL. Hp: DL tip
	acute, DL lateral margins convergent along its whole length $\ensuremath{\textit{E. purperae}}$
4	Cp surface smooth or covered with minute foveolae 5
-	Cp surface ornamented E. laesslei
5	Female CL \geq 0.9 mm
-	Female CL < 0.9 mm

6 Female Cp in dorsal view rounded (CL:W ≤ 1.2), symmetrical, with posterior margin truncate or cordate. Hp: DL tip blunt, CoP tip undivided......**7**

-	Female Cp in dorsal view elongate (CL:W > 1.2), asymmetrical, with poste-
	rior margin pointed, barely obtuse, almost acute. Hp: DL tip acute, CoP tip
	dividedE. heberti
7	Female Cp in dorsal view: posterior margin truncate, greatest width at

- mid-length. Hp: DL lateral margins divergent from base to mid-point, DL with a small distal or medial subtriangular expansion *E. bromeliarum* Female Cp in dorsal view: posterior margin cordate, greatest width at pos-
- Female CL 0.7–0.9 mm11
- 9 Female Cp symmetric in dorsal view10
- Female Cp asymmetric in dorsal view*E. inaequivalve* Female Cp in dorsal view: posterior margin truncate. Hp: right DL tip blunt, left DL tip acute, basal digitiform expansion absent*E. litoreum*
- 11 CoP tip divided......12

- Hp: DL internally with digitiform expansion......14
 Hp: DL without internal expansion or, if present, not digitiform but a short

- 15 Female Cp posterior margin pointed (obtuse) in dorsal view *E. chacoense*
- Female Cp posterior margin truncate in dorsal view16

- Female CL:W ≤ 1.2, DL finger shorter than basal seta *E. maricaoense* Female CL:W > 1.2, DL finger long (as long or longer than basal seta)......

- sition than mid-length, basal lateral margins almost parallel...... *E. purium*

Discussion

Morphology and similar species

Elpidium alarconi sp. nov. has a shell morphology that does not differ widely from those of other Elpidium species with symmetric smooth valves, shell closure with left valve embracing right valve, and truncate posterior margin in dorsal view, such as E. higutiae, E. purium, E. litoreum, E. pintoi or even the type species E. bromeliarum. Yet, some of these species are either larger, as E. bromeliarum, or more elongated (E. higutiae, E. litoreum). The remaining two species, E. purium and E. pintoi, are very similar in dorsal view and their carapace sizes overlap with that of E. alarconi sp. nov. However, both lack a basal digitiform expansion in the distal lobe of the hemipenis, which is present, and very long, in the new species. This relatively straightforward distinction between species could be established thanks to a previous review of the variability of morphological traits in the genus Elpidium by Danielopol et al. (2014). These authors highlighted the importance of valve surface (smooth or ornamented), shell size and closure (left or right valve overlapping the other one), and its outline in dorsal view, including symmetry or asymmetry of valves, shape of posterior margin (pointed, rounded, truncate, invaginated), and length/width relationship. These traits are very useful for morphological characterization of Elpidium species, and therefore for identification keys, so we also used them in the new key provided, which now includes 20 described species. However, besides the indication of a pointed shape, we used the more precise term "obtuse" for an angle > 90°, and "acute" for an angle < 90°, and rather than "invaginated", we used the term "cordate", following Hickey (1973). We call for a more general use of this terminology, well established in the literature for leaf shape, but which can be also applied to ostracod shape in dorsal or ventral view.

In some cases, carapace morphology alone is not enough to easily distinguish between similar species, and other characters may be needed. Indeed, the most diversified morphological trait in Elpidium ostracods is the shape of the hemipenis and, in particular, that of its distal lobe, copulatory process and lower ramus (Danielopol 1975; Danielopol et al. 2014; Pereira et al. 2022). Hemipenis morphology has long been considered an essential character in ostracod phylogeny, allowing species determination in lineages with similar shell structure (Danielopol 1969; Hart and Hart 1974; Bisguert-Ribes et al. 2023), and this seems to be also the case in the genus Elpidium. Besides E. alarconi sp. nov., there are other species that also have a basal digitiform expansion in the distal lobe of the hemipenis: E. chacoense, E. cordiforme, E. picinguabaense, E. merendonense, E. maricaoense, and E. higutiae. But out of these, this expansion is as long or longer than the basal seta of the distal lobe only in E. alarconi sp. nov., E. cordiforme, and E. merendonense. It is nevertheless distinctly shorter in E. merendonense than in the other two species, and this species is furthermore distinguished because of an asymmetric carapace shape in dorsal view, and a lower ramus of the hemipenis with a blunt tip. Despite the similarity of the digital expansion of E. cordiforme with that of the new species, its distal lobe has a blunt tip (pointed in the new species). In addition, E. cordiforme has a cordate posterior margin in dorsal view (hence its name), whereas E. alarconi sp. nov. has a truncate posterior margin, although a slight invagination (i.e., quasi-cordate shape) can be appreciated in some shells.

Together with the distal lobe shape, the morphology of the lower ramus is also remarkable in the new species, as it is thinner than in any other member of the genus, and L-shaped, somehow resembling a piolet or a very thin sock with an acuminate tip. The lower rami are also pointed and even almost L-shaped in other species of *Elpidium*, but always thicker at the basal part, as for instance in *E. higutiae*, *E. maricaoense*, *E. oxumae*, or *E. cordiforme*. Taking these hemipenis characters into account, *E. cordiforme* is one of the species closer to *E. alarconi* sp. nov., although the former has a twisted copulatory process, unlike any other species of the genus. Furthermore, the distal lobe of *E. merendonense* and the lower ramus of *E. maricaoense* are the most similar hemipenis structures to those of *E. alarconi* sp. nov.

Another interesting morphological trait apparently differing between species of the genus Elpidium, according to the literature, is the strength of the separation between segments 4a and 4b of the antennula. Most species have these segments only partially or weakly separated, as described for E. maricaoense, E. littlei, E. wolfi, E. litoreum, E. cordiforme, E. laesslei, E. merendonense, E. heberti, E. oxumae, E. picinguabaense, E. eriocaularum, and E. higutiae (Tressler 1941; Pinto and Jocqué 2013; Pereira et al. 2019, 2022, 2023), while others, including E. bromeliarum, E. martensi, and E. purium are described as having a single, undivided, fourth segment (Pinto and Purper 1970; Danielopol et al. 2014; Pereira et al. 2023). Consequently, most authors considered a five-segmented antennula as a diagnostic character of the genus (Pinto and Jocqué 2013; Danielopol et al. 2014; Pereira et al. 2019). However, E. alarconi sp. nov. shows a distinctly clear separation between segments 4a and 4b in most specimens (weaker in others) under standard microscopic observation in transmitted light, so that its antennula appears as having six segments, rather than five. Also six segments are apparent in the graphic description of E. heberti; although the authors indicate that the fourth segment is "partially subdivided" when describing the species in the text, it is drawn as divided with a continuous line in their figure (Pereira et al. 2019: fig. 9a), while other species described in the same publication show a dashed line. In the original description of the type species, and in a subsequent revision and establishment of neotypes, Müller (1881) and Pinto and Purper (1970) stated that the antennula usually has five segments, but that it can exceptionally have six. Pinto and Jocqué (2013) suggested this separation might not be fully functional. Later, Pereira et al. (2022), when performing a phylogenetic analysis of the genus using a list of coded characters (detailed in the Supplementary information of their publication), characterized all species for which they found information on this trait, as having a partially fused fourth segment of the antennula. They concluded, after re-examining preparations of most species, that this morphological feature was shared for all Elpidium species analyzed, and considered that, according to microscopic observations, the segmentation was most probably only occurring on one side of the segment, but not in the other (Pereira, pers. comm.). We tested this possibility in the case of E. alarconi sp. nov., and could confirm it; even if the separation was quite clear under standard transmitted light, the use of fluorescence and scanning microscopy confirmed that it was partial, and only present in the inner side of the segment for each antennula. It remains to be confirmed whether this feature is shared with all other species of the genus, and which is its functional and evolutionary significance.

Diversity and biogeography

The species described in this work represents the first member of the genus Elpidium identified to species level for the island of Hispaniola; it must be noticed that Acosta-Mercado et al. (2012) previously recorded two undetermined species collected from liverworts. Its presence in this island does not come as a surprise, considering that several Elpidium species had been found in the neighboring islands of Cuba, Jamaica, and Puerto Rico, in addition to those found in the mainland (Fig. 9). At present, Jamaica can be considered the area with the highest density of Elpidium species worldwide, most of them endemic to the island (Little and Hebert 1996; Danielopol et al. 2014; Pereira et al. 2019). The high diversity and endemicity of the genus Elpidium was previously noted by Little and Hebert (1996), based on allozyme and mitochondrial data and on partial description of hemipenis morphologies, although they did not formally describe any species. They also highlighted the role of isolation and restricted dispersal in phytotelma ostracods for speciation, although more recent works have shown how they may disperse via phoresis using mostly amphibians and snakes (Lopez et al. 1999, 2005; Sabagh and Rocha 2014; Cunha et al. 2023). The high diversity and endemicity of Elpidium has been further supported by studies in Brazil and Argentina during the past few years (Pereira et al. 2022, 2023; Díaz et al. 2024), and corroborated by the present survey. It seems therefore that the species diversity of the genus Elpidium may be much higher than previously expected. The small number of samples collected hitherto from phytotelmata in tropical countries probably caused that only 20 species of Elpidium are known to date, but we expect many more to be discovered in the future, considering the large areas in the Neotropics that have remained unexplored for this habitat (Jocque et al. 2013; Pereira et al. 2022) (Fig. 9).

The high endemicity of Elpidium species is challenged by the wide distribution of the type species E. bromeliarum, recorded from Southern Brazil to Central America and Jamaica (Fig. 9). However, we must be cautious, as probably most of the records out of Brazil might be erroneous (Pereira et al. 2023). Indeed, even if some authors cited E. bromeliarum from Costa Rica (Pinto and Jocqué 2013; Pereira et al. 2023) based on early work by Picado (1913), this author did not confirm that the Elpidium species he found was E. bromeliarum, but a similar species: "Metacypris (Elpidium) sp. (fig. 42, B). La Mica, 1500 mètres. Ce crustacé est, d'après, G. W. Müller une espèce très voisine d'Elpidium bromeliarum. Quand le Crustacé est vivant, il présente cependant une pigmentation différente de celle de l'espèce décrite par Fritz Müller..." (Picado 1913: 336). [Transl: "Metacypris (Elpidium) sp. (fig. 42, B). La Mica, 1500 metres. This crustacean is, according to G. W. Müller, a species closely related to Elpidium bromeliarum. When the crustacean is alive, however, it presents a different pigmentation from that of the species described by Fritz Müller ... "]. Picado (1913) included Elpidium bromeliarum in his list of "Animaux bromelicoles actuellement connus", but he specifically wrote that this species lived in Brazilian epiphytic bromeliads, not in Costa Rica. Later on, it was Tressler (1956) who recorded E. bromeliarum from Jamaica (Fig. 9), although he did not discuss or show diagnostic characters of the hemipenis, so it may be considered an unreliable record (Pereira et al. 2023). The presence of E. bromeliarum in Guatemala (Pérez et al. 2012) should also be considered doubtful, because the authors only provided valve pictures, and it would be necessary to check the morphology of the copulatory apparatus to confirm this de-



Figure 9. World distribution map (**A**) of *Elpidium* records, according to published information **B** detail of distribution in Jamaica. Note that some records of *E. bromeliarum* are considered doubtful (see text for further explanation).

termination. Furthermore, Pinto and Jocqué (2013) described *E. merendonense* one year later from Honduras; it would therefore be interesting to check whether or not the species determined as *E. bromeliarum* from Guatemala may actually belong to a different species, perhaps *E. merendonense*. Finally, *E. bromeliarum* has also been recorded from French Guiana (GBIF.Org 2023), but we could not find further information on morphological aspects of this record, which is quite far from other geographic locations of the species, so we consider it should be taken with caution. Actually, the confusion on the identification and distribution of *E. bromeliarum* can be traced back to its discovery; in his description of male copulatory organs, Müller (1881) included at least three different morphologies of the hemipenis distal lobe, suggesting it was very variable. However, these different morphotypes most probably belong to different species of *Elpidium*. This confusion was continued in the review of Pinto and Purper (1970), as they also showed some clearly different hemipenes as belonging to the same species, although they may actually correspond to different ones (Pereira et al. 2017, 2023).

Another potential issue for understanding the biogeography of *Elpidium* is the presence of *E. maricaoense* in Florida (Tressler 1956). Even though this record was noted by the same author who described the species earlier from Puerto Rico (Tressler 1941), and considering the high diversity of species in

the Caribbean and the lack of morphological information for the Florida specimens, the presence of *E. maricaoense* in mainland America needs to be corroborated by further sampling in Florida. In addition, undetermined species of *Elpidium* have been recorded from other locations besides Costa Rica (Picado 1913), including Brazil, Florida and Mexico (Mercado-Salas et al. 2021; GBIF. Org 2023), so we would expect the genus to be widespread in the Neotropical region, and many more species to be described in the future. Consequently, the early suggestion by Müller (1881) that *E. bromeliarum* should be widely distributed in Brazil, is not corroborated by recent data, although it has been shown that the genus *Elpidium* has probably colonised most of the Neotropical region.

The new finding of E. alarconi sp. nov. in Hispaniola should initially be considered as an endemism for the island. However, considering that it was collected from bromeliads in managed gardens or nearby secondary forests, it would not be surprising that future research may record it in other regions, considering also its morphological proximity to several mainland species, and the worldwide proliferation of exotic ostracods driven by human movements (McKenzie and Moroni 1986; Valls et al. 2014). This might be one of the reasons for the lack of congruence between the geographic distribution of Elpidium species and their phylogenetic relationship using morphological data (Pereira et al. 2022). These authors only found a clear relationship between a clade of Elpidium species and their restricted distribution in Jamaica. They suggested that the lack of a phylogeographic pattern for most of the species relies on the scarcity of studies and/or the lack of critical morphological information for some species described long ago. We agree that these are the main reasons for the unresolved Elpidium biogeography, although we would not discard human-mediated movement of Elpidium species through bromeliad trade for gardening, as shown for other ostracod species in relation to the trade of aquatic plants for cultivation, gardening or aquaculture (McKenzie and Moroni 1986; Matzke-Karasz et al. 2014; Valls et al. 2014; Smith et al. 2024). Pereira et al. (2023) proposed using the genus *Elpidium* as a model group to study biogeographic areas of endemism, but considering the issue of expanding exotic ostracods, this kind of studies should be focused on sampling bromeliads mostly in undisturbed environments, far from human-impacted sites.

Phylogeny and systematics

Our molecular phylogeny analysis placed *E. alarconi* in the same clade as *Metacypris*, *Cytheridella* and *Gomphodella*, and far from the branch where *Limnocythere* was positioned in the phylogenetic tree. Assuming that the *Limnocythere* specimen whose DNA sequence is deposited in the repository has been accurately identified, and that it is representative of the Limnocytherinae, these results provide further support for the suggestion that the former subfamilies Timiriaseviinae and Limnocytherinae should be promoted to family level (Tanaka et al. 2021). The Timiriaseviinae subfamily was established to accommodate a fossil species of the genus *Timiriasevia* by Mandelstam (in Kashevarova et al. 1960). Shortly after, the subfamily Metacyprinae was established by Danielopol (1965), initially as a tribe (Metacyprini) of the subfamily Limnocytherinae, to include the genera *Metacypris, Elpidium, Afrocythere* and *Cordocythere*. Later on, the tribe Metacyprini was promoted to subfamily, and considered a junior synonym of the Timiriaseviinae (Colin and Danielopol 1978; Danielopol et al. 2018).

Although most recent authors consider the Timiriaseviinae a subfamily included in the Limnocytheridae Sars, 1928 together with the subfamily Limnocytherinae, after considering their major differences in shell and soft parts anatomy (Martens 1995; Danielopol et al. 2018) and the long genetic distance between them (Tanaka et al. 2021), we decided to accept the proposal of these last authors to promote the Timiriaseviinae to family Timiriaseviidae, as also did earlier Mesquita-Joanes et al. (2024), although the molecular basis for this promotion needs to be further tested with more sequences of species belonging to the Limnocytheridae s.s. Nevertheless, despite some morphological similarities between both families, which might be considered large enough as to hamper the proposed change of taxonomical levels suggested by Tanaka et al. (2021) and adopted here, we consider that the differences between them are even stronger, supporting a separation in two distinct families. Regarding similarities, there are three characters that are shared between both groups (Danielopol et al. 2018), but which can be considered relatively weak or even plesiomorphic, and therefore not well founded for their use in sustaining their monophyly: i) the distal antennular aesthetasc, fused with a distal seta, shows a much longer fused zone in the Limnocytheridae s.s. than in the Timiriaseviidae, in which this fusion is very short (as in Gomphodella or Gomphocythere) or even not observed (in Elpidium, Intrepidocythere, or Metacypris); ii) the presence of three claws in the last segment of the antenna may be regarded as an important trait, but it might be considered plesiomorphic, as it appears also in the primitive Bythocytheridae, and Entocytheridae; and iii) the presence of a minute seta in the last podomere of thoracopods, this segment fused with the final claw, may be a remnant of the posterior seta that some other Cytherocopina hold in the last segment of thoracopods (when it is not fused with the claw). For instance, it is the only posterior seta present in the thoracopod endopods of some Cytherocopina (e.g., in Terrestricythere, Bythocypris, Bairdoppilata) or in Darwinuloidea (e.g., in Vestalenula). It is interesting to notice how the first thoracopod of adult males of Terrestricythere hold a small posterior seta in their modified claw, probably resulting from the fusion of the last segment with the claw, as the male endopod has only two segments, while there are three in the female (Horne et al. 2004). Furthermore, we can see a similar shape of a fused segment-claw with a tiny seta in Amnicythere prespensis (in Petkovski and Keyser 1992), a species in the family Leptocytheridae, therefore also outside the Limnocytheridae s.l. Conversely, there are some species of Limnocytheridae s.l. for which that minute seta has not been observed or illustrated, as it occurs in several Limnocythere (Martens, 1990) or in Intrepidocythere (Pinto et al. 2008), although it may have been missed by the authors when illustrating them. Consequently, it does not seem appropriate to keep Timiriaseviinae and Limnocytherinae together in the same family on the basis of such a loose character of a minute seta, considering that it is not present in all species, and that it is also present in other species outside the family Limnocytheridae s.l., suggesting it is a plesiomorphic trait.

Regarding morphological differences between Limnocytheridae s.s. and Timiriaseviidae, we consider these are more consistent and strong enough as to support their separation as two distinct families: (i) unlike the Timiriaseviidae, females of the Limnocytheridae s.s. do not have a brooding chamber in their valves. This is an important morphological trait, related to reproduction and readily observed in the female carapace of most Timiriaseviidae; (ii) another

very important trait, in our view, is the segmentation of the maxillular palp. It has only one segment in the Timiriaseviidae (Elpidium, Cytheridella, Intrepidocythere, Metacypris, Gomphodella, Gomphocythere) but two in the Limnocytheridae s.s. (e.g., in Limnocythere, Korannacythere and Leucocythere); (iii) still another important trait differing between the two families is the ventral seta on the second antennular segment, which is situated in a medial or proximal position in the Timiriaseviidae, but in the distal margin in the Limnocytheridae; in addition, (iv) the antennula is five-segmented in the Limnocytheridae, but in the Timiriaseviidae it can be five-segmented (as in Cytheridella), six-segmented (as in Metacypris and Gomphocythere) or with a partial segmentation of the 4th segment, i.e. apparently six-segmented but not completely (as in Elpidium, Gomphodella or Intrepidocythere) and (v) the distal lobe of the hemipenis is articulated in the Timiriaseviidae, but not in the Limnocytheridae. This can be considered an important trait as well, because of its potential functional role in reproduction. Furthermore, (vi) a recent review of the sieve-type pore canals (StPC) in the Limnocytheridae s.l. by Danielopol et al. (2018) concluded that these pore canals, when present, have a seta inside them in the Limnocytheridae s.s. (type C StPC) but not in the Timiriaseviidae (type B StPC). Finally, if this taxonomic scheme with two separate families (Limnocytheridae s.s. and Timiriaseviidae) is accepted, a derived conclusion should be to promote their constitutive tribes to subfamilies: Timiriaseviini Mandelstam, 1960, Cytheridellini Danielopol & Martens, 1989 and Gomphodellini Danielopol et al., 2018 would therefore change to Timiriaseviinae Mandelstam, 1960, Cytheridellinae Danielopol & Martens, 1989 and Gomphodellinae Danielopol et al., 2018, all belonging to the family Timiriaseviidae; and the Limnocytheridae s.s. would be composed by the subfamilies Leucocytherinae Danielopol & Martens, 1989 and Limnocytherinae Klie, 1938 (previously as tribes Leucocytherini Danielopol & Martens, 1989 and Limnocytherini Klie, 1938, belonging to the subfamily Limnocytherinae Sars, 1928).

Within the Timiriaseviidae, previous phylogenies using morphological traits, positioned the genus Elpidium either alone in a branch separated from another that included Gomphodella, Metacypris and Cytheridella (Karanovic 2009) or together with Metacypris in a branch separated from Gomphodella or Cytheridella (Karanovic and Humphreys 2014). In contrast, our 18S phylogenetic tree suggests Elpidium might be closer to Gomphodella than to Metacypris or Cytheridella. Such different pattern has consequences for the interpretation of the biogeographic origin of Elpidium; as Gomphodella is exclusive to the Australian region, the phylogenetic association between these two genera suggests an ancient vicariant origin from the breakage of Gondwana, when Australia became separated from Antarctica and South America, similar to the findings of Sigvardt et al. (2021) for Lynceus (Laevicaudata). Therefore, our findings do not support the alternative process of a dispersal event from Eurasia or Africa to South America, as previously proposed by Karanovic (2009) in relation to the morphological similarities between *Elpidium* and *Metacypris*, and to the rich fossil record of the latter. Yet some morphological traits point to other relationships. For instance Elpidium lack StPC, while Gomphodella or Cytheridella present this type of pores on its valves (Danielopol et al. 2018). Another interesting trait is the row of posteroventral type-A2 pores (with rim and seta) on the peripheral marginal infold of valves of Elpidium. A similar row of pores is observed in Cytheridella (Danielopol et al. 2023: fig. 11) and in Intrepidocythere ibipora

(Pinto et al. 2008), although in a more external position in this case. Still another particular trait shared by *Elpidium*, *Intrepidocythere* and *Cytheridella* is the presence of a seta at the base of the articulated distal lobe of hemipenes, not described in other species of the family. The inconsistencies between morphological and molecular phylogenetic relationships inside the family calls for further molecular analysis of other genera of Timiriaseviidae, and a more detailed morphological work, which combined would help understanding the phylogeny and early biogeography of this interesting family of non-marine ostracods.

Conclusions

With the description of a new species of Elpidium from Hispaniola, we fill the gap of the only island of the Greater Antilles for which no determined species of the genus were known to occur hitherto. Elpidium alarconi sp. nov. has a shell morphology similar to other species of the genus (e.g., with valves covered with minute foveolae, posteriorly truncated in dorsal view), but the distinct shape of its hemipenis distal lobe and lower ramus separates it from other Elpidium species. Pereira et al. (2022) classified Elpidium species in two groups: those with the hemipenis copulatory process divided, and those with it undivided, to which the new species belong. The first group is restricted to Jamaica, but the second has an inconsistent phylogeographic pattern. As these authors suggest, we need a higher coverage of taxonomic and biogeographic information of the genus in the Neotropics to be able to better understand the phylogeny and biogeographic history of *Elpidium* ostracods. At a wider phylogenetic context, the available molecular data show how Elpidium is tightly related to Gomphodella, Metacypris and Cytheridella, but not to Limnocythere, supporting the establishment of the Timiriaseviidae as a family (Tanaka et al. 2021), not as a subfamily as previously considered. Despite the still reduced molecular information, which should be increased in the future to evaluate if the pattern holds when including more sequences of other limnocytherid species, we consider that there is already a suficient number of large differences in key morphological traits that further support the promotion of the subfamily Timiriaseviinae to a family level, separated from the Limnocytheridae s.s., such as the brood space in Timiriaseviidae female carapaces, or the articulated distal lobe in male hemipenes, among others.

Acknowledgements

Personnel at the Electron Microscopy unit of the SCSIE (Univ. Valencia) helped with sample preparation and SEM pictures. We are very grateful to the subject editor of ZooKeys, Simone N. Brandão, and to Julia S. Pereira, Dan L. Danielopol, Renate Matzke-Karasz, and two anonymous reviewers, for their thorough review and their many constructive comments and suggestions that greatly contributed to improve this manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was partially supported by Ministerio de Economía, Industria y Competitividad (Spanish Government), through project CRUSTRESS (code PID2020-112959GB-I00) awarded to FP and FMJ. FP acknowledges the project "CIDEGENT/2019/028 - BIOdiversity PAtterns of Crustacea from Karstic Systems (BIOPACKS): molecular, morphological, and functional adaptations" funded by the Conselleria d'Innovació, Universitats, Ciència i Societat Digital. This work was also partially funded by the project: "Búsqueda, caracterización y evaluación de agentes ecológicamente amigables para el control de mosquitos (Diptera: Culicidae) de importancia médica en República Dominicana", supported by the Fondo Nacional de Innovación y Desarrollo Científico y Tecnológico (FONDOCyT), Ministerio de Educación Superior, Ciencia y Tecnología (MESCyT) of the Dominican Republic (Project No. 2018–19–282–043) awarded to Pedro María Alarcón Elbal and JR. Pedro Alarcón and María Altagracia Rodríguez Sosa are thanked for their collaboration in sampling and project coordination.

Author contributions

Conceptualization: FMJ, JR. Data curation: FP, FMJ, JR. Formal analysis: FP, FMJ. Funding acquisition: FP, FMJ, JR. Investigation: FP, ÁG, JR, FMJ. Methodology: ÁG, FP, FMJ, JR. Project administration: FP, JR. Resources: JR, FMJ, FP. Supervision: FMJ. Validation: JR, FMJ. Visualization: FP, ÁG, FMJ. Writing - original draft: FMJ. Writing - review and editing: FP, ÁG, JR, FMJ.

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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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Short Communication

Chironomidae (Diptera) from mountain lakes of the Eastern Carpathians, Romania: First records and insight into diversity

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Abstract

Lakes at high altitudes are extremely sensitive to environmental stressors at both local and global scales, making them important sentinels of the changing world. Chironomidae, the most diverse group of benthic macroinvertebrates inhabiting mountain lakes, respond to various environmental impacts, making them important bioindicators of the lake's ecological status. This study aimed to provide the first insight into chironomid diversity in high-altitude lakes from two mountain ranges of the Romanian Eastern Carpathians: the Maramures, and the Rodna Mountains. Floating chironomid material was collected by skimming the water surface with a hand net from 16 lakes at elevations ranging from 1378 to 1922 m a.s.l. A total of 50 species/ taxa were collected, including nine new records for Romania. Notes on newly recorded species' distribution, ecology and taxonomy are provided. In addition, an identification key for *Procladius choreus* and *P. sagittalis* based on thoracic horn characteristics is given. With our addition, the total number of chironomid species known from Romania is now 526. The study provides a baseline for future research on chironomid diversity, ecology, and biogeography in high-altitude lakes of the Carpathian Mountains.

Key words: Biomonitoring, high-altitude lakes, males, Maramures Mountains, new record, non-biting midges, *Procladius* identification key, pupal exuviae, Rodna Mountains

-4753-A4CF-Introduction

Chironomidae is the most diverse group of benthic macroinvertebrates inhabiting high-altitude lakes and ponds, where they can represent twice the diversity of all other macroinvertebrate groups and often predominate quantitatively as well (e.g., Lods-Crozet et al. 2012 and references therein). Due to these attributes, chironomids are considered a good surrogate for benthic macroinvertebrates in ecological studies and biomonitoring programs (Ruse 2010).

The sensitivity of chironomid species to various environmental impacts, such as climate change, long-range air pollution, and species introduction, makes this insect group important bioindicators in both contemporary and palaeoecological studies (Nicacio and Juen 2015 and references therein).



Academic editor: Viktor Baranov Received: 27 November 2024 Accepted: 11 February 2025 Published: 28 March 2025

ZooBank: https://zoobank. org/1738A1F8-BF8D-4753-A4CF-EA8970EF5592

Citation: Bitušík P, Slobodníková V, Novikmec M, Dudáš A, Hamerlík L (2025) Chironomidae (Diptera) from mountain lakes of the Eastern Carpathians, Romania: First records and insight into diversity. ZooKeys 1233: 107–123. https://doi. org/10.3897/zookeys.1233.142856

Copyright: [©] Peter Bitušík et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Understanding the regional chironomid fauna of mountain lakes is the first step towards using the species for lake status assessment and further monitoring. Additionally, faunistic data from contemporary limnological studies can aid in the interpretation of paleolimnological data in mountain regions (Battarbee and Bennion 2012).

Our ongoing limnological research on chironomid fauna in alpine lakes of the Eastern Carpathians has shifted from the Chornohora and Svydovets Mountains in Ukraine (Bitušík et al. 2020, 2024) to the Maramures and Rodna Mountains in northern Romania.

In this study, we provide the first insight into the diversity of the Chironomidae family in mountain lakes of these ranges, including species recorded in Romania for the first time. The results will serve as baseline information for further research on chironomid diversity, ecology, and biogeography in Carpathian lakes, as well as a basis for long-term monitoring of lake ecological status and as a prerequisite for developing appropriate management and protection strategies for these ecosystems.

Material and methods

Study area and sampling sites

The study was carried out on 16 lakes within two orographic units of the Eastern Carpathians in northern Romania: the Maramures, and the Rodna Mountains (Fig. 1).

Both massifs are extensive, covering an area of 1500 km² with a length of around 100 km (Maramures Mts) and 1300 km² with a length of almost 50 km (Rodna Mts). The Rodna Mts are the highest range in the Romanian Eastern Carpathians, with peaks exceeding 2200 m, while the Maramures Mts reach a maximum elevation of 1957 m.

The geology of both massifs primarily consists of crystalline rocks (gneiss, epimetamorphic schists, mica schists) penetrated by eruptive rocks (dacites, andesites, rhyolites) and sedimentary rocks (conglomerates, sandstone, clay schist, shale, marl, clay; Curtean-Banaduc et al. 2008; Chis 2010).

Both mountain ranges have a moderate temperate continental climate with Atlantic and Baltic influences. Based on data from the lezer meteorological station (Rodna Mts, 1785 m a.s.l.), the mean annual temperature is 1.4 °C, mean air summer temperature is 9.4 °C, mean July air temperature is 10.3 °C, and the annual precipitation is 1240 mm, applicable to lakes at or above the natural timberline (1700–1850 m) (Kucsicsa 2011). Adjusted to 1360 m a.s.l., the forest zone has an average annual temperature of 3.4 °C, a mean summer temperature of 11.6 °C, and a mean July air temperature of 12.5 °C (Farcas et al. 2013; Diaconu et al. 2017).

The Rodna Mts were heavily glaciated during the Last Glacial Maximum and show clear glacial imprints, such as glacial cirques, lakes, and peatbogs (Mindrescu and Evans 2014). There are about 23 lakes in the Rodna Mts, each with a surface area under 0.5 ha and a maximum depth of 5.2 m (Chis 2010). In contrast, Pleistocene glaciation had a lower impact on the Maramures Mts (Costea 2008). The lakes of glacial origin there are generally small, and many are in an advanced terrestrialization phase or have already turned into peat bogs (Chis 2010).


Figure 1. Geographical location of the study area and maps showing sampling sites in the East Carpathians. Star marks the Maramures Mts, spark denotes the Rodna Mts. Site codes correspond to the codes in Table 1.

The surveyed lakes in the Maramures Mts include four lakes situated in the Farcau area (Chis 2008) at altitudes ranging from 1603 m (the lower lake in the glacial cirque Vartopul Mare) to 1786 m a.s.l. (Lake Livia). The 12 studied lakes in the Rodna Mts are located at altitudes between 1378 m (Lake Taul Muced) and 1922 m a.s.l. (Lake Lala Mica) (Table 1).

Except for two forest lakes (Taul Muced, Taul Hardau), the remaining lakes are above the recent tree-line, averaging about 1600 m in the Rodna Mts (Kucsicsa 2011). The natural tree-line has been significantly impacted by deforestation and grazing, lowering the upper forest limit and fragmenting the transition between the forest and the sub-alpine zone, especially affecting dwarf pine, juniper and rhododendron growth. In the Maramures Mts, all lake catchments are treeless and heavily affected by gully erosion and shallow landslides (Balteanu et al. 2016).

The basic characteristics of the sampling sites are summarized in Table 1.

Sampling and identification

The chironomid survey was conducted during three sampling campaigns in August 2022 and July 2024 (lakes in the Maramures Mts) and in July 2023 (lakes in the Rodna Mts). Floating chironomid pupal exuviae, pupae and drowned adults were collected along the entire lake shores by skimming the water surface with a hand net (mesh size 250 μ m, frame diameter 25 cm, telescopic handle).

Table 1. Basic characteristics of the sampling sites. If we were unaware of official lake names, we named the lakes for the adjacent hills. Maximum depth was either measured in the field or abstracted from available literature: *Akinyemi et al. (2013), **Mindrescu et al. (2013). Lake area was estimated using the polygon tool in Google Earth Pro. +/- indicate presence/absence of inflow/outflow. Abbreviations of catchment characteristics: **EAP** – extensive alpine pastures, **AM** – alpine meadows, **DP** – dwarf mountain pine shrubs (*Pinus mugo*), **RS** – rhododendron shrubs (*Rhododendron myrtifolium*), **NS** – Norway spruce (*Picea abies*), **BR** – bare rocks, **P** – peatbog.

Mountain range/ Lake name	Code	Geographical coordinates	Elevation (m)	Max. depth (m)	Lake area (m ²)	Inflow/ Outflow	Catchment
Maramures Mts.					·		
Livia	M1	47°54.26'N, 24°27.59'E	1786	3.5	622	-/-	EAP
Vinderel	M2	47°54.55'N, 24°27.33'E	1677	5.0	6183	+/+	EAP
Vartopul 1	M3	47°53.82'N, 24°27.63'E	1647	0.8	843	+/-	EAP
Vartopul 2	M4	47°53.59'N, 24°27.76'E	1603	0.8	246	+/-	EAP
Rodna Mts.							
Lala Mare	R1	47°31.68'N, 24°53.99'E	1805	1.5	6665	+/+	AM, DP
Lala Mica	R2	47°31.60'N, 24°53.46'E	1922	0.6	5332	+/+	AM, RS
Taul Hardau	R3	47°34.93'N, 24°49.20'E	1552	*3.5	568	-/-	EAP, NS
Taul Stiol	R4	47°34.35'N, 24°48.82'E	1657	5.0	10790	+/+	EAP, DP
Gargalau 3	R5	47°34.42'N, 24°47.64'E	1911	0.9	263	-/-	EAP
Gargalau 2	R6	47°34.55'N, 24°47.32'E	1887	1.2	120	-/-	EAP
Gargalau 1	R7	47°34.56'N, 24°47.18'E	1894	0.4	601	-/-	EAP
Lacul lezer	R8	47°35.90'N, 24°38.78'E	1822	**4.5	3883	+/+	AM, BR, DP
Buhaescu 1	R9	47°35.21'N, 24°38.71'E	1825	3.0	783	+/+	AM, BR, DP
Buhaescu 2	R10	47°35.30'N, 24°38.58'E	1892	5.2	1529	+/+	AM, BR, DP
Buhaescu 3	R11	47°35.33'N, 24°38.58'E	1911	2.0	659	-/+	AM, BR, DP
Taul Muced	R12	47°34.44'N, 24°32.70'E	1378	1.0	593	-/-	P, NS, DP

Onshore, each sample was transferred to a labelled 100 ml plastic bottle and preserved in 4% formalin. In the laboratory, samples were placed in Petri dishes and all specimens were sorted under a stereomicroscope at a magnification of 7.5–50×.

Pupal exuviae were examined and classified to at least the genus level. All pupal exuviae of the least abundant morphotypes, as well as pharate adults and males associated with pupal skins, were mounted on microscopic slides, while at least 10 exuviae were prepared for the most abundant ones.

Berlese solution was used as the mounting medium. Chironomid pupal exuviae were identified using Langton and Visser (2003), while adults were identified with Langton and Pinder (2007a, 2007b). In some cases, more detailed keys were used, including Fittkau (1962); Reiss (1969); Säwedal (1976); and Langton et al. (2013). Species nomenclature and distribution follow Ashe and O'Connor (2009, 2012); and de Jong (2016).

All the slides and samples are archived in the Department of Biology and Environmental Studies, Faculty of Natural Sciences, Matej Bel University in Banská Bystrica, Slovakia.

Statistical analysis

To improve the separation of *Procladius* pupal exuviae, we focused on thoracic horn parameters of the exuviae which were associated with males of *P. sagittalis* from Lakes Vartopul 1 and Vartopul 2 and compared them with those of *P. choreus* from Western Carpathian reservoirs. Our previous research showed that only

thoracic horn characteristics were statistically significant for the identification of *Procladius* exuviae (Langton et al. 2013). We measured thoracic horn length, maximum breadth, and plastron plate diameter on a total of 30 thoracic horns from 15 *P. sagittalis* specimens and 81 thoracic horns from 41 *P. choreus* specimens.

For automatised classification, a decision tree classifier of Custode and lacca (2023) was selected for its decision-making quality and interpretability.

Results and discussion

A total of 1118 chironomid pupal exuviae, six pupae, seven pharate adults (six males, one female), 40 males and one female were collected and identified, representing 50 chironomid species/ taxa from 26 genera across 5 subfamilies. Nine Chironomidae species were recorded for the first time in Romania.

A list of all species/ taxa recorded is provided below; sampling site codes refer to Table 1; "**Pe**" after the genus name refers to a morphotype not associated with an adult by Langton (1991); * denotes the first record of a species from Romania. For detailed data on collected specimen abundance and life stages, see Suppl. materal 1.

CHIRONOMIDAE

Tanypodinae

Procladius (Holotanypus) choreus (Meigen, 1804): M1, M2, R3 *Procladius (Holotanypus) sagittalis (Kieffer, 1909): M3, M4 *Procladius (Holotanypus) simplicistilus Freeman, 1948: R5 Procladius (Holotanypus) Pe3 Langton 1991: R7, R12 Macropelopia nebulosa (Meigen, 1804): R1, R4 Monopelopia tenuicalcar (Kieffer, 1918): R12 *Zavrelimyia punctatissima (Goetghebuer, 1934): R4

Diamesinae

Diamesa Pe 5? Langton 1991: R4 *Pseudodiamesa (Pseudodiamesa) nivosa (*Goetghebuer, 1928): R8

Prodiamesinae

Prodiamesa olivacea (Meigen, 1818): R1, R8

Orthocladiinae

Brillia bifida (Kieffer, 1909): R9 Bryophaenocladius sp./ Gymnometriocnemus sp.: R2 Corynoneura celeripes Winnertz, 1852: R3 Corynoneura celtica Edwards, 1924: R4 Corynoneura lobata Edwards, 1924: R12 Cricotopus (Cricotopus) cf. albiforceps (Kieffer, 1916): R1 Cricotopus (Cricotopus) curtus Hirvenoja, 1973: R4 Cricotopus (Isocladius) sylvestris (Fabricius, 1794): R4 Cricotopus (Isocladius) trifasciatus (Meigen, 1810): M2 Eukiefferiella coerulescens Kieffer, 1926: R4 Eukiefferiella cf. dittmari Lehman, 1972: R4 Heterotrissocladius marcidus (Walker, 1856): R2, R8, R9, R10, R11 Krenosmittia camptophleps (Edwards, 1929): R4 Limnophyes cf. asquamatus Andersen, 1937: M1, M2, R5, R6, R7, R8 Limnophyes cf. gelasinus Saether 1990: R2 Orthocladius (Mesorthocladius) frigidus (Zetterstedt, 1838): R8 Psectrocladius (Allopsectrocladius) obvius (Walker, 1856): M4, R4 *Psectrocladius (Allopsectrocladius) platypus (Edwards, 1929): M4, R12 *Psectrocladius (Psectrocladius) oligosetus Wuelker, 1956: R3, R7, R12 Rheocricotopus (Rheocricotopus) effusus (Walker, 1856): R4 Thienemanniella Pe1 Langton 1991: R4

Chironominae

Chironomus (Chironomus) cf. aberratus Keyl, 1961: M2, M4, R3, R4, R5 Chironomus (Chironomus) cf. holomelas Keyl, 1961: R6 Chironomus (Chironomus) cf. longistylus Goetghebuer, 1921: M2, M3, M4, R3, R7 Chironomus (Lobochironomus) dorsalis Meigen, 1818: R5, R6 Chironomus (Lobochironomus) Pe2 Langton 1991: R3, R5, R6, R7 Chironomus (Chironomus) sp.: R3 Cladopelma goetghebueri Spies et Saether, 2004: R12 Polypedilum (Pentapedilum) cf. uncinatum (Goetghebuer, 1921): R3 *Synendotendipes lepidus (Meigen, 1830): R5 Synendotendipes sp.: M3, M4, R3, R5, R6, R7, R12 *Micropsectra bodanica Reiss, 1969: M2 Micropsectra junci (Meigen, 1818): R9 Micropsectra lindrothi Goetghebuer, 1931: M2 *Micropsectra notescens (Walker, 1856): R10, R11 Paratanytarsus austriacus (Kieffer, 1924): M2, R4 Tanytarsus bathophilus Kieffer 1911: R4, R8 Tanytarsus gregarius Kieffer, 1909: R1, R2, R4 *Tanytarsus miriforceps (Kieffer, 1921): R2, R4 Tanytarsus Pe 4c Langton 1991/ debilis (Meigen, 1830): M2, M3

Comments on new records of Chironomidae from Romania

Zavrelimyia punctatissima (Goetghebuer, 1934)

Material examined. • 6 pupal exuviae, Taul Stiol (R4), 3 July 2023.

Distribution. West Palaearctic. The species is known from a few European countries: Austria, France, Germany, Italy, Norway, and Slovakia (Ashe and O'Connor 2009).

Habitat. It is a cold-stenothermal species adapted to live in oligotrophic waters with high oxygen concentrations (Boggero and Lencioni 2006). Boggero (2018) considers it strictly rheophilous. The species is a typical inhabitant of the littoral, inlets and outlets of alpine lakes (Rossaro et al. 2006; Hamerlík and Bitušík 2008; Steingruber et al 2013).

Remarks. Pupal exuviae closely resemble those of *Zavrelimyia hirtimana* (Kieffer, 1918), but all collected specimens exhibit very small plastron plates. The plastron plate diameter to thoracic horn length (0.054–0.055) aligns with the diagnosis of Langton and Visser (2003) for *Z. punctatissima*.

Procladius (Holotanypus) sagittalis (Kieffer, 1909)

Material examined. • 11 pupal exuviae, 1 male, Lake Vartopul 1 (M3), 1 July 2024 • 25 pupal exuviae, 2 pupae, 1 pharate adult – male, Lake Vartopul 2 (M4), 1 July 2024.

Distribution. Palaearctic and Oriental. Distributed from Europe and North Africa through Iran to Japan and the Russian Far East. One record is known from China (Ashe and O'Connor 2009; de Jong 2016).

Habitat. Generally, larvae of the subgenus *Holotanypus* are dwellers of stagnant and slow flowing waters regardless of size or volume. Langton (1991) noted that *P. sagittalis* typically occurs in shallow water under 2 m deep, which aligns with the findings from small-volume habitats (e.g., Velasco et al. 1993; Hirabayashi et al. 2004). However, the species has also been recorded from artificial ponds and reservoirs, as well as from backwaters, and large rivers (Bitušík 1993; Evrard 1994; Móra et al. 2010; Quintana et al. 2018). It should be noted that ecological information on the species could be more accurate if the identification of the preimaginal stages were more reliably resolved.

Remarks. Identification of the pupal exuviae, and even adult males of *Procladius* (*Holotanypus*) is extremely challenging (Vallenduuk and Moller Pillot 2007). The extended key for exuvia (Langton et al. 2013) is not reliably applicable to *Procladius* material collected from the Maramures lakes due to the variability of the tergite armament. Notably, the distinctive "fish scale" armament typical of *P. choreus* can also appear in some specimens of *Procladius* Pe3. The parameters of the thoracic horns appear to be more reliable characteristics for identification.

Thus, we propose a model that classifies input data with 97% accuracy, achieving 100% for *P. sagittalis* and 96% for *P. choreus*. Based on the decision tree trained on our dataset, we constructed an identification key for distinguishing the aforementioned *Procladius* species (Table 2). We are aware of the tentative nature of the key and acknowledge that a larger dataset would improve the tuning and evaluation of the proposed system. Therefore, the proposed key should be used with great caution.

Question	Our sting tout	Result		
number	Question text	Yes	No	
1	Is the length of the thoracic horn \leq 469 μ m?	P. sagittalis	2	
2	Is the diameter of the plastron plate \leq 93 µm?	3	4	
3	Is the length of the thoracic horn \leq 478 µm?	5	P. choreus	
4	Is the breadth of the thoracic horn \leq 144 µm?	P. choreus	P. sagittalis	
5	Is the length of the thoracic horn \leq 476 µm?	P. choreus	P. sagittalis	

Table 2. A tentative identification key for *Procladius choreus* and *P. sagittalis* based on thoracic horn characteristics, using the decision tree classifier of Custode and Iacca (2023).

Procladius (Holotanypus) simplicistilus Freeman, 1948

Material examined. • 1 pharate adult – male, Lake Gargalau 3 (R5), 6 July 2023. **Distribution.** Palaearctic. The species was recorded only from a few countries in West and North Europe, but also from the Far East of Russia (Ashe and O'Connor 2009). **Habitat.** The ecological requirements of this species are not sufficiently known because of the problematic identification of the pre-imaginal stages. Generally, larvae inhabit stagnant waters, they are resistant to low pH values (Murray and Baars 2006; Perova 2008; Baars et al. 2014) and salinity (Kawai et al. 2000).

Remarks. An adult male with associated exuviae confirms the presence of the species in Romania.

Psectrocladius (Allopsectrocladius) platypus (Edwards, 1929)

Material examined. • 64 pupal exuviae, 1 pharate adult – male, Lake Vartopul 2 (M4), 1 July 2024 • 1 pupal exuviae, Taul Hardau (R3), 6 July 2023.

Distribution. Palaearctic. Known from several European countries, as well as Turkey and Algeria (Ashe and O'Connor 2009).

Habitat. The species is typical of small, acidic, stagnant waters in moorlands and peat bogs. In addition to tolerating low pH, it can withstand low oxygen levels in polyhumic waters; however, larvae are also found in lake littorals and small streams with slow currents (Moller Pillot 2013 and references therein). In the Western Carpathians, pupal exuviae were collected from a small, non-acid sub-alpine lake (Bitušík et al. 2006). The species is frequently recorded in temporary pools and ponds (e.g., Bazzanti et al. 1997; Puntí et al. 2007), as well as ephemeral waters (Moller Pillot 2003), indicating relatively high dispersal potential of females.

Remarks. The findings indicate the humic conditions of Taul Hardau and suggest at least partial drying of Lake Vartopul 2.

Psectrocladius (Psectrocladius) oligosetus Wuelker, 1956

Material examined. • 26 pupal exuviae, Taul Hardau (R3), 6 July 2023; 59 pupal exuviae, Taul Muced (R12), 7 July 2023 • 1 pupal exuviae, Lake Gargalau 1 (R7), 6 July 2023.

Distribution. Palaearctic. Recorded from several European countries ranging from the south (Sicily) to the north (Scandinavia) and from the west (Ireland) to the eastern part of Russia (Ashe and O'Connor 2009).

Habitat. Cold-stenothermic species occurring in lakes in mountain regions (e.g., Laville and Vinçon 1986, Bitušík et al. 2007, Boggero 2018), although Rieradevall et al. (2007) found it in intermittent mountain headstreams. The species shows an apparent affinity for low pH humic waters (e.g., Ruse 2002; Bitušík and Svitok 2006; Moller Pillot 2013; Bitušík et al. 2020).

Remarks. This finding, along with an earlier record from Ukraine (Bitušík et al. 2020), partially fills the distribution gap of the species extending from the Baltic republics across Poland to the Balkans.

Synendotendipes lepidus (Meigen, 1830)

Material examined. • 4 males, 1 female, Lake Gargalau 3 (R5), 6 July 2023.

Distribution. Palaearctic. Widespread in Europe (Ashe and Cranston 1984, Moller Pillot 2009), and it has been reported from Turkey (Ozbek et al. 2018) and the Russian Far East (Orel 2016).

Habitat. The species has been recorded mainly from stagnant waters regardless of size and trophic status. Lundström et al. (2010) collected adults from temporary wetlands, and there are data from lowland brooks (Ozbek et al. 2018). Like other species of the genus, it tolerates acid conditions of peatland pools (Plóciennik et al. 2018). According to Moller Pillot (2009), the larvae are miners in the tissues of *Nuphar lutea*. However, they evidently utilize other types of littoral vegetation, such as sedges, since *N. lutea* does not occur in the studied lakes.

Remarks. Species of the genus *Synendotendipes* are indistinguishable as pupal exuviae, so it is not possible to confirm if *Synendotendipes* pupal exuviae recorded in other lakes also belong to *S. lepidus*.

Micropsectra bodanica Reiss, 1969

Material examined. • 1 male, Lake Vinderel (M2), 1 July 2023.

Distribution. Palaearctic. The species has so far been recorded from only a few countries, such as Germany, Austria, and Portugal, with its occurrence in Corsica and Slovakia not yet confirmed (Moubayed-Breil and Ashe 2012; Novikmec et al. 2015).

Habitat. Ecological requirements of the species are still inadequately understood. Reiss (1969) considered the species (together with *M. attenuata*) as cold stenothermic and polyoxybiontic, typically inhabiting mosses on stones in springs and the upper stretches of streams (see also Langton and Visser 2003). Records of the pupal exuviae of *M. attenuata/bodanica* in the Western Carpathians come from headwater streams (one even artificially modified) with stony bottoms but without moss growths (Novikmec et al. 2015). It can be assumed that the collected adult male comes from a spring or small stream flowing in Lake Vinderel.

Remarks. Since the pupal exuviae of *M. bodanica* are indistinguishable from those of *M. attenuata* (Langton and Visser 2003), the first record of *M. bodanica* based on an adult male in Romania is particularly valuable.

Micropsectra notescens (Walker, 1856)

Material examined. • 26 pupal exuviae, Lake Buhaescu 2 (R10), 5 July 2023 • 22 pupal exuviae, Lake Buhaescu 3 (R11), 5 July 2023.

Distribution. Palaearctic. Widespread in Europe including the Canary Islands (Langton and Visser 2003); also recorded in Morocco (Kettani and Langton 2011).

Habitat. Traditionally, the species is considered cold stenothermic and polyoxybiontic (Säwedal 1976). It has been documented in mountain, boreal and woodland springs and spring brooks (Orendt 2000; Ilmonen et al. 2009; Lencioni et al. 2012), as well as alpine lakes and ponds (Bitušík et al. 2006; Oertli et al. 2010; Lods-Crozet et al. 2012). However, data from low-altitude streams suggest a wider temperature tolerance (Móra and Szivák 2012). Its presence in temporary habitats, such as fountains (Oboňa et al. 2017) and temporarily flooded wetlands (Lundström et al. 2010), indicates a high distribution potential of females. **Remarks.** The presence of *M. notescens* in Romania has already been reported by Säwedal (1976) based on two males collected by Andrzej Kownacki from the Fagaras Mts. However, the species is not listed in the latest checklist of Romanian Chironomidae (Tatole 2023).

Tanytarsus miriforceps (Kieffer, 1921)

Material examined. • 13 pupal exuviae, 1 adult – male, Taul Stiol (R4), 3 July 2023 • 110 pupal exuviae, 1 pharate adult – male, 1 adult – male, Lake Lala Mica (R2), 4 July 2023.

Distribution. Holarctic. The species is widespread across Europe, primarily in northern and western countries (Ashe and Cranston 1984; de Jong 2016), with recent records in Poland, Montenegro and European Russia (Gilka and Dominiak 2007; Krasheninnikov 2014; Gadawski et al. 2022). It is also known from Canada (de Jong 2016); and the Far East (Orel 2018).

Habitat. Current data indicate that this species is a limnobiont inhabiting lakes mainly at high altitudes and high latitudes (except for Lake Skadar), suggesting a preference for low temperatures (e.g., Verneaux and Aleya 1999).

Remarks. The species exhibits symptoms of glacial relictualism as already suggested by Reiss and Fittkau (1971) and Reiss (1984).

The collection of floating chironomid pupal exuviae from the lakes in this study provides an excellent basis for the chironomid inventory of the area. For species identification, exuviae are sometimes even more useful than adults (Prat et al. 2016). However, it should be noted that our species inventory from a "snapshot" survey cannot be comprehensive, as not all species present in a site emerge simultaneously. Even though the collection was conducted during a period suitable for recording most species (Wilson and Ruse 2005; own data), we believe that the absence of cold-stenothermic species/ genera in our collection is due to their early spring emergence.

Compared to some Central and East European countries, such as Hungary, Ukraine, Czechia, Slovakia, and Poland, the Romanian chironomid fauna is relatively well-studied. The latest checklist of the family from Romania (Tatole 2023) includes 517 species, with recent records of nine additional species raising this total to 526. This number could be even higher if species within the genus *Limnophyes* and some *Chironomus* species could be reliably identified.

A detailed examination of the chorological data in the aforementioned checklist reveals a lack of records from the Rodna and Maramures Mountains. Chironomids are also absent from the list of Diptera collected in Maramures Mountains Nature Park (Parvu 2008). The only available information on chironomids associated with the studied lakes comes from the sediment core of Lake Taul Muced, where subfossil larval remains were identified to morphotype level (Diaconu et al. 2017).

Here, we provide the first information about chironomid occurrence within the protected areas of Maramures Mountains Nature Park and Rodna Mountains National Park, offering potential value to use by the administrations of both parks.

Acknowledgements

We gratefully acknowledge the support of the Administrations of the Maramures Mountains Nature Park and Rodna Mountains National Park. We are especially indebted to Vasile Ciolpan, Ioan Coman and Claudiu Iusan for valuable advice, providing field collection assistance and transport to some sampling sites.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The study of mountain lakes in the Carpathians was supported by the Slovak Research and Development Agency (project No. APVV-20-0358) and Grant Agency VEGA (project No. 1/0400/21).

Author contributions

Conceptualization: PB. Data curation: PB. Formal analysis: MN, AD. Funding acquisition: PB. Investigation: AD. Software: AD. Validation: LH. Visualization: MN. Writing – original draft: MN, PB, LH, AD, VS. Writing – review and editing: PB, MN, VS, LH.

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Data availability

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

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Supplementary material 1

List of chironomid taxa collected from studied lakes in the Maramures Mts. and the Rodna Mts.

Authors: Peter Bitušík, Veronika Slobodníková, Milan Novikmec, Adam Dudáš, Ladislav Hamerlík

Data type: docx

- Explanation note: Sampling site codes refer to codes in Table 1; numbers refer to number of chironomid pupal exuviae collected; P – pupa, M – male, F – female (in case of adult specimens), PhM – pharate adult male, PhF – pharate adult female, * – first record of species for Romania.
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Link: https://doi.org/10.3897/zookeys.1233.142856.suppl1



Research Article

Contribution to the knowledge of the genus *Calcyopa* Stüning, 2000 (Lepidoptera, Geometridae, Ennominae, Boarmiini), with description of a new species

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Abstract

The genus *Calcyopa* Stüning, 2000, is briefly reviewed. A new species, *Calcyopa hainana* Liu, **sp. nov.**, is described from Hainan Province, China. Within the genus *Calcyopa*, two species groups are identified, characterized by shared traits yet distinguished by a set of consistent features. The *difoveata*-group, comprises *C. difoveata*, *C. fansipana* and *C. hainana* **sp. nov.**, and the *rosearia*-group, includes *C. rosearia*, *C. prasina* and *C. sub-prasina*. The relationship of both species groups is discussed, and an identification key of all known *Calcyopa* species is presented. Illustrations are provided for adult males and females of the *difoveata*-group, along with their genitalia, except for *C. fansipana*, which is known only from males. DNA barcodes are provided for the type species and the newly described species.

Key words: *Calcyopa hainana*, DNA barcode, identification key, new species, species groups, taxonomic history

Introduction

The genus-group name *Calichodes* was first published by Warren (1897: 246) with the description of *Calichodes foveata* from Penang (Peninsular Malaysia). However, Warren did not mention it as a new genus, as the author usually did. Therefore, Wehrli (1943: 544) considered the name to be merely a manuscript name ("Warren M. S.") and proposed it for a new subgenus of *Boarmia*, with a different type species, *B.* (*Calichodes*) *difoveata* Wehrli. Subsequently, Fletcher (1979: 32) only referenced *Calichodes* Wehrli, 1943, and raised it to the rank of a genus. Holloway ([1994]: 251) redefined *Calichodes* Warren as a monotypic genus, distinct from *Calichodes* Wehrli, and emphasized, based on a personal communication with I. W. B. Nye, that "the earlier usage is valid under the International Code of Zoological Nomenclature and therefore has priority". Parsons et al. (1999: 100) followed this treatment and mentioned only *Calichodes* Warren, 1897, but included the type species *difoveata* of *Calichodes* Wehrli, without addressing the later invalid usage of Wehrli.



Academic editor: Gunnar Brehm Received: 28 November 2024 Accepted: 5 March 2025 Published: 28 March 2025

ZooBank: https://zoobank. org/34D5074B-916B-4414-B326-A29C4809C6C6

Citation: Liu B (2025) Contribution to the knowledge of the genus *Calcyopa* Stüning, 2000 (Lepidoptera, Geometridae, Ennominae, Boarmiini), with description of a new species. ZooKeys 1233: 125–138. https://doi. org/10.3897/zookeys.1233.142955

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Consequently, the generic name *Calcyopa* was proposed by Stüning (2000: 134) as a replacement name for *Calichodes* Wehrli, 1943, with *Boarmia* (*Calichodes*) *difoveata* Wehrli, 1943 designated as the type species. Additionally, *Calcyopa prasina* Stüning, 2000 was described from Thailand, with its distribution extending from Vietnam, Peninsular Malaysia, and Myanmar to northern India and Nepal. Furthermore, the latter author included two other species, *Ectropis rosearia* Joannis, 1929 from "Tonkin" (N. Vietnam), Yunnan (China), Thailand, and Sumatra (Indonesia), and an undescribed species from Vietnam in this genus. Two decades later, two new species – *Calcyopa fansipana* Sato, 2022 from N. Vietnam and *Calcyopa subprasina* Sato, 2022 from Laos – were added to the genus. Rajaei et al. (2022) mistakenly considered *Calcyopa* Stüning as a replacement name for *Calichodes* Warren and included all the species mentioned under *Calichodes* Warren in the catalogue of Parsons et al. (1999) under *Calcyopa* Stüning, 2000.

Currently, five species of the genus *Calcyopa* are known, and this paper describes a new species from Hainan Island, China.

Material and methods

Specimen collection

The study is based on moth specimens housed in the following collections: Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang, China (CRICATAS); Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS); Natural History Museum, London, United Kingdom (NHMUK); Institute for Agro-Environmental Sciences, NARO, Tsukuba, Japan (NIAES); Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK); Zoologische Staatssammlung München, Germany (ZSM).

Morphology

Terminology of the wing venation follows the Comstock-Needham System (Comstock, 1918) as adopted for Geometridae by Scoble (1992) and Hausmann (2001), while genitalia terminology is based on Klots (1970) and Skou and Sihvonen (2015). For genitalia examination, abdomens were removed and placed in a hot 10% KOH solution. Genitalia were then dissected in 10% ethanol and stained with Chlorazol Black E. Photographs of adult moths were taken using a Nikon camera (D750) equipped with a Nikon lens (AF-S Micro 60 mm f/2.8G ED). Photos of genitalia were taken with a digital camera (KUY NICE E31SPM) attached to a Nikon microscope (SMZ745T). Focus-stacked images were generated using Helicon Focus software (version 8.2.2 Pro).

DNA barcoding

Genomic DNA was extracted from the legs of dried adult specimens, and the barcode fragments were amplified using primers pairs: LCO-1490 and HCO-2198 or LepF1 and LepR1 (Folmer et al. 1994; Hebert et al. 2004). The obtained sequences (658 bp) were deposited in the Barcode of Life Data Systems (BOLD: Ratnasingham and Hebert 2007; http://www.boldsystems.org).

All sequences mentioned in this study were obtained from the BOLD Systems. Genetic distances between species are reported as uncorrected pairwise distances (p-distance).

Taxonomic account

Calcyopa Stüning, 2000

Calcyopa Stüning, 2000, *Moths of Nepal, Part 6*. Tinea Vol. 16 (Suppl. 1):134. Type species: *Boarmia* (*Calichodes*) *difoveata* Wehrli, 1943.

Generic characters. Small ennomine geometrid moths with forewing length 11-14 mm. Ground color light to dark grey or brown, with distinct black antemedial and postmedial lines on both wings. Head. Male antennae fasciculate, with two pairs of shortly conical, sclerotized, ciliate projections latero-ventrally on each segment. Cilia curved ventrad, 3-4 times longer (depending on species) than diameter of flagellomeres. Female antennae with cilia much shorter, the sclerotized projections absent. Vertex covered with lamellar scales. Frons smooth-scaled, not protruding. Labial palpi slightly extending beyond frons, basally with long scales. Proboscis rather short. Chaetosemata present, near eye margin. Thorax. Patagia and tegulae densely covered with somewhat longer lamellar scales, distal end of tegulae with a few long hair-scales. Legs slender, index of spurs 0-2-4, hind tibia not dilated, without scent brush (= hair-pencil of authors) in males. Forewings moderately elongate, apex angled, termen smoothly curved, with a double fovea in males. Antemedial and postmedial lines conspicuous on both wings. Postmedial line on forewing curved outward between M₁ and M₂, with the extent of curvature varying among species; outside postmedial line between veins M₃ and CuA₁ or even CuA₂ with a dark, round, oval or squarish patch (absent in C. rosearia only) and a faint, square pale patch outside the strongly dentate submarginal line (absent in C. rosearia). Marginal line consisting of small, black dots in the middle between veins, alternating with groups of dark fringe-scales. Hindwing with apex rounded, termen minutely concave between vein-ends, marginal dots rather lunulate. Tornus without distinct spot (elongate black spot present near tornus in C. prasina and C. subprasina) Postmedial line straighter than on forewing. Discal dot distinct, visible on both wings, but larger on forewing. Underside with pattern paler. Venation (Fig. 1). R1 and R2 coincident (distal branch of R1 reduced, only R2 reaching costa closer to apex), the base of the combined veins running closely parallel to vein Sc and the stem of R_{3-5} (R_1) + R_2 and the stalk of R_{3-5} both arising from the same position, shortly before anterior angle of cell. M₂ from the middle of the discoidal vein. CuA, from shortly before posterior angle of cell. Hindwing: Sc+R, running closely parallel to upper vein of cell for a short distance; Rs arising widely before anterior angle of cell; M₂ absent, represented by a fold; CuA₁ from shortly before posterior angle of cell; 3A absent. Folds through cells of both wings and those replacing CuP in forewings and M₂ in hindwings very vague. Pregenital abdomen. Tympanal organs of moderate size, without lacinia. Sterno-tympanal process and setal comb on third sternite absent. Lateral coremata on the posterior portion of the third segment only present in C. difoveata and C. hainana (very small and easily brushed off during abdominal preparation). Seventh segment



Figure 1. Wing venation of Calcyopa difoveata.

strongly modified, short, sternite membranous, with a pair of external corematous lobes, both laterally with a dense row of fine setae; anterior invaginated coremata, with intersegmental opening between segments 6 and 7 absent (not "weak, scarcely invaginated" as described in Stüning (2000) for *C. difoveata*) in all *difoveata*-group species (present in *rosearia*-group species).

Male genitalia. Uncus deeply divided, stout, triangular, apically straight, slightly curved ventrad in species of the *difoveata*-group (elongate, more deeply divided, distally slender, apical part strongly bent ventrad in *rosearia*-group species). Gnathos with broad, flattened lateral arms, central part strong, thorn-shaped, elongate, slightly curved, pointed in *difoveata*-group species (central part delicate, spine-shaped in *rosearia*-group species). Juxta small, basally a rounded plate, extended into a narrow arm distally, slightly cup-shaped and laterally bifurcate at apex. Valvae acutely triangular. Basal costal arm free, standing obliquely upright towards the upper valva margin, apically dilated, with long bristles. Saccular process stick-like, spined at apex only (*C. difoveata*) or

without spines (*C. hainana* and *C. fansipana*) (spined over half or more of its length in *rosearia*-group species). Aedeagus short and stout, apically with a long, acutely triangular, sclerotized process, shaft distally with short spines or groups of strong teeth, vesica with a small row of cornuti or a round diverticulum covered with numerous spicules in *difoveata*-group species (aedeagus smaller, narrower, without distal extension and without teeth on shaft and vesica without cornuti in *rosearia*-group species).

Female genitalia. Ovipositor short, papillae anales rounded, scarcely setose. Needle-like sclerotization between their bases present. Anterior apophyses short, about ½ length of posterior apophyses. Lamella postvaginalis a rounded, rather small plate in *difoveata*-group species (a large, sclerotized plate with a wide rounded incision anteriorly in *rosearia*-group species). Introitus small, sclerotized; posterior part of bursa sclerotized, scobinate inside; anterior part of bursa swollen, broader than posterior part, signum consisting of 6 to 8 chains of small dentate projections in *difoveata*-group species (bursa short, rounded, signum built of dentate projections arranged to small chains or distributed over larger areas of the bursa surface in *rosearia*-group species).

Diagnosis. The genus Calcyopa currently comprises a total of six species, including the newly described species presented in this study. These species can be distinctly divided into two groups, mainly based on the genitalia (both male and female) characters and the abdominal coremata. Despite their differences, all Calcyopa species share a deeply divided uncus, similarly triangular-shaped valvae, a free basal costal arm that is strongly setose at the apex, and a unique stick-like saccular process with or without spines in the male genitalia. These characters collectively set them apart from other related genera such as Paracalcyopa Sato, Myrioblephara Warren, and Chrysoblephara Holloway. The closely related genus Necyopa Walker exhibits most of these traits; however, its saccular process is replaced by a strong, longitudinal fold that extends close to the apex of the valva. In the present study, we separate all the known Calcyopa species into two groups: the C. difoveata species group, which includes C. difoveata, C. fansipana and C. hainana; and the C. rosearia species group, which comprises C. rosearia, C. prasina and C. subprasina. The main diagnostic characters of these two species groups are outlined in Table 1. While the two species groups share several common characters, they are also distinguished

Diagnostic characters	C. difoveata species group	C. rosearia species group	
Intersegmental abdominal coremata between ST 6 and ST 7	absent	present	
Uncus	moderately long, apex slightly curved ventrad	rather long, apex strongly bent ventrad	
Saccular process	without spines or spined at apex only	spined over half its length	
Aedeagus	rather stout, apical sclerotization long, tapering; cornuti spine-like	thin, apical sclerotization absent or indistinct, cornuti absent	
Lamella postvaginalis	rather small, rounded	large, with a large, round incision medio- anteriorly	
Introitus	small, sclerotized	large, membranous	
Corpus bursa	long, cylindrical, proximally slightly inflated	short, oval or squarish	

Table 1. Diagnostic characters separating the Calcyopa difoveata and C. rosearia species groups.

by a set of stable features. For the time being, we tentatively retain all of these species within the genus *Calcyopa*. However, depending on further molecular evidence, there might be a possibility of establishing a new genus for the *C*. *rosearia* species group or merging it with the genus *Necyopa* Walker.

Calcyopa difoveata (Wehrli, 1943)

Figs 2-5, 14, 15, 20

- Boarmia (Calichodes) difoveata Wehrli, 1943, in: Seitz, Gross-Schmett. Erde 4 (Suppl.): 544. Type-locality: "West-Tien-Mu-Shan, Chekiang" (West Tianmushan, Zhejiang Province, China)
- *Calichodes difoveata* Wehrli: Fletcher, 1979, In: Nye IWB (ed.), The Generic Names of Moths of the World 3: 32; Parsons et al. 1999, in: Scoble, Geometrid Moths of the World, A Catalogue, 1: 100.
- Aethalura lushanalis Sato, 1987, Japan Heterocerists' J. 144: 289, 290, figs 3, 4, 8. Synonymized with *C. difoveata* by Stüning (2000).
- *Calcyopa difoveata*: Stüning, 2000, Tinea 16 (Suppl. 1): 135, fig. 1507; Sato 2020, in: Kishida Y, Moths of Laos, Part 1, Tinea 25 (Suppl. 2): 81, pl. 28, fig. 30; Sato 2022, Tinea 26 (3): 228, 230, figs 9, 10, 24, 36.

Type material examined. *Lectotype:* CHINA – **Zhejiang Province** • ♂; Pz. Chekiang, West-tien-mu-shan (= Zhejiang, West Tianmushan); 1600 m; 26 Apr. 1932; H. Höne leg.; gen. prep. slide no. 2451-DS; ZFMK (des. Stüning, 2000).

Paralectotypes: CHINA – **Zhejiang Province** • 2 ♂♂, 1 ♀; same locality and collector as lectotype; 30 Apr. 1932; ZFMK. – **Guangdong Province** • 1 ♀; Lienping (Heyuan City, Lianping County); May; H. Höne leg.; coll. Wehrli, ZFMK. **Remark**: Wehrli (1943: 544) mentioned seven syntypes that were collected between 23 April and 13 June. Two female paralectotypes not included in the preceding material examined are not in coll. ZFMK, but should be the two specimens given to coll. NHMUK in exchange (1961).

Additional material examined. CHINA – Zhejiang Province • 4 ♂♂, 2 ♀♀; same locality and collector as lectotype; 3 Apr., 4 Apr., 10 Apr., 17 Apr. (2), 18 Apr. 1932; ZFMK • 5 ♂♂, 2 ♀♀; same locality and collector as lectotype; 400 m; mid-April 1936; gen. glycerol no. 6/B1♂; ZFMK • 1 ♀; Chekiang, Wenchow (= Zhejiang, Wenzhou); 18 Apr. 1939; H. Höne leg.; ZFMK • 3 ♂♂, 1 ♀; same locality and collector as for preceding; June 1939; ZFMK • 1 ♂, 2 ♀♀; same locality and collector as for preceding; July 1939; gen. glycerol no. 6/B32; ZFMK. – Fujian Province • 1 ♂; Fukien, Kuatun (= Fujian, Guadun); 27.40°N, 117.40°E; 2300 m; 3 Apr. 1938; J. Klapperich leg.; ZFMK. – Guangdong Province • 2 ♂♂, 3 ♀♀; Canton or Lienping; ZFMK. – Taiwan Province ・ 2 ごう (paratypes of Aethalura lushanalis Sato, 1987); Nantou Hsien, Lushan-wenchuan; 13–14 Aug. 1983; R. Sato leg.; ZFMK • 2 ♀♀; Kaohsiung, Liukuei Sanping; 650 m; 24–26 Jul. 1987; R. Sato leg.; ZFMK • 1 ♂; Nantou Co., Hueisun Forest; 600 m; 22 Jun. 1993; F. Aulombard & J. Plante leg.; ZFMK • 1 3, 1 ♀; same locality and collectors as for preceding; 570–800 m; 28/29 Sep. 1992; ZFMK. – Hainan Province • 1 ♂, 1 ♀; Wuzhishan; 1333 m; 10 Jan. 2008; V. Siniaev leg.; ZSM • 5 ♂♂, 3 ♀♀; Wuzhishan; 756 m; 25 Mar. 2023; Bo Liu leg.; CATASCRI.

Diagnosis. The diagnostic characters are given under the newly described species.



Figures 2–13. Adults of *Calcyopa* species. 2–5 *C. difoveata*, Hainan, China, CRICATAS 2 male, upperside 3 ditto, underside 4 female, upperside 5 ditto, underside 6 *C. fansipana*, holotype, male, upperside, N. Vietnam, NIAES 7 ditto, underside 8–11 *C. hainana* sp. nov., Hainan, China, CRICATAS/ IZCAS 8 holotype, male, upperside 9 ditto, underside 10 paratype, female, upperside 11 ditto, underside 12, 13 specimens of *C. hainana* sp. nov. in resting position, type series, Hainan, China 12 male 13 female. Scale bar: 1 cm.

Distribution. China (Zhejiang, Fujian, Jiangxi, Guangdong, Hainan, Taiwan), Vietnam, Laos.

Genetic data. The Barcode Index Number for *Calcyopa difoveata* is BOLD: AAH0723 (*N* = 3, Sample IDs: CRICATAS00104, BC ZSM Lep 16015, BC ZSM Lep 15995).

Remarks. This species is now recorded for the first time from Hainan Island, China. Some specimens from Hainan show minor differences in genitalia compared to those from Taiwan and Vietnam, including variation in the thickness of the gnathos in male genitalia as well as the shape of the lamella postvaginalis in female genitalia. At present, we treat these as infrasubspecific variations.

Calcyopa fansipana Sato, 2022

Figs 6, 7, 16, 17

Calcyopa fansipana Sato, 2022, *Tinea* 26 (3): 226, figs 1, 2, 23. Type-locality: Mt Fan-si-pan, N. Vietnam.

Type material examined. *Holotype:* VIETNAM • ♂; N. Vietnam, Cha-pa (Sa Pa), Mt Fan-si-pan, N. Seite; 22°17'N, 103°44'E; 1600 m; 21–23 Apr. 1995; leg. Sinjaev & Sammler, ex coll. A. Schintlmeister; Prim Urwald; NIAES.

Paratype: VIETNAM • 1 ♂; same data as holotype; NIAES.

Additional material examined. VIETNAM • 5 ♂♂; same locality and elevation as holotype; 01–05 Mar. 1995; ex coll./ leg. Dr. R. Brechlin; ZFMK • 1 ♂; same locality and collector as for preceding; 1520 m; ZFMK.

Diagnosis. Diagnostic characters are given under the newly described species. The male adult and genitalia are illustrated in Sato (2022), but we depict them here again for comparison with the other two species of *difoveata*-group. The female is still unknown at present.

Distribution. Vietnam.

Genetic data. No data available.

Remarks. Stüning (2000: 135) already mentioned this species as "an undescribed species also from Vietnam", which was later described as *fansipana* by Sato (2022: 226). He wrote in the description: "Third abdominal sternite with setal comb" and "Hind tibia with hair-pencil". These two characters were mentioned by an unexplainable error (Sato 2023, pers. comm.). Both setal comb and hair-pencil are absent in all species of the genus *Calcyopa*, which, of course, was known to Sato.

Calcyopa hainana sp. nov.

https://zoobank.org/8AC4C54D-B7E3-4F34-B811-E5AB81F959CF Figs 8-13, 18, 19, 21

Type material. *Holotype:* CHINA – **Hainan Province** • ♂; Lingshui, Diaoluoshan; 922 m; 16–19 Apr. 2024; Bo Liu & Wei Yan leg.; CRICATAS/ IZCAS.

Paratypes: (12 ♂♂, 21 ♀♀) CHINA – Hainan Province • 1 ♂; Wuzhishan; 1333 m; V. Siniaev leg.; 10 Jan. 2008; ZSM • 2 ♂♂, 2 ♀♀; same locality as holotype; 20 Apr. 2023; Bo Liu leg.; gen. prep. nos. CRICATAS00101, CRICA-TAS00103; CRICATAS/ IZCAS • 4 ♀♀; same locality and collectors as holotype; 05–07 Mar. 2024; CRICATAS/ IZCAS • 1 ♂, 1 ♀; same locality as holotype; 01– 03 Apr. 2024; Bo Liu, Wei Lin & Miaofeng Xu leg.; CRICATAS/ IZCAS • 4 ♂♂, 5 ♀♀; same data as holotype; CRICATAS/ IZCAS • 4 ♂♂, 9 ♀♀; same locality and collectors as holotype; 07–12 May 2024; CRICATAS/ IZCAS/ ZFMK.

Diagnosis. This species closely resembles *C. difoveata* and, though much less, also *C. fansipana*, but can be distinguished by several morphological characters: The postmedial line of the forewing between veins M_1 and M_3 exhibits a more pronounced outward curve than in *C. difoveata*, similar to that in *C. fansipana*. Moreover, the light-brown ground color of the upperside and the absence of broad, dark marginal bands on the underside of the latter are also diagnostic. The free costal arm of the valva is longer and narrower in *C. hainana* than in



Figures 14–19. Male genitalia of *Calcyopa* species. 14, 15 *C. difoveata*, Hainan, China 14 genitalia capsule 15 aedeagus, close-up of cornuti at the bottom left 16, 17 *C. fansipana*, holotype, N. Vietnam, gen. prep. no. RS8731 16 genitalia capsule 17 aedeagus 18, 19 *C. hainana* sp. nov., paratype, Hainan, China, gen. prep. no. CRICATAS00101 18 genitalia capsule 19 aedeagus. Scale bar: 1 mm.

the other two congeners and the saccular process lacks spines. The triangular apex of the aedeagus is more pointed and elongated compared to *C. difoveata* and *C. fansipana*. Additionally, the cornuti in *C. hainana* are composed of a group of long juxtaposed spines, while in *C. difoveata* they consist of shorter spines, and in *C. fansipana* they form a dense cluster of minute spines covering a round diverticulum of the vesica. A row of rather large spines and a big single tooth present apically on the shaft of aedeagus of *C. difoveata* are replaced by a large number of smaller denticles in *C. hainana*.

Description. Forewing length 11.1-11.9 mm in males, 11.7-14.3 mm in females. Ground color brownish-green in fresh specimens, but gradually fading to brown over time, with numerous scattered dark scales and distinct dark antemedial and postmedial lines on both wings. Head. Male antennae fasciculate, with two pairs of shortly conical, sclerotized, ciliate projections latero-ventrally on each segment. Female antennae with cilia much shorter, and sclerotized projections absent. The longest cilia are about 3 times the diameter of the flagellum segments in males and about 0.5 times the diameter of the flagellum segments in females. Vertex covered with lamellar, pale brown scales. Frons smooth-scaled, not protruding. Labial palpi slightly extending beyond frons, basally with long, dark scales, third segment small. Proboscis rather short. Chaetosemata present, near eye margin. Thorax. Patagia and tegulae densely covered with somewhat longer lamellar scales, distal end of tegulae with a few long hair-scales. Wings brownish, scattered with numerous dark scales. Forewings moderately elongate, apex angled, termen smoothly curved, with a double fovea in males. Antemedial line fine, visible. Discal spot small, streak-like. Postmedial line outcurved between veins M₁ and M₃, at CuA₂, and at 1A+2A. Outside postmedial line between M₂ and CuA₁ with a round black patch anteriorly and a square pale patch posteriorly. Subterminal line zigzag, white, with a dark inner edge. Terminal line black, with a series of dark spots between the veins. Hindwings with apex rounded, termen minutely concave between veinends. Basal area densely covered with numerous black scales. Antemedial line broad, outcurved at lower cell vein and inner margin. Discal spot small, streaklike. Postmedial line outcurved between veins M_1 and M_3 , and at inner margin. Subterminal zigzag, white, with a dark edge inside. Terminal line black, with dark spots between the veins. Underside blurry and paler, with a broad, dark band outside postmedial line on both wings. Antemedial line faint on forewing, but clearly visible on hindwing. Postmedial line barely outcurved on both wings. Legs slender, index of spurs 0-2-4, hind tibia not dilated, without scent brush in males. Pregenital abdomen. Tympanal organs, sterno-tympanal process, setal comb, and abdominal coremata as mentioned in the generic description. Abdomen laterally with several pairs of scale brushes on segments 2, 3, 4, 5, 6 and 7.

Male genitalia. Uncus deeply divided, triangular, apical part straight, slightly curved ventrad. Gnathos with broad, flattened lateral arms, central part strong, thorn-shaped, elongate, slightly curved. Valvae acutely triangular. Basal costal arm free, standing obliquely upright towards the upper valva margin, apically dilated, with long bristles. Saccular process stick-like, without spines. Juxta small, basally a rounded plate, extended into a narrow arm distally, slightly cup-shaped and laterally bifurcate at apex. Aedeagus short and stout, apically with a long, acutely triangular, sclerotized process; shaft distally with a group of small serrate projections; vesica with a small row of long-spined cornuti. Bulbus ejaculatorius long, about four times as long as the aedeagus shaft.



Figures 20, 21. Female genitalia of *Calcyopa* species. 20 *C. difoveata*, Hainan, China 21 *C. hainana* sp. nov., paratype, Hainan, China, gen. prep. no. CRICATAS00103. Scale bar: 1 mm.

Female genitalia. Ovipositor short, papillae anales scarcely setose. Anterior apophyses short, about ½ length of posterior apophyses. Ventral longitudinal sclerotization needle-like, basally dilated, slightly triangular. Lamella postvaginalis small, round or oval, bottom concave at center. Introitus bursae small, sclerotized, significantly narrower than the posterior part of bursa, setting into a small sternite pocket. Posterior part of bursa slightly longer than anterior part, posterior one-third sclerotized, scobinate inside. Anterior part of bursa swollen, triangular, noticeably broader than posterior part, consisting of 7 to 8 chains of small serrate projections on both opposed inner sides.

Etymology. The specific name of *"hainana"* is derived from the type locality, Hainan Island, China.

Distribution. China (Hainan).

Genetic data. The Barcode Index Number for *Calcyopa hainana* is BOLD: AAH2362 (*N* = 2, Sample IDs: CRICATAS00101, BC ZSM Lep 16053). The genetic distance of *C. hainana* from *C. difoveata* (*N* = 3, Sample IDs: CRICATAS00104, BC ZSM Lep 16015, BC ZSM Lep 15995) ranges from 5.32% to 5.62% (p-distance).

Key to all known Calcyopa species, based on male and female genitalia

- Uncus rather long and slender, deeply bifid until ¾ of its length, apically strongly bent ventrad; saccular process spined over half its length; aedeagus small, finer, apical sclerotization indistinct; cornuti absent; corpus bursae short, inflated, triangular or squarish; lamella postvaginalis large, rectangular, with a large, round incision medio-anteriorly......4 (rosearia-group)
- 2 Saccular process spined at the tip...... Calcyopa difoveata

Acknowledgements

I would like to express my sincere thanks to Dieter Stüning (ZFMK, Germany) who sent me images and data of the original specimens used by Eugen Wehrli for the description of "*Calichodes*" *difoveata* (including type-material), to Rikio Sato (Niigata, Japan) for providing me with valuable literature and images of the holotype of *Calcyopa fansipana*, and to Axel Hausmann (ZSM, Germany) who sent me the data of the *Calcyopa* specimens. I would also like to thank Wei Yan (CRICATAS, China) and Jiexiong Fu (Diaoluoshan Division of Hainan Tropical Rainforest National Park Bureau, Lingshui, China) for their assistance in specimen collection.

Additional information

Conflict of interest

The author has declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the Science and Technology Special Fund of Hainan Province (ZDYF2022XDNY214) and the Hainan Tropical Rainforest Conservation Research Project, ZDYF2023RDYL01 (supported by the Hainan Institute of National Park, HINP, KY-24ZK02).

Author contributions

Writing - Original draft: BL. Writing - Review and Editing: BL.

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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

Taxonomic revalidation of *Selenobrachys* Schmidt, 1999 and *Chilocosmia* Schmidt & von Wirth, 1992 based on morphological and molecular analyses (Araneae, Theraphosidae), with the description of a new species from Romblon Island, Philippines

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Academic editor: Chris Hamilton Received: 22 May 2024 Accepted: 7 October 2024 Published: 31 March 2025

ZooBank: https://zoobank.org/ E82A9CA6-EC67-4050-A3A9-2A40AFB528FE

Citation: Acuña DC, Dumbrique MMU, Ranido MC, Ragasa LRP, Noriega CNC, Mayor ABR, Florendo Jr GA, Fadri MJA, von Wirth V, Santiago-Bautista MR, Guevarra Jr LA (2025) Taxonomic revalidation of *Selenobrachys* Schmidt, 1999 and *Chilocosmia* Schmidt & von Wirth, 1992 based on morphological and molecular analyses (Araneae, Theraphosidae), with the description of a new species from Romblon Island, Philippines. ZooKeys 1233: 139–193. https://doi.org/10.3897/ zookeys.1233.128056

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Abstract

Selenobrachys Schmidt, 1999 and Chilocosmia Schmidt & von Wirth, 1992 were considered junior synonyms to Orphnaecus Simon, 1892 without further morphological investigation nor the use of molecular methods of analysis. Herein, the type specimens are reexamined with newly collected samples of currently known Orphnaecus species, including new specimens from Romblon Island, Philippines. Morphological and molecular analyses were performed, utilizing cytochrome oxidase I (COI) and ribosomal genes (12S-tRNA-Val-16S). Synapomorphies in the structure of maxillary lyra, spermathecae, and male palpal morphology were observed in O. philippinus and the Romblon specimen which are distinct from other Orphnaecus species. In addition, lyrate morphology, setae structure on the patella of palp dorsal, and the male palpal organ morphology of O. dichromatus differ from other Orphnaecus species. Cladistic separation observed in molecular phylogenetic analyses supports morphological observations. Our findings suggest that the genus Selenobrachys is distinct from Orphnaecus; hence, the genus Selenobrachys Schmidt, 1999, stat. rev. and its type species Selenobrachys philippinus Schmidt, 1999, comb. rest., are restored and the new species from Romblon Island, Selenobrachys ustromsupasius sp. nov., be identified as the second Selenobrachys species. Furthermore, the genus Chilocosmia Schmidt & von Wirth, 1992, stat. rev. and the original combination of its type species, Chilocosmia dichromata Schmidt & von Wirth, 1992, comb. rest. are restored. Male specimens of S. philippinus and C. dichromata were described for the first time. Insights on the biogeography of Philippine tarantulas are discussed.

Key words: Asian tarantulas, biogeography, *Orphnaecus*, Philippine spiders, phylogeny, Selenocosmiina

Introduction

Tarantulas of the family Theraphosidae Thorell, 1869, are large-sized spiders that currently comprise 168 genera and over 1000 species (World Spider Catalog 2024). These spiders currently have colonized every continent on Earth, except Antarctica, and have a diverse array of terrestrial, burrowing, and arboreal species that exhibit unique defense mechanisms, predatory behaviors, reproductive strategies, and ecological adaptations (Pérez-Miles 2020; WSC 2024).

Orphnaecus Simon, 1892 is one of the 11 recognized tarantula genera of the subfamily Selenocosmiinae Simon, 1899 found in Asia and Australia (following the transfer of *Poecilotheria* Simon, 1885 from Selenocosmiinae to the revalidated subfamily Poecilotheriinae Simon, 1892 (Lüddecke et al. 2018; Foley et al. 2019). This genus currently has five accepted species (WSC 2024), which include *O. adamsoni* Salamanes et al., 2022, *O. dichromatus*, *O. kwebaburdeos* (Barrion-Dupo et al., 2015), *O. pellitus* Simon, 1892, and *O. philippinus* (Schmidt, 1999). Aside from *O. dichromatus*, which was discovered in Western New Guinea, Indonesia, the rest of the *Orphnaecus* species are endemic to the Philippines (Schmidt and von Wirth 1992; Nunn et al. 2016).

Simon (1892) distinguished the genus *Orphnaecus* in having smaller and more separated eyes. West et al. (2012) noted the unique structure of the maxillary lyra of *Orphnaecus* by its patch of similar short bacilliform rods in reniform shape and with rows of large clavate bacillae. Nunn et al. (2016) transferred *Phlogiellus kwebaburdeos* to *Orphnaecus* by its lyrate morphology description that obviously fits the genus. Recently, Salamanes et al. (2022) described a new species, *O. adamsoni*, from the Dinagat Islands, Philippines.

Two species of Orphnaecus, namely O. philippinus and O. dichromatus, which are currently known only from female specimens, became part of this genus after Selenobrachys and Chilocosmia were synonymized with Orphnaecus (West et al. 2012). Selenobrachys philippinus was transferred to Orphnaecus despite its structural difference in lyrate morphology (West et al. 2012). This species has an oval with a proximally truncate and distally tapering lyrate patch with rows of strong paddles, which is atypical of the characteristic reniform shape with rows of clavate bacillae generally found in Orphnaecus species (Schmidt 1999; West et al. 2012; Nunn et al. 2016). This characteristic of O. philippinus was considered, particularly the variation in lyrate morphology, as an autapomorphy, disregarding the recognition of Selenobrachys as a separate genus (West et al. 2012). Chilocosmia dichromata was transferred to Selenocosmia Ausserer, 1871 after the synonymy of Chilocosmia to Selenocosmia due to the lack of comparison which can adequately support the recognition of the genus (Raven 2000). Later, Chilocosmia was synonymized with Orphnaecus based on its lyrate morphology, median carapace line, spermathecal shape, male embolus keel, and cheliceral striker morphology (West et al. 2012). West et al. (2012) transferred only the type species, C. dichromata, to Orphnaecus and the other species formerly placed in Chilocosmia were continued to be treated as Selenocosmia (Se. arndsti, Se. barensteinerae, Se. peerboomi, and Se. samarae) (WSC 2024). Schmidt (2015) disagreed with this and suggested that genetic investigations be made to confirm the validity of the revision.

Molecular biology methods such as DNA barcoding and sequence analysis are techniques that have become useful in identifying organisms and resolving issues related to taxonomic identity (Hebert et al. 2003a, 2003b, 2004, 2016; Hebert and Gregory 2005; Antil et al. 2022). DNA barcoding has been a complementary method that is used as a rapid method to identify organisms and resolve taxonomic ambiguities (Tyagi et al. 2019). This technique allows rapid identification of organisms at the species level based on the sequences of genes with a length of between 400 and 800 base pairs (Kress and Erickson 2008). Typically, the mitochondrial markers cytochrome oxidase I (COI) and 16S rRNA are the genes used in DNA barcoding for animals (Amer 2021). The ultimate objective of this technique is to produce and report a DNA sequence that represents an organism in a global database and use this as taxonomic information for comparison with unknown organisms for identification (Hebert et al. 2003a, 2003b, 2004, 2016; Hebert and Gregory 2005; Wilson et al. 2018).

In this study, we restored two genera: *Chilocosmia* stat. rev. with type species *C. dichromata* comb. rest. and *Selenobrachys* stat. rev. with type species *S. philippinus* comb. rest. A new *Selenobrachys* species collected from Romblon Island, Philippines is described. Males of *C. dichromata* comb. rest. and *S. philippinus* comb. rest. are described for the first time. Also, we utilized the concept of Pleistocene Aggregate Island Complexes (PAICs), which identify the seven major biogeographical regions of the Philippines, to discuss our insights on the biogeography of Philippine theraphosid fauna.

Materials and methods

Specimen collection

The newly collected specimens used in this study were collected through opportunistic sampling, mostly after midday to night, from different site localities of tarantula spiders in the Philippines. Specimens of *O. dichromatus* examined were from the Staatliches Museum für Naturkunde Stuttgart collection in Germany. For field collection, permits and consent were acquired from respective local government units and Protected Area Management Bureau (PAMB). Gratuitous permit was secured from the Department of Environment and Natural Resources- Biodiversity Management Bureau (DENR-BMB) prior to sampling. Newly collected specimens were preserved in 80% ethanol.

Species concept

The unified species concept proposed by de Queiroz (2005, 2007) was followed, which utilizes pluralistic evidence and a pragmatic approach. The best available evidence we have for species delimitation used in this study is morphological and genetic criteria. Species delimitation was conducted using morphological differences and then verified using genetic data based on the result of phylogenetic analysis.

The undescribed putative species used in the phylogenetic analysis were morphospecies initially identified based on their distribution and morphological differences, such as in lyra, genitalia, legs, and setation. They are denoted by their island locality and species number (e.g., "L1" = "Luzon Island species 1").

Morphological analysis

The descriptive format generally follows West et al. (2012). Species and genera described in this study are diagnosed and compared using type specimens and newly collected samples. Observations and documentation were made using an Olympus SZ61 stereomicroscope with a Touptek camera attachment or with a 3.0 USB C-mount Touptek microscope camera adapted with a Nikon SMZ 18 stereo microscope. Illumination was a Starlight RL 5 ring light (daylight) and a Starlight IL 11 incident light (pure white). Images were stacked with Helicon Focus 8.2.0 and post-processed with Adobe Photoshop CS2 9.0. Micro measurements were measured using ToupView software version X64, and macro measurements were taken using digital calipers. All measurements are given in millimeters to the nearest 0.01 mm. Measurement of total body length includes chelicerae but does not include spinnerets. Carapace length is measured from the anterior tip to the posterior longest point. Cephalic height is measured from the base of the carapace to the highest point of the cephalic region laterally. The cheliceral strikers are counted and categorized into three rows: the primary rows are the strikers found at the lowest rows which are distinctly longer, secondary rows are in the middle rows which are often shorter than the primary strikers, and the tertiary rows are above the secondary rows which are the very tiny strikers. Pseudostrikers are rows of long and pallid setae found below the cheliceral strikers. The length of leg segments was measured on the lateral aspect up to the longest point and did not include trochanter and coxa. Leg width is measured through the widest point and includes both lateral and dorsal aspects. The length of the tarsus does not include claws and tufts. The width of the tarsus and metatarsus does not include scopulae. The length and width of the coxae and trochanter were measured ventrally. The leg formula is the order of legs based on leg length given in descending order. The orientation of the eye rows is described by connecting the center or midpoint of each eye. Eye diameter is measured through the widest point or the major axis. The length of curve structures (e.g., fangs, claws, etc.) is measured by their arc/curve length. Fovea width and curve length were both measured. Palpal bulb structure followed Bertani (2000) based on the position of the keels. Measurements of tegulum and embolus followed Bertani (2023). Spermathecae are cleaned using lactic acid, and emboli are bleached using hydrogen peroxide (see von Wirth and Hildebrandt 2022, 2023). Variation in measurements, if available, is provided as 'total sample size: minimum-maximum' (n: min-max). Indices herein were created to ease comparative morphometrics between theraphosid species for future referencing.

Indices used herein

- **CI** Carapace Index = Car. width/ Car. length × 100, the resulting value shows the ratio of carapace width to length
- **CLI** Cephalic Region Length Index = Cephalic region length/ Car. length × 100, the resulting value shows the length ratio of the cephalic region within the carapace
- **CHI** Cephalic Region Height Index = Car. height/ Car. length × 100, the resulting value shows the ratio of the cephalic height to carapace length

- **EI** Eye Index: EI(AME) = AME diameter/Car. length × 100; EI(ALE) = ALE diameter/Car. Length × 100; the resulting value shows the diameter ratio of AME/ALE to carapace length
- **DLI** Dorsal Leg Index = Leg dor. width/ Leg length × 100, the resulting value shows the ratio of dorsal width to length of a leg
- **LLI** Lateral Leg Index = Leg lat. width/ Leg length × 100, the resulting value shows the ratio of lateral width to the length of a leg
- RF~ Leg Relation Factor (von Wirth and Striffler 2005) = Leg I length/Leg IV length × 100, the resulting value shows the length ratio of Leg I to Leg IV
- **MI** Metatarsal Index = Met. length / Tib. length × 100, the resulting value shows the length ratio of the metatarsus to the tibia of the same leg
- **TI** Tarsal Index = Tar. length/ Met. length × 100, the resulting value shows the length ratio of tarsus to metatarsus of the same leg
- **EMI** Embolic Index = Embolus length/ tegulum length × 100, the resulting value shows the length ratio of the male embolus to its tegulum
- **POI** Palpal Organ Index = (Embolus + tegulum length)/ Palp tib. length × 100, the resulting value shows the length ratio of the male palpal organ to the palpal tibia.

Setation followed Guadanucci et al. (2020) with additional terminologies. Terminologies on setation used in this study herein are designated as:

- **TS** Tactile setae: hard mechanosensory setae for touch perception, which are the most common body sensilla of spiders (Guadanucci et al. 2020) and are diverse in form
- **SC** Cuticular scales: flattened lanceolate or acicular (some are wavy and cotton-like) light-reflective covering setae or setal mat that have weak pedicel, almost parallel to the cuticle (Townsend and Felgenhauer 1998; Guadanucci et al. 2020)
- **ETB** Epitrichobothria: tactile-like setae but very short, intermix with trichobothria on clusters (Guadanucci et al. 2020)
- **TB** Trichobothria: clavate or filiform, ground or air movement sensitive sensilla (Guadanucci et al. 2020)
- **CHS** Chemosensory sensilla: tiny translucent erect sensilla tapering distally (Guadanucci et al. 2020), usually found singly on the legs and abdomen
- **FS** Femoral Setation: a field of modified tactile setae (TS) on the prolateral surface of femora I which varies in form (e.g., sword-like, acicular, filiform, etc.), that can be diagnostic in identifying selenocosmiine genera
- **PB** Palpal Brush (first mentioned in West et al. 2012): a layer of modified elongated flat scales (SC) that is present on the male palpal patella and tibia dorsally; long and dense in *Orphnaecus*. They are classified as scales herein due to their weak pedicel and scale properties.

Abbreviations and acronyms

car. carapace; dor. dorsal; lat. lateral; fem. femur; met. metatarsus; OT ocular tubercle; tib. tibia; troch. trochanter; PS prolateral superior keel; PI prolateral inferior keel; A apical keel; BL basal lobe; Op embolic opening; StR subtegular ridge; AME anterior median eye; ALE anterior lateral eye; PME posterior median eye; PLE posterior lateral eye; PMS posterior median spinneret; PLS posterior lateral spinneret; MNHN Muséum National d'Histoire Naturelle, Paris; PASI Philippine Arachnological Society, Inc. Reference Collection, Manila; PNM Philippine National Museum, Manila; SMF Senckenberg Museum, Frankfurt am Main; SMNS Staatliches Museum für Naturkunde, Stuttgart; UPLB-MNH University of the Philippines Los Baños - Museum of Natural History, Laguna; UST-ARC University of Santo Tomas - Arachnid Reference Collection, Manila; ZMB Museum für Naturkunde, Berlin; PAIC Pleistocene Aggregated Island Complex.

DNA Isolation, amplification, and analysis

DNA samples were isolated from tissue collected from the right leg III of the tarantulas. DNA extraction was performed using the QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's protocol. For amplification of the Cytochrome oxidase I (COI) gene, degenerate primer pairs LCO1490_PH (forward)-HCO2198_PH (reverse) and C1-J- 2123-PH (forward)-C1-N-2776-PH (reverse) were used after modifications from the reference primers (see Table 1). The PCR mix consisted of a final concentration of 0.4uM each of forward and reverse primer, < 10 ng/µL DNA, 12.5ul 2X GoTaq G2 Master Mix, and water for a final volume of 25 µL. PCR conditions included an initial denaturation at 94 °C for 3 min, followed by five cycles consisting of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, extension at 72 °C for 1 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min.

Amplification of the ribosomal genes, a continuous stretch of partial 12S, complete tRNA- Val, and partial 16S (12S-tRNA-Val-16S), was performed using our newly designed primer pair 12SUST-PHTarantula (forward)-16SUST-PHTarantula (reverse) (Table 1). PCR mix content is the same as with the COI primers above. PCR was performed with initial denaturation at 94 °C for 1 min, followed by 25 cycles consisting of denaturation at 94 °C for 1 min, annealing at 68 °C for 30 s, and extension at 72 °C for 1 min. This was followed by five cycles of denaturation at 94 °C for 30 s, and extension at 72 °C for 1 min. This was followed by five cycles of cor 1 min. The final extension step was performed at 72 °C for 10 min.

After PCR amplification, the products were separated and visualized on a 1.2% agarose gel with a 1kb DNA ladder. The resulting amplicons were sent for sequencing by Macrogen (Seoul, Republic of Korea). Sequence data was

Primers	Primer sequence	Source				
COI						
LCO1490_PH (forward)	5'-TTTCWACTAATCATARGGATATTGG-3'	modified from LCO1490aphonopelma (Hamilton et al. 2011)				
HCO2198_PH (reverse)	5'-TAAACCTCCGGATGWCCAAAAAAYCA-3'	modified from dgHCO-2198 (Meyer 2003)				
C1-J-2123_PH (forward)	5'- GATCGAAATTTTAATACTTCKTTYTTTGA-3'	modified from C1-J-2123 (Vidergar et al. 2014)				
C1-N-2776_PH (reverse)	5'-GGATAATCAGAATATCGTCGAGGTATTCCAT-3'	modified from C1-N-2776 (Hedin and Maddison 2001)				
12S-tRNA-Val-16S						
12SUST_PHTarantula (forward)	5'-CGTCACCCTCGTCCAAAGAT-3'	this study				
16SUST_PHTarantula (reverse)	5'-CGATAGGGTCTTGTCGTCCC-3'	this study				

Table 1. List of primers used to amplify and sequence the DNA barcoding regions COI and rRNA genes.
processed using PREGAP4 and GAP4 (Staden Package, http://staden.sourceforge.net/; Bonfield et al. 1995). Raw sequences were trimmed based on base quality. Forward and reverse reads were aligned and manually checked for ambiguous sites. A consensus sequence was then generated for each individual. Sequences from all COI primer pairs were combined to generate the COI consensus sequence for each individual for an extended length. Sequences were aligned with Theraphosidae sequences from databases using the MUSCLE algorithm. The hypervariable regions in rRNA gene alignment were removed using GBLOCKS v. 0.91b online tool (Castresana 2000) under all of the less stringent set of conditions. The rRNA genes were not separately analyzed due to the short lengths of 12S and tRNA-Val genes after cleaning.

Maximum-likelihood trees with 10,000 bootstrap replicates were generated in MEGA11 for both markers using the General Time Reversible model with gamma-distributed rates among sites with invariant sites (GTR+G+I), chosen in MEGA11 as best models for both markers based on the lowest Bayesian Information Criterion (BIC) score (Tamura et al. 2021). All sequences were used in generating the group (genus rank) mean and pairwise genetic distances using the Maximum Composite Likelihood model (Tamura et al. 2004) in MEGA11. Percent similarity is computed by getting the difference of 100% and the percent distance (similarity% = 100% - distance%). The two markers are not concatenated due to the unsuccessful ribosomal gene sequencing for many of the samples and many of the outgroups do not have corresponding COI or ribosomal gene sequences, hence analyzed separately. DNA sequences of outgroups were acquired from GenBank. The new sequences in this study are deposited to GenBank (Suppl. material 1).

Accession numbers of the sequences obtained from GenBank: **COI**-• JN018124 = Aphonopelma seemanni MNHN-JAC96 • JN018125 = Chilobrachys huahini MNHN-JAC48 • JF884459 = Chilobrachys sp. 2GAB_PAK (iBOL) • KJ744742 = Selenotholus sp. MYG381-T113579 • KT022078 = Grammostola sp. JEA-2015 • KT995328 = Brachypelma verdezi IBUNAM:CNNA Ar003417 • MF804598 = Chilobrachys sp. USNM ENT 01117339 • MK234708 = Grammostola rosea NCA 2017/394 ; **12S-tRNA-Val-16S-** • MG273466 = Brachypelma sp. ZSM-A 2017/0058 • MG273467 = Chilobrachys fimbriatus ZSM-A 2017/0059 • MG273468 = Cyriopagopus lividus ZSM-A 2017/0060 • MG273470 = Lampropelma nigerrimum ZSM-A 2017/0063 • MG273471 = Orphnaeus [Orphnaecus] sp. ZSM-A 2017/0064 • MG273476 = Lyrognathus giannisposatoi ZSM-A 2017/0070 • MG273477 = Phlogiellus sp. [?] ZSM-A 2017/0071 • MG273480 = Selenocosmia javanensis ZSM-A 2017/0074 • MG273484 = Poecilotheria formosa PONAL1 • MG273485 = Grammostola pulchripes GRGOL1.

Materials of subject species described in this study are placed on their respective taxonomic treatments (see Results).

Comparative materials examined

Type materials

 Orphnaecus adamsoni Salamanes et. al, 2022: PHILIPPINES: Dinagat Island— Dinagat Islands Prov. • holotype ♂, PNM 14889; Loreto, Mt. Mangkuno; Oct 2018, J Santos, GG Villancio leg., forest grounds • allotype ♀, PNM 14888 [Ornithoctoninae sp.*]; Basilisa-Cagdianao, Mt. Arayat; Oct 2018, J Santos, GG Villancio leg.; PNM

*Remarks: The allotype \bigcirc (PNM 14888) described in Salamanes et al. (2022) and herein examined was misidentified and misplaced in *Orphnaecus*. It belongs to Ornithoctoninae due to the presence of plumose setal field on retrolateral chelicerae and has a stridulatory organ with rows of thorn setae on the prolateral maxilla, which characteristics are absent in Selenocosmiinae but synapomorphy to Ornithoctoninae. A taxon cannot be assigned for this specimen due to its poor condition.

- Orphnaecus kwebaburdeos (Barrion-Dupo et al., 2015): PHILIPPINES: Polillo Island—**Quezon Prov.** • paratypes, 3 ♂♂, BPB 2112012-4, BPB 2112012-12, BPB 2112012-13 and 1 ♀, BPB 2112012-2; Burdeos, [Brgy. Aluyon], Puting Bato Cave 3-4; 02 Nov 2012, J. Rasalan leg.; UPLB-MNH
- Orphnaecus pellitus Simon, 1892: PHILIPPINES: Luzon Island- Camarines Sur Prov. • syntypes ♂♀, AR4678; Libmanan, Calapnitan Caves [Culapnitan Caves] (now Libmanan Caves National Park); MNHN

Other materials

- Orphnaecus kwebaburdeos (Barrion-Dupo et al., 2015): PHILIPPINES: Polillo Island- Quezon Prov. • 4 ♂♂ and 14 ♀♀, 7 j, UST- ARC 0059-0083; Burdeos, Brgy. Aluyon, inside Puting Bato Cave 2 and 3; 150 m horiz. depth, 13-14 May 2023, DC Acuña, JD Fornillos leg.; UST-ARC
- Orphnaecus pellitus Simon, 1892: PHILIPPINES: Luzon Island Camarines Sur Prov. 2 ♂♂, 3 ♀♀, 12 j, UST-ARC 0031–0047; Libmanan, Brgy. Sigamot, Libmanan Caves National Park (Culapnitan Caves), inside Kalangkawan Cave; 50–300 m horiz. depth, 20 Apr 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos leg. 4 j, UST-ARC 0048–0051; [same general locality data as above], inside Alinsanay Cave; 50 m horiz. depth, [same collection data as above] 2 ♂♂, 4 ♀♀, 1 j, UST-ARC 0052–0058; [same general locality data as above], inside Laya Cave; 30–50 m horiz. depth, 20 Jul 2023, LA Guevarra, DC Acuña, JD Fornillos leg.; UST-ARC
- Orphnaecus sp. 'C1': PHILIPPINES: Catanduanes Island− Catanduanes Prov.
 4 ♀♀, 1 j, PNM 18829−18833; Aug 1990, P Gonzales et al. leg.; PNM
- Orphnaecus sp. 'L1': PHILIPPINES: Luzon Island Laguna-Quezon Prov. border
 1 ♂, 7 ♀♀, 1 j, UST-ARC 0105–0111; UP Sierra Madre Land Grant; 450 m a.s.l., burrows under rocks and logs, 19 Aug 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos, EP Maglangit leg. Laguna Prov. 1 j, UST-ARC 0084; Siniloan, Brgy. Magsaysay, Tulay na Bato Falls trail; 330 m a.s.l., burrows under logs, 04 Feb 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos leg.; UST-ARC
- Orphnaecus sp. 'L2': PHILIPPINES: Luzon Island Laguna Prov. 5 ♀♀, 9 j, UST-ARC 0088–0101; Siniloan, Brgy. Halayhayin; 15–405 m a.s.l., 05 Feb 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos leg. 3 j, UST-ARC 0085–0087; Brgy. Magsaysay-Galalan, Southern Sierra Madre; 470 m a.s.l., under logs, 04 Feb 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos leg. Quezon Prov. 3 ♀♀, UST-ARC 0102–0104; Real, Brgy. Llavac; 300 m a.s.l., 14 May 2023; DC Acuña, CN Noriega, JD Fornillos leg.; UST-ARC

- Orphnaecus sp. 'L3': PHILIPPINES: Luzon Island- Camarines Sur Prov. · ♂♀ UST-ARC 0132-0133; Libmanan, Brgy. Sigamot, Libmanan Caves National Park; burrows under and inside logs on forest slope near Kalangkawan Cave, 20 July 2023, LA Guevarra, DC Acuña, JD Fornillos leg. ♂♀ UST-ARC 0130-0131; [same locality and natural history data as above]; 20 Apr 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos leg. ♀ UST-ARC 0134; Libmanan, Brgy. Malinao; burrow under limestone rock, 20 Apr 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos leg. ♀ UST-ARC 0134; Libmanan, Brgy. Malinao; burrow under limestone rock, 20 Apr 2023, LA Guevarra, DC Acuña, CN Noriega, JD Fornillos leg. ౩ ♂♂, 4 ♀♀, UST-ARC 0135-0139; [same locality data as the latter]; 20 Jul 2023, burrows under piles of coconut husks, LA Guevarra, DC Acuña, JD Fornillos leg.; UST-ARC
- Orphnaecus sp. 'L4': PHILIPPINES: Luzon Island Camarines Sur Prov. 4 ♀♀, UST-ARC 0141-0144; Libmanan, Brgy. Sigamot, Libmanan Caves National Park; burrows under logs on forest slope near Laya Cave, 20 July 2023, LA Guevarra, DC Acuña, JD Fornillos leg.; UST-ARC
- Orphnaecus sp. 'L5': PHILIPPINES: Luzon Island— Nueva Ecija Prov. ♀, UST-ARC 0145; Bongabon; 2019, G Bathan leg. —Pangasinan Prov. • 3 ♂♂, 3 ♀♀, PASI ara0014–ara0019; Calasiao, Brgy. Nalsian, Sitio Centro; neglected residential lot and mango orchard, burrows under leaf litter, 08 Nov 2022, DOR Mapile leg.; UST-ARC/ PASI
- Orphnaecus sp. 'L6': PHILIPPINES: Luzon Island Pangasinan Prov. ♀ PASI-0020; Sison, Brgy. Poblacion Sur; residential lot, burrows under rock, 08 Apr 2023, DC Acuña leg.; PASI
- Orphnaecus sp. 'M1': PHILIPPINES: Mindanao Island Agusan del Sur Prov.
 ♀, UST-ARC 0146; Prosperidad, Brgy. Poblacion, Puting Buhangin Cavelevel 3; 2019, GD Petros leg.; UST-ARC
- Orphnaecus sp.: PHILIPPINES: Luzon Island−**Metro Manila** ♀, ZMB 32341; Manila; Scheteley leg.; ZMB

Results

DNA sequence and phylogenetic analysis

A total of 56 individuals were sampled which resulted in 45 new COI sequences and 28 new 12S-tRNA-Val-16S sequences. Additional 8 COI sequences and 10 12S-tRNA-Val-16S sequences were obtained from GenBank (Lüddecke et al. 2018). After alignment, a total of 53 sequences with 467 base pairs were used for COI, and 38 sequences with 605 base pairs were used for 12S-tRNA-Val-16S. The sequences under investigation include representatives from different genera, including *Orphnaecus*, *Selenobrachys* stat. rev., *Chilocosmia* stat. rev., and outgroups (Suppl. material 1). The difference between *Orphnaecus*, *Selenobrachys* stat. rev., and *Chilocosmia* stat. rev. is evident in genetic similarity matrices as well as their distinct placements in the phylogenetic tree (Fig. 1).

Intergeneric genetic relationship

The phylogenetic tree topologies of both COI and ribosomal genes (12S-tRNA-Val-16S) depict the segregation of *Orphnaecus*, *Selenobrachys*, *Chilocosmia* into different clades (Fig. 1A, C). In the rRNA phylogenetic tree, *Orphnaecus*, *Selenobrachys*, Selenocosmiini genera (*Lyrognathus* and *Selenocosmia*), and



Figure 1. Maximum-Likelihood phylogenetic tree of **A** COI gene **C** rRNA genes (12S-tRNA-Val-16S). Group mean percent similarity heatmap of **B** COI gene **D** rRNA genes (12S-tRNA-Val-16S), using the Maximum Composite Likelihood model. Abbreviations: *O. = Orphnaecus, S. = Selenobrachys, C. = Chilocosmia, Ch. = Chilobrachys, P. = Phlogiellus, Se. = Selenocosmia, Sel. = Selenotholus.* Note: dashes (-) in B and D denote values that are not computed because they only contain one gene sequence.

Ornithoctoninae are among the clades that have moderate to good support, with bs = 76-99 (Fig. 1C). However, it is noticeable that all clade separations in the COI phylogenetic tree (Fig. 1A) have very low support (except Theraphosinae outgroups) despite having a good alignment during the analysis. This gene

probably cannot resolve cladistic distinctions for these taxa or might need more gene samples to have better support to clades. The separation of Orphnaecus and Selenobrachys in the rRNA phylogenetic tree has moderate support with bs = 76. On the other hand, this separation is well supported by the genetic similarity matrices, indicating substantial genetic divergence between Orphnaecus and Selenobrachys clades, with an average of 88% similarity in COI and an average of 85% similarity in rRNA genes (Fig. 1B, D). The percent similarity of the two genera using both markers shows their close affinity suggesting that they have common ancestry and that Selenobrachys might have recently diverged into a distinct clade. Percent pairwise distance among individual samples between Orphnaecus and Selenobrachys ranges from 10.20%-15.50% in COI and from 12.05%-18.33% in rRNA genes (Suppl. material 2). Chilocosmia is more distant at a lower average similarity value of 86% to both Orphnaecus and Selenobrachys, as well as to Selenotholus, using the COI marker (rRNA gene sequences for Chilocosmia are not available) (Fig. 1B, D). Pairwise percent distances among individual samples of Chilocosmia from Orphnaecus individuals using the COI gene range from 10.59%-16.76%, and from Selenobrachys samples range from 11.81%-18.11% (Suppl. material 2).

Interspecific genetic relationship

The percent similarity values between putative 9 species (only 7 putative species were successfully sequenced for rRNA genes) within Orphnaecus range from 89.14%-95.10% (at 4.9%-10.86% distance) in COI sequences and 86.46%-95.02% (at 4.98%-13.54% distance) in 12S-tRNA-Val-16S sequences using Maximum Composite Likelihood model (Tamura et al. 2004) (Suppl. material 2). Some species within Orphnaecus have relatively high pairwise distance values for COI. For instance, between O. pellitus (the type species) and the clade of the putative species Orphnaecus spp. 'L1', 'L2', 'L5', and 'L6' have 9.63%-10.86% pairwise distance (Suppl. material 2). Some species of this group have a closer genetic distance from other Orphnaecus species which does not support separation from Orphnaecus (Suppl. material 2). On the other hand, O. kwebaburdeos and Orphnaecus sp. 'L3' recorded the closest genetic affinity having a 4.90%-5.09% distance in COI, but heterospecificity is well supported by their distinct genital structures. In the rRNA genes, the furthest distance recorded with 12.03%-13.54% distance is between O. pellitus and Orphnaecus spp. 'L2', and 'L3' ('L6' has no successful sequence), except for Orphnaecus sp. 'L1' which has 8.47%-9.46% distance to O. pellitus. The closest distance is also between O. kwebaburdeos and Orphnaecus sp. 'L3' with 4.98% distance in all rRNA gene sequences.

The close relationship between the two *Selenobrachys* species, *S. philippinus* comb. rest. and *S. ustromsupasius* sp. nov., is evident in both the COI and rRNA genes (12S-tRNA- Val-16S) genetic matrices (Suppl. material 2). In the phylogenetic tree, their relationship has enough support in COI (bs = 95) and rRNA genes (bs = 93) (Fig. 1A, C). In the COI matrix, these two species exhibit percent similarity values of 90.57%–91.74% (8.43%–9.26% distance), while in the rRNA genes matrix, they show values of 90.18%–90.84% (9.16%–9.82% distance) (Suppl. material 2). These higher similarity values compared to other sequences suggest a relatively reduced genetic divergence between them.

The phylogenetic tree placement further corroborates this observation. The COI sequences of *S. philippinus* comb. rest. and *S. ustromsupasius* sp. nov. cluster together within a distinct clade, suggesting a shared evolutionary history.

Taxonomy

Family Theraphosidae Thorell, 1869 Subfamily Selenocosmiinae Simon, 1889

Tribe Selenocosmiini Simon 1889

Phlogiini Simon, 1892 (synonymized by Simon 1903).

Included genera. *Chilocosmia* Schmidt & von Wirth, 1992 stat. rev. (provisionally placed), *Coremiocnemis* Simon, 1892, *Lyrognathus* Pocock, 1895, *Psednocnemis* West, Nunn & Hogg, 2012, *Selenocosmia* Ausserer, 1871.

Remarks. The genus *Chilocosmia* does not fit to any of the three currently recognized selenocosmiine tribes (Chilobrachini, Selenocosmiini, and Yamiini) and probably belongs to a tribe currently in synonymy with Selenocosmiini (probably Phlogiini Simon, 1892); hence, we provisionally placed it in Selenocosmiini until further investigation is conducted on the proper systematic placement of most of Papuan and Australian taxa.

Genus Chilocosmia Schmidt & von Wirth, 1992, stat. rev.

Type and included species. *C. dichromata* Schmidt & von Wirth, 1992, comb. rest., by original designation and monotypy.

Diagnosis. *Chilocosmia* stat. rev. differs from all known selenocosmiine genera (i) in having a palpal organ with twisted tegulum (Figs 4A–C, 5A–D) and (ii) in having a stridulatory organ on the prolateral maxilla with short bacilliform rods that form an arcuate strip of a lyrate patch and with club-shaped bacillae at lowest row (Fig. 3D, E). The lyra of *Chilocosmia* stat. rev. (Fig. 3D, E) quite resembles the lyra of *Orphnaecus* (Fig. 20G) but differs in the shape of the largest stridulating setae which lacks a pointed apex (Fig. 20C, F). It further differs from *Orphnaecus* in the male palpal organ morphology in having a twisted tegulum and a thicker embolus with shorter PS and lacking a basal lobe (Fig. 5A–E), and in lacking palpal brush on dorsal palp in males (Fig. 4D).

Distribution. Indonesia: West New Guinea.

Etymology. A combination of two Greek words *chilos* (*cheilos*; χείλος), which means lip, and *kosmein* (κοσμείν), which means arrange or keep in order (Schmidt and von Wirth 1992). Gender is feminine.

Chilocosmia dichromata Schmidt & von Wirth, 1992, comb. rest. Figs 2–6, 20C, F, 21I, J

Type material examined. INDONESIA− **West New Guinea** • holotype ♀, SMF 37099-84; Sorong; SMF.

Other material examined. INDONESIA – **West New Guinea** • 2 ♂♂ SMNS Aran-004182 and Aran-004183, 5 ♀♀, SMNS Aran-004184, Aran-004185 and Aran-004162–004164, 3 j, SMNS Aran-004186; Sorong; 00°47'36.4"S, 131°26'32.8"E, F. Schneider leg., 2014; SMNS.

Diagnosis. See genus diagnosis.

Description. Male (SMNS Aran-004182). Body length 38.50 (n = 2 38.50-38.62). *Carapace* (Fig. 2B). 17.2 long, 14.9 wide, anterior width 8,95, cephalic height 4.9, cephalic region 11.3 long, thoracic region 4.79 long. Cl 86.62, CLI 65.69, CHI 28.48. Fovea width 1.85, curve length 2.11, procurved (Fig. 2E). Carapace has four pairs of furrows, mainly covered with yellowish brown setae directed towards fovea, lateral profile low and flat, and integument dark brown. Setation: TS(a), rows of very short pale yellowish brown setae covering entire carapace, directed towards fovea and OT anteriorly. Posterior surface of OT with few long pale brown TS. TS(b), long pale yellowish brown setae at carapace margin, anterior margin pale brown. SC(a), brown flat scales sparsely covering OT. SC(b), sparse, pale yellowish brown, acicular scales covering carapace, anteriorly.

Eyes (Fig. 2D). Ocular Tubercle 3.12 long, 2.28 wide, OT integument entirely dark. AME round, rest of the eyes ovoid. Anterior row of eyes slightly procurved, posterior row of eyes recurved. Eyes: AME 0.69, ALE 0.79, PME 0.46, PLE 0.61. Interocular distances: AME-AME 0.38, ALE-ALE 1.94, PME-PME 1.48, PLE-PLE 2.21, AME-ALE 0.13, AME-PME 0.22, AME-PLE 0.56, ALE-PLE 0.23, ALE-PME 0.47, PME-PLE 0.14. **EI** (AME) 4.01, **EI** (ALE) 4.59.

Chelicerae (Fig. 3A–C). length 9.72, dorsal width 3.27, lateral width 5.99, fang curve length 7.29. Teeth 12, mesoventral denticles sparse 45. Setation: **TS**, long pale brown setae on dorsal and upper 1/2 retrolateral surface, longer anteriorly. Lower 1/2 of retrolateral surface posteriorly with rows of pallid filiform setae and anteriorly with patch of pallid needleform setae. Mesoretrolateral surface with rows of very sparse setae basally spiniform and anteriorly needleform. Mesoprolateral surface with intercheliceral setae, arcuate strip of rows of pallid needleform setae originating basally. Lower 1/2 prolaterally sparsely covered with setae, brown needleform at lower rows, and pale brown filiform at upper rows. **SC**, flat, translucent, pale brown scales, covering dorsal and upper retrolateral surface. Integument mostly dark brown. Cheliceral strikers (Fig. 3C): 111, 0.20–1.03, dark, long spiniform with filiform ends.

Maxillae (Fig. 3D, F). Prolateral maxilla 6.33 long, 4.02 wide laterally, 3.58 wide ventrally. Maxilla prolaterally planoconvex, anterior lobe well pronounced, integument orangey brown, dark dorsally, basoventral cuspules 212. Lyra (Fig. 3D): lyrate patch, 2.24 long, 1.38 high, total rods 190, 10 or 11 rows, surrounded by very fine setae, denser above and distally. Short rods form an arcuate strip of patch. Longest club-shaped rods in lowest row 13, 0.27–0.78. Setation: **TS**, pale brown setae, longer ventrally, stronger at distodorsal margin. Lyrate patch surrounded by fine setae. Above maxillary suture, rows of ~ 13 stiff dark TS. **SC**, brownish white flat scales covering dorsally. Retrolateral surface smooth, with rows of short semi-transparent bristles at the lower margin.

Labium and sternum (Fig. 2C, F). Labium: 2.30 long, 3.12 wide. Integument orangey brown, dark at posterior margin, anterior 1/3 with cuspules 371. Setation: **TS**(a), long, pale brown with pale filiform ends, covering labium anterior-ly and laterally except on cuspule cluster, longer and greater at anterior edge, all pointing anteriorly. **TS**(b), brown, short needleform below cuspule cluster.



Figure 2. Chilocosmia dichromata comb. rest. ♂, SMNS Aran-004182 A habitus, in situ B prosoma, dorsal view C ventral view D ocular tubercle, dorsal view E fovea, dorsal view F labium, ventral view. Photo credit: Frank Schneider (Fig. 2A).

Sternum: 8.2 long, 6.93 wide integument yellowish brown. Posterior sternal corner acuminate, lateral corners weakly acuminate. Setation: **TS**(a), long pale brown spiniform setae, pale apically, on entire sternum, but very sparse. **TS**(b), short pale brown setae covering sternum entirely. **TS** (c) fine, pallid, and short spiniform, at sternal margin. **SC**, brownish white flat scale mat densely covering entire sternum. Labiosternal sigilla 1.07 long, 0.34 wide, 0.83 apart. Sternal sigilla 2 pairs, median sigilla 0.69 long, 0.35 wide, 3.91 apart and 0.58 away from sternal margin adjacent to coxa II, posterior sigilla 1.08 long, 0.42 wide, 1.59 apart and 1.77 away from sternal margin adjacent to coxa III (Fig. 2C).



Figure 3. Chilocosmia dichromata comb. rest. ♂, SMNS Aran-004182 A left chelicera, retrolateral view B prolateral view C cheliceral strikers, retrolateral view D maxillary lyra on left prolateral maxilla E left maxilla, prolateral view.

Abdomen and spinnerets. Abdomen: 17.56 long, 10.09 wide. ovular elongated, integument pale brown. Setation: **TS**(a), long, pale brown with darker bases, needleform, on entire abdomen, shorter ventrally, pallid on book lungs. **TS**(b), pallid short paddle-like, on book lungs and sparse on epigynal plate. **SC**, overlapping pale brown translucent scales covering entire abdomen. Spinnerets: PMS 2.39 long, 0.84 wide, PLS, anterior 2.30 long, 0.71 wide, median 1.82 long,



Figure 4. *Chilocosmia dichromata* comb. rest. ♂, SMNS Aran-004182 **A** left pedipalp, prolateral view **B** ventral view **C** retrolateral view **D** left palpal patella and tibia, prolateral view.

0.99 wide, posterior 3.65 long, 1.39 wide. Setation: TS(a), long, pale brown with darker bases, needleform, on dorsal PMS and PLS. TS(b), dark and short, pale brown paddle-like, intermixed with spigots, on PMS and PLS ventrally. **SC**, brownish white flat scales covering PLS dorsally.

Genitalia (Figs 4A–C, 5A–E). Palpal Organ: almost 3/4 of palp tibia length (**POI** 73.70). Tegulum 2.67 long, 2.14 wide, twisted, widest medially, subtegular projection very weakly pronounced (Fig. 5A–D). Embolus length 3.30, width 0.54 basally, tip 0.10 wide (**EMI** 123.56), base robust, tapering distally, curved retrolaterally and ending to a weakly tip. There is a prolateral inferior keel (PI)



Figure 5. *Chilocosmia dichromata* comb. rest. ♂, SMNS Aran-004182 **A** left palpal organ, prolateral view **B** retrolateral view **C** ventral view **D** apical view **E** tip of embolus, prolateral view **F**, **G** claws on left tarsus IV of two ♂♂ of *C. dichromata* **F** SMNS Aran-004182 **G** SMNS Aran-004183, retrolateral view. Abbreviations: PS- prolateral superior keel, PI- prolateral inferior keel, A- apical keel, Op- embolic opening.

and prolateral superior keel (PS) in the apical third of the embolus (Fig. 5E). The apical keel (A) is located at the tip of the embolus and is semicircular in shape (Fig. 5E). Embolic opening (Op) located between PS and PI near the tip. Basal lobe is not present.

Legs. Leg formula: IV, I, II, III. **RF** ~ 91.77, **LLI** (I) 26.54, **LLI** (IV) 21.15, **DLI** (I) 23.18, **DLI** (IV) 18.49, **MI** (I) 76.25, **MI** (IV) 132, **TI** (I) 69.67, **TI** (IV) 45.45. Leg lengths (fem, pat, tib, met, tar/cym): Palp 26 (10, 4.2, 8.1, -, 3.7) Leg I 61.3 (16.5, 8.1, 16, 12.2, 8.5) Leg II 55.6 (15, 7.1, 13, 12.5, 8) Leg III 49.5 (13, 6, 9, 14.5, 7) Leg IV 66.8 (17, 6, 15, 19.8, 9). Leg lateral width (fem, pat, tib, met, tar/cym): Palp (3.24, 2.92, 3.21, -, 2.46) Leg I (4.15, 3.51, 3.62, 2.82, 2.17) Leg II (4.33,

3.36, 3.16, 2.37, 1.94) Leg III (4.88, 3.45, 3.04, 2.45, 1.91) Leg IV (3.99, 3.47, 3, 1.96, 1.71) Leg dorsal width (fem, pat, tib, met, tar/cym): Palp (3.19, 2.93, 3.21, -, 2.88) Leg I (3.38, 3.30, 2.84, 2.18, 2.51) Leg II (3.40, 3.13, 2.59, 2.05, 2.07) Leg III (4.22, 3.17, 2.42, 1.90, 2.13) Leg IV (3,40, 3,06, 2.54, 1.89, 1.46). Cymbium bipartite. Tarsi IV transversely cracked apically.

Leg setation and spines. Setation (femur to tarsus): **TS** on all legs pale brown and short, longer on all metatarsi dorsally, femur of rear legs ventrally and on all tibia ventrally, dense on tibiae I and II, ventrally. There are only pale brown TS. **PB** is also not present on the palps. There are only pale brown TS. **SC**, brownish. Other sensory setae: **ETB**, pair of thin inverted L-shaped cluster of short dark brown setae, basoretrolateral on Met. I and II, single cluster on Met III. Cymbium with single cluster dorsally that broadens basally. **TB**(a), long and short filiform TB intermix with ETB in two rows, longest dorsally. **TB**(b), rows of unordered clavate TB, varying in size, present in all tarsi, and intermix with tarsal ETB. **CHS**, tiny, pale brown translucent erect sensilla tapering apically, present on the palpal and all leg femora to tarsi and intermixes with tarsal and metatarsal scopulae. Spines (dorsal-dorsoprolateral-dorsoretrolateral-ventral): Met II (0-0-0-2). Met III (0-1-1-2). Met IV (0-0-1-4).

Coxae and trochantera. Coxae: Length (coxa I, II, III, IV), 8.68, 6.05, 6.76, 6.83. Width (coxa I, II, III, IV), 4.46, 3.68, 4.17, 4.59. Setation: **TS**, long brown setae, dorsally and ventrally; strong and short spiniform setae, prolaterally and retrolaterally on all coxae. Trochantera: Length (troch. palp I, II, III, IV), 3.20, 3.21, 2.92, 3.38, 4.03. Width (troch. Palp I, II, III, IV), 2.77, 4.15, 3.88, 3.55, 3.72.

Scopulae and claws. Scopulae: cymbium scopulated ventrally. Tar. I, entire, with very few longer setae. Tar. II, entire, with very few longer setae. Tar. II, entire, with very few longer setae. Tar. IV, in the basal 1/4 divided by three or four long setae. The tarsus is cracked in the apical 1/4. Met. I covered almost all ventral surfaces, entire, but with a few very sparse long setae. Met. II, almost all ventral surface covered, entire, but with a few very sparse long setae. Met. III, almost all ventral surface covered, entire, but with a few very sparse long setae. Met. III, almost all ventral surface covered, entire, but with a few very sparse long setae. Met. III, almost all ventral surface covered, entire, but with a few very sparse long setae. Met. IV, covering 90% of ventral surface, divided by two or three rows of strong long setae. Claws: longest tarsal IV claw 1.70, no inferior third claw (Fig. 5G) (but present on some specimens; Fig. 5F), but there are a few large teeth on the claws whose number can vary from one claw to another.

Color in life. The opisthosoma and legs, except for the coxa and trochanter, are black in color (Fig. 2A). Coxa and trochanter as well as the carapace and the chelicerae basal segment are bronze-colored (Fig. 2A, C). The underside of the cephalothorax is also black. The area around the eye tubercle, as well as two diverging hairless stripes on the carapace, which frame the head, are dark brown in color. On each chelicerae basal segment there is also an elongated dark brown stripe in the middle of the bronze coloration.

Variation. The third inferior claw is present or almost absent on some specimens and varies in length if present, which is also observed in other species in this study.

Etymology. Greek *dýo chrómata* (δύο χρώματα) means two colors, which refers to the bicolored orange and black coloration of this species (Schmidt and von Wirth 1992).

Natural history and distribution. The spiders live in tubes in the primary forest whose entrances are well camouflaged and difficult to find (Fig. 6B). In the



Figure 6. *Chilocosmia dichromata* comb. rest. natural habitat **A** *C*. *dichromata* comb. rest., female, in situ **B** burrow entrance camouflaged with leaf litter **C**, **D** primary forest habitat in Sorong, West New Guinea, Indonesia. Photo credits: Frank Schneider.

habitat of *C. dichromata* comb. rest., there is apparently only this species of tarantula (Fig. 6). All tubes were filled with water to a depth of ~ 10 cm. The temperature in the tubes was 24–26 °C. The water in the tubes was ~ 2 °C cooler. Probably the spiders flee into the water when disturbed. Known only from Sorong, West New Guinea (now Southwest Papua Province), Indonesia.

Tribe Yamiini Kishida, 1920

Phlogiellini West et al., 2012 (synonymised by Nunn et al. 2016).

Included genera. *Orphnaecus* Simon, 1892, *Phlogiellus* Pocock, 1897, *Seleno-brachys* Schmidt, 1999, stat. rev.

Genus Orphnaecus Simon, 1892

Type species. Orphnaecus pellitus Simon, 1892, by monotypy.

Included species. *O. adamsoni* Salamanes et al., 2022, *O. kwebaburdeos* (Barrion-Dupo et al., 2015), *O. pellitus* Simon, 1892.

Diagnosis. Orphnaecus differs from all selenocosmiine genera (including Chilocosmia stat. rev.), except Phlogiellus and Selenobrachys stat. rev., by

having a long prolateral superior keel (PS) (= retrolateral keel (R) in West et al. 2012) from tip to base with pronounced basal lobe (BL) on the embolus of males. *Orphnaecus* differs from *Phlogiellus* and *Selenobrachys* stat. rev., (i) in having a reniform patch, proximally broader, of short bacilliform rods, whereby the bacillae in the lowest row are larger, club-shaped, and rounded at the tip, prolaterally (Fig. 20A, D, G). It can also be distinguished from *Selenobrachys* stat. rev. in having long and dense dorsal palpal brush of setae on patella in males (Fig. 15A), in having a palpal tibia in males proximally swollen and distally tapering (Fig. 21A–D).

Remarks. Raven (1985) and Sivayyapram et al. (2020) distinguished Orphnaecus from other selenocosmiine genera in lacking an unpaired third claw at least on leg IV. However, the absence of a third claw cannot stand alone as the only diagnostic and defining characteristic of Orphnaecus against its known species that possess a third claw, while their genital and lyrate morphology is the same. In the Orphnaecus specimens (including syntypes of O. pellitus) mentioned and examined herein, we were able to find adult specimens within one species and one locality with a developed third claw, as well as those with a very reduced 3rd claw, and even a few specimens that did not have a third claw at all (Fig. 5G). Raven (1985) may have examined a type specimen from the same type series of O. pellitus with no third claw. Most of the syntypes and newly collected specimens of O. pellitus possess an unpaired third claw on leg IV (Fig. 15E). Congeners, herein directly examined (types and non-types), also possess a third claw on leg IV. Simon (1892) distinguished Orphnaecus in having tiny and more separated eyes caused by troglomorphic adaptation. However, this is only phenotypic plasticity and cannot serve as a defining characteristic of the genus, as it arises solely from prolonged habitation in completely dark cave environments.

Distribution. Philippine endemic: Luzon Is. (Simon 1892), Polillo Is. (Barrion-Dupo et al. 2015), Catanduanes Is., Masbate Is. (West et al. 2012), Negros Is. (Lüddecke et al. 2018), Dinagat Is. (Salamanes et al. 2022), and Mindanao Is. (Fig. 22). Probably widespread from Luzon PAIC, West Visayas PAIC, to Mindanao PAIC.

Etymology. Orphnaeus, one of the four horses that drew the golden chariot of Hades, the king of the underworld in Greek mythology, attached with the suffix *-cus* (probably to avoid homonymy with a centipede genus, *Orphnaeus* Meinert, 1870). The type species *O. pellitus* is possibly a troglobitic species, exhibiting troglomorphism and spending its whole life inside the cave (Simon 1892). Gender is masculine.

Genus Selenobrachys Schmidt, 1999, stat. rev.

Type species. *Selenobrachys philippinus* Schmidt, 1999, comb. rest., by original designation and monotypy.

Included species. (2 species) *S. philippinus* Schmidt, 1999, comb. rest., *S. ustromsupasius* sp. nov.

Diagnosis. Selenobrachys stat. rev. differs from all other selenocosmiine genera (including *Chilocosmia* stat. rev.), except sister genera *Orphnaecus* and *Phlogiellus*, in having a long prolateral superior keel (PS) (= retrolateral keel

in West et al. 2012) from base to tip with a pronounced basal lobe (BL) on the embolus of males (Figs 10A-F, 14A-E). Selenobrachys stat. rev. differs from Orphnaecus, and Phlogiellus (i) in having an ovoid proximally truncated and distally mildly tapering lyrate patch on the prolateral maxilla, with rows of strong paddle-shaped bacillae possessing thick and strong shafts (Figs 7F, G, 1E, F, 17E, F, 20E) (reniform lyrate morphology for Orphnaecus; see above and Fig. 20G; rudimentary patch of needleform rods, if present, for Phlogiellus; see Nunn et al. 2016) and where the largest ones in the lowest row have a more pointed tip in prolateral view (Fig. 20E); (ii) in having greater number of cheliceral strikers (< 150; excluding tertiary rows) (Figs 8C, 13C, D, 17C, D); (iii) in having a long and cylindrical palpal tibia in males (Figs 9A-D, 15B, C) (proximally swollen and distally tapering in Phlogiellus and Orphnaecus; see Fig. 15A and Nunn et al. 2016); and (iv) in having a broad and short, not reduced ends, almost symmetrical, tombstone-shaped spermathecal lobe in females (Fig. 18A, B). It further differs from Orphnaecus in lacking long and dense dorsal scopulate palpal brush in adult males (Figs 9D, 15B, C) and in having short sword-like femoral setation on prolateral femur I (Fig. 21E-H). It also differs from Phlogiellus in having a greater number of labial cuspules (~ 331-760) and a wider fovea than the ocular tubercle (Nunn et al. 2016; Sivayyapram et al. 2020).

Distribution. Philippine endemic: Negros Is. (Schmidt 1999; West et al. 2012) and Romblon Is. (this study; Fig. 22). Probably restricted to West Visayas PAIC and Romblon PAIC.

Etymology. A combination of two generic names, *Selenocosmia* and *Chilobrachys* (*Seleno- + -brachys*) (Schmidt 1999). Gender is masculine.

Selenobrachys philippinus Schmidt, 1999, comb. rest.

Figs 7–10, 18B, 20B, E, 21G, H

Type material examined. PHILIPPINES: Negros Island− **Negros Occidental Prov.** • holotype ♀, SMF 39202-84; Mambucal (now Mambucal Resort and Wildlife Sanctuary); SMF.

Other material examined. PHILIPPINES: Negros Island – Negros OccidentalProv.• ♂, PASI ara0006, 9 ♀♀, 8 j, UST-ARC 0112–UST-ARC 0127 (field#NO-M1A-01–NOM1A-16); Mambucal Resort and Wildlife Sanctuary; 365 m a.s.l.,26 Jun 2023, burrows under metamorphic rock boulders and crevices, LA Gue-varra, DC Acuña, CN Noriega, R Enguito, LJS Villaflor leg.; PASI/ UST- ARC • ♀,SMNS Aran-004192, 5 ♂♂, SMNS Aran-004187–Aran-004191; Mount Canlaon;2010, JM Verdez leg.; SMNS.

Diagnosis. Selenobrachys philippinus comb. rest. can be distinguished from its congener, *S. ustromsupasius* sp. nov., (i) in having longer leg IV than leg I (**RF~** 89–98); (ii) in having broader posterior sigilla on the sternum (Fig. 7E); (iii) in having a palpal organ in males with lower palpal organ index (**POI** < 42), with embolus with narrower basal lobe (BL) (Fig. 10B), with thinner prolateral superior keel (PS) (Fig. 10A, B) but broader at the tip (Fig. 10E, F), and with less pronounced subtegular ridge (StR) (Fig. 10A, B); and (iv) in having lesser number of maxillary (< 305) and labial (< 471) cuspules. It also differs in color, having an orange to orangey brown general body coloration (Fig. 7A; Schmidt 1999).

Description. Male (PASI ara0006). Body length 42.07 (*n* = 6: 41.43–43.98).



Figure 7. Selenobrachys philippinus comb. rest. ♂, PASI ara0006 A dorsal habitus B prosoma dorsal view C ventral view D ocular tubercle, dorsal view E posterior sternal sigilla F left maxilla, prolateral view G lyra on left prolateral maxilla.

Carapace (Fig. 7B). 17.51 long, 14.94 wide, anterior width 10.24, cephalic height 3.8, cephalic region 11.6 long, thoracic region 5.91 long. **CI** 85.32, **CLI** 66.25, **CHI** 21.70. Fovea width 2.27, curve length 2.43, procurved. Carapace has four pairs of weak furrows, lateral profile low and flat, and integument orangey brown, darker anteriorly. Setation: **TS**(a), rows of very short pale yellowish brown setae covering entire carapace, directed towards fovea and anteriorly to OT. Posterior surface of OT with pale brown short TS(a). **TS**(b), long pale yellowish brown setae at carapace margin, anterior margin light brown. **SC**(a), very short light brown flat scales sparsely covering OT. **SC**(b), pale yellowish brown acicular scales covering carapace, dense anteriorly, very sparse medially.



Figure 8. Selenobrachys philippinus comb. rest. ♂, PASI ara0006 A left chelicera, retrolateral view B prolateral view C cheliceral strikers, retrolateral view.

Eyes (Fig. 7D). Ocular tubercle 2.09 long, 2.73 wide, integument dark, paler anteriorly. AME round, rest of the eyes ovoid. Anterior row of eyes slightly procurved, posterior row of eyes recurved. Eyes: AME 0.61, ALE 0.76, PME 0.51, PLE 0.51. Interocular distances: AME- AME 0.26, ALE-ALE 1.55, PME-PME 1.25, PLE-PLE 1.98, AME-ALE 0.18, AME-PME 0.13, AME-PLE 0.64, ALE-PLE 0.31, ALE-PME 0.35, PME-PLE 0.14. *El* (AME) 3.48, *El* (ALE) 4.34.

Chelicerae (Fig. 8A–C). length 8.92, dorsal width 3.68, lateral width 6.28, fang curve length 7.48. Teeth 11, mesoventral denticles sparse ~ 35. Setation: **TS**, long pale brown setae on dorsal and upper 1/2 retrolateral surface, longer anteriorly. Lower 1/2 of retrolateral surface posteriorly with a patch of pallid filiform setae, anteriorly needleform. Mesoretrolateral surface with rows of very sparse setae basally spiniform, anteriorly needleform. Mesoprolateral surface with intercheliceral setae in arcuate strip of rows of pallid needleform setae originating basally. Lower 1/2, prolaterally, sparsely covered with brown needleform setae, filiform above. **SC**, flat, translucent, brownish white scales,



Figure 9. Selenobrachys philippinus comb. rest. ♂ PASI ara0006, left pedipalp A tibia, cymbium, and palpal organ, prolateral view B ventral view C retrolateral view D left patella to tibia, retrolateral view.

covering dorsal and upper retrolateral surface. Cheliceral strikers (Fig. 8C): Primary rows, ~ 16, 0.65–0.91 dark, long spiniform with filiform ends. Secondary rows, ~ 136, 0.21–0.55, dark long and short spiniform. Tertiary rows, ~ 114, 0.16–0.23, pallid very short needleform setae. Pseudostrikers long and pallid, and present ventrally.

Maxillae (Fig. 7F, G). Prolateral maxilla 7.47 long, 4.53 wide laterally, 3.75 wide ventrally. Maxilla prolaterally planoconvex, anterior lobe well pronounced, integument orangey brown, darker dorsally, basoventral cuspules ~ 305. Maxillary lyra (Fig. 7G): lyrate patch dense, in ovoid shape, truncated proximally, mildly tapering distally, 3.90 long, 2.10 high, total rods ~ 539, on 10–11 rows, surrounded by very fine setae, denser above and distally. Short bacilliform rods ~ 496, 0.24–0.39, needleform. Longer rods ~ 43, 0.49–0.85, paddle-shaped with pointed ends, which ~ 12 have well-defined paddle blades, and thick strong shafts slightly curved outward, located at the lowest rows. Setation: **TS**, pale brown spiniform setae, longer ventrally, stronger at distodorsal margin. Lyrate patch surrounded by fine setae. Above maxillary suture, two rows of ~ 15 stiff dark spiniform TS. Retrolateral surface is smooth, with rows of short semi-transparent bristles at lower margin. **SC**, flat whitish yellow-brown scales covering dorsal surface.

Labium and sternum (Fig. 7C, E). Labium: 2.32 long, 3.15 wide. Integument orangey brown, darker posteriorly, anterior 1/3 with cuspules ~ 471. Setation: **TS**(a), long, pale brown with pale filiform ends, covering labium anteriorly and laterally except on cuspule cluster, longer and greater at anterior edge, all pointing anteriorly. **TS**(b), dark, short needleform below cuspule cluster. Sternum: 8.20 long, 6.93 wide, integument yellowish brown. Posterior sternal corner acuminate and lateral corners weakly acuminate. Setation: **TS**(a), long pale brown spiniform setae, pale apically, on entire sternum but sparse medially. **TS**(b), fine, pallid, and short spiniform, at sternal margin. **SC**, white flat scale mat covering entire sternum. Labiosternal sigilla 1.49 long, 0.38 wide, 0.75 apart. Sternal sigilla 3 pairs, anterior sigilla 0.34 long, 0.22 wide, 4.53 apart, and 0.26 away from sternal margin adjacent to coxa I, median sigilla 0.81 long, 0. 34 wide, 3.93 apart, and 0.50 away from sternal margin adjacent to coxa II, posterior sigilla broad (Fig. 7E), 1.40 long, 0.58 wide, 1.60 apart, and 1.17 away from sternal margin adjacent to coxa III.

Abdomen and spinnerets. Abdomen: 18.69 long, 7.65 wide. ovular elongated, integument pale citron brown. Setation: **TS**(a), long, citron brown with darker bases, needleform, on entire abdomen, shorter ventrally, and pallid on book lungs. **TS**(b), rows of pallid and short paddle-like on book lungs and sparse on epigynal plate. **SC**, dense, overlapping flat, translucent, pale brown scales lightened by pale citron integument producing an orangey brown mat covering entire abdomen. Spinnerets: PMS 2.24 long, 0.74 wide, PLS, anterior 2.56 long, 1.52 wide, median 2.84 long, 1.12 wide, posterior 3.76 long, 1.00 wide. Setation: **TS** (a), long, citron brown with darker bases, needle form, on dorsal PMS and PLS. **TS**(b), dark and short, pale brown paddle-like TS, intermixed with spigots, on PMS and PLS ventrally. **SC**, flat brownish white scales covering PLS dorsally.

Genitalia (Figs 9A–C, 10A–E). Palpal Organ: approximately 2/5 of palp tibia length (**POI** 41.45). Tegulum 1.92 long, 1.81 wide, globular, widest medially, subtegular ridge (StR) weakly pronounced (Fig. 10A, B). Embolus length 2.42, width 1.20 basally and 0.37 medially, tip 0.16 wide, Embolus length 1.26 times longer than tegulum length (**EMI** 126.04), base robust, tapering distally, curved retrolaterally and ending to a broad tip. Embolus has a long and stout prolateral superior keel (PS) (Fig. 10A, B) but broad at tip (Fig. 10E, F); has prolateral inferior keel (PI) short, emerged from the tip to rear at apical 1/5, below PS and embolic opening (Op) (Fig. 10E, F); apical keel (A) very short, emerged almost at the tip; embolic opening (Op) located between PS and PI near the tip (Fig. 10E, F). Basal lobe pronounced but not broad, projected proximally (Fig. 10A, B).

Legs. Leg formula: IV, I, II, III. **RF**~ 91.94, LLI (I) 20.93, LLI (IV) 16.39, DLI (I) 19.97, DLI (IV) 16.23, MI (I) 80.99, MI (IV) 124.10, TI (I) 59.78, TI (IV) 44.41. Leg lengths (fem, pat, tib, met, tar/cym): Palp 31.96 (11.08, 6.46, 10.47, -, 3.95) Leg I 66.41(18.11, 9.92, 16.73, 13.55, 8. 1) Leg II 58.56 (15.7, 8.16, 14.59, 12.86, 7.25) Leg III 51.81(13.04, 6.59, 11.54, 13.63, 7.01) Leg IV 72.23 (17.64, 7.68, 16.8, 20.85, 9.26). Leg lateral width (fem, pat, tib, met, tar/cym): Palp (2.64, 2.34, 2.56, -, 2.35) Leg I (4.05, 3.61, 3.06, 1.9, 1.28) Leg II (3.49, 3.35, 2.58, 1.45, 1.37) Leg III (3.61, 2.8, 2.39, 1.75, 1.2) Leg IV (3.77, 3.08, 2.34, 1.55, 1.1) Leg dorsal width (fem, pat, tib, met, tar/cym): Palp (2.63, 2.27, 2-, 2.38) Leg I (3.75, 3.08, 2.96, 1.84, 1.63) Leg II (3.31, 2.69, 2.54, 1.65, 1.35) Leg III (3.43, 2.61, 2.44, 1.72, 1.31) Leg IV (3.25, 2.76, 2.48, 1.96, 1.27). Cymbium bipartite. Tarsi I–IV transversely cracked, shows transverse weakening or pallid region, tar. I and tar. II more anteriorly, tar. III and tar. IV medially.

Leg setation and spines. Setation (femur to tarsus): TS(a), brown spiniform setae on all legs, longer TS pale brown, longer on all femora, ventrally and on palp patella and tibia, thicker on leg III and IV, dense citron brown on tibiae I and II, ventrally. TS(b), short and pallid paddle-like setae, dense on ventral femur I, sparse on ventral palpal femur, ventral patella I, and ventral femur II. FS, prolateral femur I with dense field (less dense than females) of elongated sword-like TS. PB, thin layer of short, flat, pale brown, scales, present on dorsal palpal patella and dorsal palpal tibia but very sparse (Fig. 9D). SC, flat whitish scales, darkened by reddish brown integument, covering all legs. Other sensory setae: ETB, pair of thin inverted L-shaped clusters of short pale brown setae, starting from basolateral to dorsal Met. I and II, single cluster on Met III and IV. Cymbium with a single cluster dorsally that broadens basally. **TB**(a), long and short filiform TB intermix with ETB in two rows, longest dorsally. TB(b), rows of unordered clavate TB, varying in size, present in all tarsi, and intermix with tarsal ETB. CHS, tiny, pale brown translucent erect sensilla tapering apically, present on the palpal and all leg femora to tarsi and intermixes with tarsal and metatarsal scopulae. Spines (dorsal-dorsoprolateral-dorsoretrolateral-ventral): Met I (0-0-0-1), Met II (0-0-0-3). Met III (0-1-1-4). Met IV (0-1-1-3).

Coxae and trochantera. Coxae: Length (coxa I, II, III, IV), 8.16, 7.88, 6.78, 7.31. Width (coxa I, II, III, IV), 4.49, 3.97, 4.27, 4.21. Setation: **TS**(a), long pale brown setae, covering dorsal and ventral surfaces; **TS**(b), strong and short spiniform setae, prolaterally on all coxae; **TS**(c), patches of fine setal fringe present laterally on coxae, intermixed with TS; **TS**(d), Coxae I–IV have rows of short semi-translucent bristles, prolaterally, denser on coxae I and II. **SC**(a), flat, grayish brown scales, covering the ventral to retrolateral 1/2; **SC**(b), white, cottony, and acicular, covering the dorsal of all coxae. Trochantera: Length (troch. palp I, II, III, IV), 2.78, 3.95, 3.02, 1.84, 2.77. Width (troch. palp I, II, III, IV), 2.59, 3.99, 3.52, 3.44, 3.62.

Scopulae and claws. Scopulae: cymbium scopulated ventrally. Tar. I, entire, but intermixed with one or two longitudinal rows of very sparse short spiniform setae. Tar. II, entire, but intermixed with one or two longitudinal rows of very sparse short spiniform setae. Tar. III, entire, but intermixed with two or three longitudinal rows of short spiniform setae. Tar. IV, divided by four rows



Figure 10. Selenobrachys philippinus comb. rest. *A*, PASI ara0006, left palpal organ **A** prolateral view **B** retrolateral view **C** ventral view **D** apical view **E** tip of embolus, dorsal view **F** prolateral view. Abbreviations: PS- prolateral superior keel, PI- prolateral inferior keel, A- apical keel, BL- basal lobe, Op- embolic opening, StR- subtegular ridge.

of strong and long spiniform setae. Met. I ventral surface almost completely scopulated, entire, but with one or two longitudinal rows of very sparse long setae. Met. II, almost all ventral surface covered, entire, but intermixed with one or two longitudinal rows of very sparse long setae. Met. III, covering 4/5 distally, entire, but intermixed with one or two longitudinal rows of very sparse long setae. Met. IV, covering 3/4 distally, divided by two or three rows of strong long setae. Claws: pair of claws present on all leg tarsi, with one to three teeth on each claw. Tarsal IV claw, 2.27, with unpaired inferior third claw. 0.20.

Color in life. Monochromatic. The reddish brown integument is lightened by whitish scales creating a uniform orange to orangey brown body coloration (Fig. 7A).

Variation. The third inferior claw is absent or almost absent on some specimens and varies in length if present, which is also observed in other species in this study.

Natural history and distribution. Spiders are found roaming outside near their burrows at night. Burrows, not self-dug, are found near streams under metamorphic rock boulders and crevices on mountain slopes. Known only from Mambucal Resort and Wildlife Sanctuary (at the foot of Mt. Canlaon) and Sipalay City (West et al. 2012) in Negros Occidental, Negros Island, Philippines. Reports from the internet in the islands of Panay, Guimaras, and Cebu are unverified and may represent a separate island endemic species of *Selenobrachys*.

Etymology. The specific epithet is a masculine adjective derived from the country locality, the Philippines (Schmidt 1999).

Selenobrachys ustromsupasius sp. nov.

https://zoobank.org/96F1B924-D25E-48A5-AEB9-4548078E2E15 Figs 11-14, 15B-D, 16, 17, 18A, 21E, F

 Type material examined.
 PHILIPPINES: Romblon Island Romblon Prov.

 holotype ♂, UST-ARC 0002 (field#R01-02), *paratypes* 4 ♂♂, 8 ♀♀, 1 j, UST-ARC 0001, 0003-0014 (field#R01-01, R01-03-R01-14); Municipality of Romblon, Brgy. Tambac; 12°32.0106'N, 122°17.6595'E, 200 m a.s.l., Sep 2022, AB Mayor, L De Capiz, RM De Capiz leg.
 paratypes 2 ♂♂, 4 ♀♀, 10 j, UST-ARC 0015-0030 (field#R02-01-R02-16); Municipality of Romblon, Brgy. Guimpingan; 12°35.2478'N, 122°17.2416'E, 80 m a.s.l., 24 Feb 2023, DC Acuña, LA Guevarra Jr., CN Noriega, MJ Fadri, GA Florendo Jr., CJ Cabisuelas leg.; UST-ARC.

Diagnosis. Selenobrachys ustromsupasius sp. nov. can be distinguished from its congener, *S. philippinus* comb. rest, (i) in having longer leg I than leg IV, with **RF** ~ 104–111; (ii) in having narrower posterior sigilla (Figs 12B, 16C); (iii) in having a palpal organ in males with greater palpal organ index (**POI** > 48), with embolus with broader basal lobe (Fig. 14B), with thicker prolateral superior keel (PS) (Fig. 14A, B) but stouter at tip (Fig. 14E), and with less pronounced subtegular ridge (StR) (Fig. 14A, B); and (iv) in having greater number of maxillary (> 365) and labial (> 608) cuspules. It also differs in color, having a brown to dark brown general body coloration (Figs 11A, B, 19G, H).

Description. Holotype ♂, UST-ARC 0002 (field#R01-02). Body length 43.18. (*n* = 7: 43.00-45.19) Figs 11A, 12-14, 15B-D.

Carapace (Fig. 12A). Length, 17.41, median width 15.34, **CI** 88.11, **CLI** 65.88, **CHI** 22.75. Integument dark reddish brown. Caput profile low and flat, cephalic region slightly higher. Fovea procurved (Fig. 12D), width 1.72, curve length 1.89. Distance of fovea to posterior carapace margin 4.55; distance to OT 9.68. Setation: **TS**(a) long, thin, pale brown setae at carapace margin, pointing anteriorly. **TS**(b) long, strong, dark brown setae, pale brown apically, intermixed with TS(a) and SC, at clypeus, and few at the top of OT between AMEs. **TS**(c) rows of short pale or dark brown setae, pale brown apically, that run along radial grooves pointing to fovea. **SC**(a), very short, thick, flat, pale brown scales covering anterior 1/2 surface of OT. **SC**(b), mat of long, dense, whitish acicular scales, covering lateral sides and front of OT, pointing towards OT and carapace, sparse to none on middle surface, denser on coastal surface, mostly pointing anteriorly.

Eyes (Fig. 12C). OT length 1.94, width 2.86. Clypeus 0.5, integument dark. Eyes anterior row slightly procurved, posterior row recurved. Eyes: AME 0.61 (round), ALE 0.78 (ovoid), PME 0.44 (ovoid), PLE 0.47 (ovoid). Interocular



Figure 11. Selenobrachys ustromsupasius sp. nov. dorsal habitus A holotype ♂, UST-ARC 0002 B paratype ♀, UST-ARC 0005.

distances: AME-AME 0.31, PLE-PLE 1.97, ALE-ALE 1.59, PME-PME 1.24, ALE-PLE 0.21, AME-ALE 0.20, AME-PME 0.12, ALE-PME 0.32, PME-PLE 0.09, AME-PLE 0.55. **EI**(AME)3.50, **EI**(ALE) 4.48.

Chelicerae (Fig. 13A-D). Dorsal length 8.51, dorsal width 3.79, lateral width 6.49. Fang curve length 6.68. Prolateral surface: more than upper 1/2 integument dark, lower surface reddish brown, with long brown fine setae, lowest portion above teeth darker and longer. Intercheliceral pegs absent but with rows of stiff setae. Retrolateral surface: integument upper 2/3 dark, lower surface reddish brown to amber, flat whitish scales (SCa), fine, white, cottony acicular scales (SCb), and long strong setae (TS) covering dorsal and upper retrolateral surfaces, greater anteriorly, patch of fine and long, mostly slightly curved setae at posterior area of lower retrolateral surface, patch of very fine setae with curved ends at anterior area of retrolateral surface, proximomedial setae needleform, very sparse. Long, pale reddish brown brush of setae ventrally. Teeth 11, mesoventral denticles sparse, ~ 41, on three or four rows. Lyrate region (Fig. 13C, D): 237-248 total strikers on six to seven horizontal rows, unordered, the strongest and longest strikers on lower rows. Primary rows ~ 11 (0.80-0.83 long) long dark brown with long curved filiform ends pointing distally. Secondary rows ~ 142 (0.17-0.66), dark spiniform with short curved filiform ends pointing inward. Tertiary rows ~ 90 (0.09-0.18), short, pallid, and needleform, located above secondary rows. Long, pallid pseudostrikers present ventrally.

Maxillae (Fig. 13E, F). Prolaterally planoconvex, anterior lobe well pronounced, cuspules on inner basoventral surface ~ 365. Stridulatory organ (Fig. 13F): ~ 465 total bacilliform rods, in nine or ten rows, form long and dense ovoid patch, proximally truncated and distally mildly tapering (3.31 long, 1.70 high). Short bacilliform rods ~ 424, 0.22–0.53, needleform. Longer rods ~ 41, 0.55–0.77,



Figure 12. Selenobrachys ustromsupasius sp. nov., holotype ♂, UST-ARC 0002 A prosoma, dorsal view B ventral view C ocular tubercle, dorsal view D fovea, dorsal view E labium, ventral view F spinnerets, ventral view.

paddle-shaped with thick and strong shafts and pointed ends, ~ 10 of which have well-defined paddle blades, located at lowest rows. Paddles flattened perpendicularly to maxillary surface. Setation: **TS**, brown setae, with upper part paler, present dorsally and ventrally, longest ventrally. Proximodorsal with erect



Figure 13. Selenobrachys ustromsupasius sp. nov., holotype ♂, UST-ARC 0002, left chelicera and maxilla A left chelicera retrolateral view B prolateral view C cheliceral strikers retrolateral view D ventrolateral view E left maxilla, prolateral view F maxillary lyra on left prolateral maxilla.

fine pallid setae. Lyrate patch surrounded by fine setae. Above maxillary suture, two rows of ~ 20 stiff dark TS. **SC**, flat whitish scales covering dorsal surface. Retrolateral surface smooth, with rows of short semi-transparent bristles at lower margin.

Labium and sternum (Fig. 12B, E). Labium (Fig. 12E): length 2.30, width 3.30, integument reddish brown, paler anteriorly. Labial cuspules \sim 608, dark, at apical 1/3. Setation: **TS**(a), long brown with filiform ends, covering labium anteriorly and laterally except on cuspule cluster, longer and denser at anterior edge, all pointing anteriorly. **TS**(b), dark, spiniform, on posterior surface. Sternum (Fig. 12B): sternum length 7.68, width 6.80, integument reddish brown, slightly

darker at margin. Posterior sternal corner acuminate, lateral corners weakly acuminate. Labiosternal sigilla ovoid, 1.12 long and 0.40 wide, 0.72 apart. Sternal sigilla 3 pairs, ovoid. Anterior pair 0.28 long, 0.16 wide, 4.72 apart, and 0.16 away from the sternal margin adjacent to coxa I. Median pair 0.88 long, 0.24 wide, 3.88 apart, and 0.20 away from the sternal margin adjacent to coxa II. Posterior pair 1.44 long, 0.44 wide, 1.48 apart, and 1.16 away from the sternal margin adjacent to coxa III. Setation: **TS**(a), long and short dark spiniform setae on entire sternum, sparse on middle surface. **TS**(b), dark and pallid, short spiniform with filiform ends, at sternal margin. **SC**, flat grayish brown scales mat covering entire sternum.

Abdomen and spinnerets. Abdomen 14.70 long, 8.25 wide, ovular elongated, integument pale brown. Pedicel 1.8, pale brown, dorsally striated. Setation: **TS**, long brown setae, paler apically, covering entire abdomen, except book lungs and epigynal plate, denser laterally. Book lungs covered with short, pale brown spiniform setae. **PTS**, dark and short, covering book lungs and epigynal plate, intermixed with semi-transparent thin and short sensilla. **SC**, grayish brown, covering entire, sparse on book lungs and epigynal plate. All setae pointing distad. Spinnerets (Fig. 12F): PMS 2.02 long, 0.60 wide. PLS 9.52 long (ant. 3.12, mid. 2.84, pos. 3.56), and 2.88 wide (ant. 1.32, med. 0.92, post. 0.64). Setation: **TS**, long brown setae, paler apically, covering dorsally and laterally. **PTS**, dark and short, covering ventrally, intermixed with spigots. **SC**, grayish brown, sparse, present dorsally.

Genitalia. Palpal organ (Fig. 14A–E): almost 1/2 of palp tibia length (**POI** 48.35). Tegulum length 1.91, width 1.78. Embolus length 2.79, basal width 1.11, middle length 0.33, tip 0.21 wide. Tegulum globular, widest medially, subtegular ridge (StR) pronounced (Fig. 14A, B). Embolus 146 times longer than tegulum (**EMI** 146.07), robust at the base (Fig. 14A, B), tapering distally, Embolus has long and thick prolateral superior keel (PS) from base to tip (Fig. 14A, B) but stout at the tip (Fig. 14E); prolateral inferior keel (PI) short, emerged from the tip to rear at apical 1/5, located below PS and embolic opening (Op) (Fig. 14E); apical keel (A) very short, emerged almost at the tip (Fig. 14E); embolic opening (Op) located between PS and PI near the tip (Fig. 14E). Basal lobe pronounced and broad, projected proximally (Fig. 14B). Palpal tibia long and cylindrical (Fig. 15B, C).

Legs. Leg formula: I, IV, II, III. **RF** ~ 110.99, **LLI** (I) 20.90, **LLI** (IV) 18.83, **DLI** (I) 20.85, **DLI** (IV) 18.67, **TI**(IV) 44.03, **MI**(IV) 108.80. Leg lengths total (fem., pat., tib., met., tar. /cym.): Palp 31.51 (11.24, 6.82, 9.72, n/a, 3.73), Leg I 64.31 (17.72, 9.63, 16.01, 13.19, 7.76), Leg II 53.39 (14.59, 7.88, 12.36, 11.65, 6.91), Leg III 45.83 (12.24, 6.31, 9.63, 11.51, 6.14), Leg IV 57.94 (15.47, 6.89, 13.86, 15.08, 6.64). Leg lateral widths (fem., pat., tib., met., tar. /cym.): Palp (2.53, 2.38, 2.24, n/a, 2.61), Leg I (4.23, 3.21, 2.85, 1.76, 1.39), Leg II (3.85, 3.16, 2.40, 1.58, 1.32), Leg III (4.19, 2.94, 2.47, 1.56, 1.25), Leg IV (3.52, 2.89, 2.17, 1.43, 0.91). Leg dorsal widths (fem., pat., tib., met., tar. /cym.): Palp (2.74, 2.50, 2.07, n/a, 2.50), Leg I (3.56, 3.36, 2.99, 1.93, 1.57), Leg II (3.38, 3.04, 2.45, 1.78, 1.40), Leg III (3.66, 2.75, 2.54, 1.73, 1.29), Leg IV (3.20, 2.52, 2.33, 1.65, 1.12). Cymbium bipartite. Tarsi I–IV transversely cracked, shows transverse weakening or mild pallid region, tar. I and tar. II more anteriorly, tar. III and tar. IV medially.

Leg setation (femora to tarsi) and spines. Setation: **TS**, long, dark brown setae with contrasting pale brown filiform ends, present on palp and to all legs, but sparse to all tarsi, prolateral femora I and II, and retrolateral femur IV, all



Figure 14. Selenobrachys ustromsupasius sp. nov., holotype ♂, UST-ARC 0002, left palpal organ A prolateral view B retrolateral view C apical view D tip of embolus, retrolateral E prolateral view. Abbreviations: PS- prolateral superior keel, PI- prolateral inferior keel, A- apical keel, BL- basal lobe, Op- embolic opening, StR- subtegular ridge.

pointing distad, with some erect rows on leg1 dorsally. **PTS**, short and pallid, dense on ventral femur I, sparse on ventral palpal femur, prolateral to ventral patella I, and ventral femur II. **FS**, prolateral femur I with dense field (less dense than females) of elongated sword-like setae. **SC**, reflective grayish brown, covering all legs, longer and fine on all femora, pale brown on all patellae. **PB**, grayish, present on dorsal palpal patella but not long and dense, also present on dorsal palpal tibia but very sparse (Fig. 15B, C). **ETB**, short brown setae in pair of thin inverted L-shaped clusters, starting from basolateral to dorsal Met I to II, single cluster on Met III to IV. Cymbium with single cluster dorsally that broadens basally. **TB**, long and short filiform intermix with ETB in two rows, longest dorsally. Rows of clavate TB, unordered, varying in size, present in all tarsi and

cymbium, and intermix with tarsal ETB. **CHS** tiny, pale brown translucent erect sensilla tapering apically, present on the palpal and all leg femora to tarsi and intermix with tarsal and metatarsal scopulae. Spines: (dorsal-dorsoprolateral-dorsoretrolateral-ventral): Met I (0-0-0-1), Met II (0-0-0-3). Met III (0-1-1-3). Met IV (0-1-1-5).

Coxae and trochantera. Coxae: Palp coxa (see *Maxillae*). Lengths (coxa I, II, III, IV) 7.52, 5.80, 5.20, and 5.60. Widths (coxa I, II, III, IV) 4.00, 3.48, 3.44, 3.88. Setation: **TS**, long brown setae, dorsally and ventrally; strong and short spiniform setae, prolaterally on all coxae. **SC**(a), flat scales covering the ventral to retrolateral 1/2. **SC**(b), white, cottony, acicular scales covering the dorsal of all coxae. Patches of fine setal fringe present laterally on coxae, intermixed with short spiniform setae. Coxae I–IV have rows of short semi-translucent bristles, prolaterally, denser on coxae I and II. Trochantera: Lengths (troch. palp, I, II, III, IV) 2.12, 3.52, 3.32, 3.08, 2.72. Widths (coxa palp, I, II, III, IV) 2.48, 3.40, 2.96, 3.12, 3.28.

Scopulae and claws. Scopulae: cymbium scopulated ventrally. Tar. I, entire, but intermixed with longitudinal one or two rows of very sparse short spiniform setae. Tar. II, entire, but intermixed with longitudinal one or two rows of very sparse short spiniform setae. Tar. III, entire, but intermixed with longitudinal two or three rows of strong, long setae. Tar. IV, divided by four rows of strong, long setae. All tarsi with a bald spot ventrobasally. Met. I, almost all ventral surface covered, entire, but intermixed with longitudinal one or two rows of very sparse long setae. Met. II, most ventral surface covered, entire, but intermixed with longitudinal one or two rows of very sparse long setae. Met. II, most ventral surface covered, entire, but intermixed with longitudinal one or two rows of very sparse long setae. Met. III, covering 4/5 distally, entire, but intermixed with longitudinal one or two rows of very sparse long setae. Met. IV, covering 3/4 distally, divided by two or three rows of strong long setae. Claws: pair of claws present on all leg tarsi with one to three teeth on each claw. Unpaired inferior third claw 0.13, very short, present on tarsus IV (Fig. 15D).

Color in life. Slightly dichromatic, dark on carapace and all femora, and covered with pale brown setae on leg patellae, tibiae, metatarsi, and tarsi, including trochantera, sternum, abdomen, and spinnerets (Fig. 11A). Almost uniformly dark brown with a mild purplish blue sheen reflected by scales just after ecdysis, which fades in a few days.

Paratype ♀, UST-ARC 0005 (field#R01-05). Body length 53.85 (*n* = 12: 42.56− 55.13) Figs 11B, 16, 17, 18A, 21E, F

Carapace (Fig. 16A). Length 18.82, median width 15.61, anterior width 9.47, **CI** 82.94, **LI** 66.84, **CHI** 27.42. Integument reddish brown. Caput profile low and flat, and cephalic region slightly higher. Fovea procurved (Fig. 16E), width 2.13, with curve length 2.22. Distance of fovea to posterior carapace margin 4.74, distance to ocular tubercle 10.67. Setation: **TS**(a), long, thin, pale brown setae at the margin of carapace, directed anteriorly; **TS**(b), long, strong, dark brown setae and pale brown apically, intermixed with TS(a) and SC(b), at clypeus, and one or two at top of OT between AMEs; **TS**(c), rows of short pale brown or dark brown setae, pale brown apically, that runs to radial grooves pointing to fovea; **SC**(a), short, flat, pale brown scales covering anterior 1/2 surface of OT; **SC**(b), mat of satiny white acicular scales covering carapace but sparse to absent medially and greater on margin, most directed anteriorly (denser and longer on male).



Figure 15. B−D Selenobrachys ustromsupasius sp. nov., holotype ♂, UST-ARC 0002, pedipalp and tarsus B Left palpal patella and tibia, prolateral view C dorsal view D claws on left tarsus IV, retrolateral view A, E Orphnaecus pellitus, syntype ♂, MNHN AR4678 A left pedipalp, prolateral view E claws on left tarsus IV, retrolateral view.

Eyes (Fig. 16A, B). Ocular tubercle (OT) length 2.16, width 2.99. Clypeus 1.2. Eyes anterior row slightly procurved, posterior row recurved. Eyes: AME 0.68 (round), ALE 0.85 (ovoid), PME 0.54 (ovoid), PLE 0.52 (ovoid). Interocular distances: AME-AME 0.34, PLE- PLE 2.16, ALE-ALE 1.69, PME-PME 1.34, ALE-PLE 0.20, AME-ALE 0.19, AME-PME 0.06, ALE-PME 0.27, PME-PLE 0.20, AME-PLE 0.57. **EI**(AME)3.61, **EI**(ALE) 4.52.



Figure 16. Selenobrachys ustromsupasius sp. nov., paratype ♀, UST-ARC 0005 A, B ocular tubercle, dorsal view C prosoma, ventral view D labium, ventral view E fovea, dorsal view.

Chelicerae (Fig. 17A–D). Dorsal length 11.48, dorsal width 4.79, lateral width 67.46. Fang curve length 8.86. Prolateral surface (Fig. 17B): reddish brown, darker distally and paler proximally, long brown fine setae at lower surface, darker and longer at lowest portion above teeth. Intercheliceral pegs are absent but with rows of stiff setae. Retrolateral surface (Fig. 17A): integument, upper 2/3 dark brown, lower surface reddish brown to amber, flat whitish scales (**SC**a), fine, white, cottony acicular (**SC**b), and long, strong tactile setae (**TS**) covering dorsal and upper retrolateral surface, greater anteriorly, patch of fine and long, mostly slightly curved setae at posterior area of lower retrolateral surface, proximomedial setae needle form, very sparse. Long pale reddish brown brush of setae, ventrally. Teeth 16 (including 4 uprooted and 5 smaller teeth) (0.13–0.51), mesoventral denticles dense, ~ 77, on three or four rows. Lyrate region



Figure 17. Selenobrachys ustromsupasius sp. nov., paratype Q, UST-ARC 0005, left chelicera and maxilla **A** left chelicera, retrolateral view **B** prolateral view **C** cheliceral strikers, retrolateral view **D** ventrolateral view **E** left maxilla, prolateral view **F** maxillary lyra on left maxilla.

(Fig. 17C, D): 234–245 strikers on six or seven horizontal rows, unordered. Strongest and longest strikers on lower rows. Primary rows ~ 11 (0.86–0.95) long and dark brown, with long, curved filiform ends pointed distally. Secondary rows ~ 140 (0.19–0.67) dark and lanceolate with short curved filiform ends pointed downward. Tertiary rows ~ 89 (0.12–0.15) short, pallid, and needleform. Long, pallid pseudostrikers present ventrally.

Maxillae (Fig. 17E, F). Prolaterally planoconvex, anterior lobe well pronounced, cuspules on inner basoventral surface ~ 365. Stridulatory organ (Fig. 17F): ~ 370 bacilliform rods, in nine or ten rows, form a long and dense ovoid patch, proximally truncated, distally mildly pointed (3.47 long, 1.71 high). Short bacilliform rods 0.21–0.51, with pointed ends. Longer rods 0.49–0.84, paddle-shaped, with paddle blades 0.23–0.36 long and pointed ends, with thick, strong shafts slightly curved outward located at lowest rows. Paddles are flattened perpendicularly to maxillary surface. Total number of rods with paddle blades ~ 40, ~ 10 of them have well-defined paddle blades. Setation: **TS**, brown setae, with upper part paler, present dorsally and ventrally, longest ventrally. Proximodorsal surface with erect pallid fine setae. Lyrate patch surrounded by fine setae. Above maxilla suture with two rows of ~ 24 stiff dark TS. **SC**, flat whitish scales covering dorsal surface.

Labium and sternum. Labium (Fig. 16D) length 2.76, width 3.45, integument reddish brown, paler anteriorly. Labial cuspules ~ 759 (0.05–0.08), dark, at apical 1/3. Setation: **TS**(a), long, brown with filiform ends, covering labium anteriorly and laterally except on cuspule cluster, longer and greater at anterior edge, all pointing anteriorly. **TS**(b), dark, spiniform, on posterior surface. Sternum (Fig. 16C) length 8.04, width 7.88, integument pale brown, slightly darker at margin. Posterior sternal corner acuminate, lateral corners weakly acuminate. Labiosternal sigilla ovoid, 1.36 long and 0.49 wide, 1.15 apart. Sternal sigilla three pairs, ovoid. Anterior pair 0.48 long, 0.30 wide, 4.60 apart, and 0.67 away from sternal margin adjacent to coxa I. Median pair 0.48 long, 0.30 wide, 4.92 apart, and 0.52 away from sternal margin adjacent to coxa II. Posterior pair 1.64 long, 0.55 wide, 1.90 apart, and 1.38 away from sternal margin adjacent to coxa III. Setation: **TS**(a), long and short dark spiniform setae on entire sternum, less concentrated on middle surface. **TS**(b), dark and pallid, short spiniform with filiform ends, at sternal margin. **SC**, grayish brown flat scale mat covering entire sternum.

Abdomen and spinnerets. Abdomen 23.55 long, 16.33 wide, ovular elongated, integument pale brown. Pedicel 1.87, brown, dorsally striated. Setation: **TS**(a), long brown setae, paler apically, covering entire abdomen except book lungs and epigynal plate, shorter ventrally. Book lungs covered with short brown spiniform setae. **TS**(b), dark short paddle-like, covering entire epigynal plate densely, intermixed with semi-transparent thin and short sensilla, and long spiniform setae anteriorly. **SC**, flat grayish brown scales, covering entire area, darker on epigynal plate. All setae pointing distad. Spinnerets: PMS 2.40 long, 0.84 wide. PLS 10.76 long (ant. 3.72, mid. 3.08, pos. 3.96), 4.08 wide (ant. 1.68, mid. 1.32, pos. 1.08). Setation: **TS**(a), long brown setae, paler apically, covering dorsally and laterally. **TS**(b), dark short paddle-like, covering ventrally. Spigots present on all segments ventrally. **SC**, flat grayish brown scales, sparse, present dorsally.

Genitalia. Spermathecae unilobed, not fused. Lobe length 1.25, width 0.93, basal width 1.10, very broad and short tombstone-shaped spermathecal lobe with rounded ends and almost parallel lateral margins (Fig. 18A). Entirely sclerotized, greater apically and weaker basally. Lobes close to each other, separated by 0.07. Epigastric fold 3.96 long.

Legs. Leg formula: I, IV, II, III. **RF~** 104.28., **LLI** (I) 24.43, **LLI** (IV) 21.84, **DLI** (I) 23.37, **DLI** (IV) 20.48, (**MI** (IV) 126.80, **TI** (IV) 50.92. Leg lengths total (fem., pat., tib., met., tar./cym.): Palp 32.07 (10.72, 6.28, 7.92, n/a, 7.15), Leg I 61.35 (16.42, 10.08, 14.29, 11.65, 8.91) Leg II 48.54 (13.13, 8.06, 10.90, 10.10, 6.35), Leg III 42.60 (11.33, 6.30, 8.48, 10.35, 6.14), Leg IV 58.83 (15.07, 7.66, 12.39, 15.71, 8.00). Leg lateral widths (fem., pat., tib., met., tar./cym.): Palp (3.95, 2.50, 2.68, n/a, 0.93), Leg I (4.31, 3.50, 3.02, 2.49, 1.67), Leg II (4.13, 3.28, 2.54, 1.78, 1.41), Leg III (3.69, 3.05, 2.66, 1.74, 1.33), Leg IV (4.06, 3.11, 2.86, 1.61, 1.21). Leg



Figure 18. Spermathecae, dorsal view **A** Selenobrachys ustromsupasius sp. nov., paratype \bigcirc , UST-ARC 0005 **B** Selenobrachys philippinus comb. rest., \bigcirc , UST-ARC 0117 **C** Orphnaecus kwebaburdeos, \bigcirc , UST-ARC 0060 **D** Orphnaecus pellitus, \bigcirc , UST-ARC 0031.

dorsal widths (fem., pat., tib., met., tar./cym.): Palp (2.67, 2.42, 2.46, n/a, 1.70), Leg I (3.42, 3.66, 3.04, 2.31, 1.91), Leg II (3.20, 2.94, 2.51, 2.04, 1.73), Leg III (3.54, 2.60, 2.45, 1.89, 1.64), Leg IV (3.34, 2.79, 2.62, 1.75, 1.55). Tarsus I–V with transverse weakening, tar. I and tar. II more anteriorly, tar. III and tar. IV medially.

Leg setation (femora to tarsi) and spines. Setation: TS(a), long and short brown spiniform setae, paler on upper part, covering all over palp and legs except to median to lower prolateral palpal femur surface and leg I and II prolateral femora, longest on palpal tibia and ventral palpal femur, and all leg femora, tibiae, metatarsi, and tarsi. Leg retrolateral femur IV with stout setae. All pointing distad but erect on all ventral femora. TS(b), dark paddle-like setae, present on palp (prolateral to ventral distal femur, patella, and proximal tibia), leg I (dorsolateral and ventral femur, ventral to prolateral patella, and proximal tibia), leg II (prolateral and ventral femur, patella, and proximal tibia), and leg III and IV (ventral femora). Very dense on ventral femora I and II and prolateral tibia I and femora II. FS, prolateral femur I with a dense field of short sword-like TS (Fig. 21F), similar to TS(b) but shorter and with pointed ends. SC, reflective, flat, grayish brown scales, pale brown on all dorsal patellae, covering all legs and palp except prolateral palpal femur proximally, which leaves an ovoid smooth surface. Other sensory setae: ETB, short brown setae in two thin inverted L-shaped clusters, starting from basolateral to dorsal Met I to II, and a single cluster on Met III to IV. **TB**(a), long and short filiform TB intermix with ETB in two rows,

longest dorsally. **TB**(b) rows of unordered clavate TB, varying in size, present to all tarsi, and intermix with tarsal ETB. **CHS**, tiny pale brown translucent erect sensilla tapering apically, present on palpal and all leg femora to tarsi, and also intermixes with tarsal and metatarsal scopulae. Spines: (dorsal-dorsoprolateral-dorsoretrolateral-ventral): Met I (0-0-0-1), Met II (0-0-0-3). Met III (0-1-1-3). Met IV (0-1-1-3).

Coxae and trochantera. Coxae: Palp coxa (see *Maxillae*). Lengths (coxa I, II, III, IV) 8.42, 7.28, 5.72, 6.12. Widths (coxa I, II, III, IV) 4.40, 4.04, 3.88, 4.28. Setation: **TS**, long brown setae dorsally and ventrally, strong and short spiniform setae prolaterally on all coxae. **SC**(a), flat scales covering ventral to retrolateral 1/2. **SC**(b), white cottony acicular covering dorsal of all coxae. Patches of fine setal fringe present laterally on coxae, intermixed with short spiniform setae. Coxae I–IV have rows of short semi-translucent bristles, prolaterally, denser on Coxae I and II. Trochantera: Lengths (troch. palp, I, II, III, IV) 2.84, 4.32, 3.88, 3.12, 3.64. Widths (coxa palp, I, II, III, IV) 2.92, 3.76, 3.20, 3.04, 3.52.

Scopulae and claws. Scopulae (left): Palp tarsus undivided but parted. Tarsus I, entire, but intermixed with longitudinal one or two rows of very sparse short spiniform setae. Tarsus II, entire, but intermixed with very sparse longitudinal one or two rows of short spiniform setae. Tarsus III, entire, but intermixed with longitudinal two or three rows of strong long setae. Tarsus IV, entire, divided by four rows of strong long setae. All tarsi with a bald spot ventrobasally. Met. I covered almost all ventral surfaces, entire, but with longitudinal one or two rows of very sparse long setae. Met. II, covered almost all ventral surface, entire, but with longitudinal one or two rows of very sparse long setae. Met. III, covered 4/5 distally, entire, but with longitudinal one or two rows of very sparse long setae. Met. IV, covered 3/4 distally, divided by two or three rows of strong long setae. Claws: pair of claws present on all leg tarsi with one to three teeth on each claw. Tarsus IV with a short unpaired third inferior claw.

Color in life. Females are mildly dichromatic (Fig. 11B), with dark brown on carapace, abdomen, palp, and all legs, contrasted by dark femora on all legs and palp. Dark brown legs (except femora) are topped with pale brown setae, sparsely hirsute. Legs and abdomen reflect (by scales) a deep, mild purplish blue sheen. Generally, uniformly dark with a mild bluish sheen after ecdysis, which fades in a few days. Coloration changes, becoming pale brown, as the exoskeleton ages before ecdysis.

Natural history and distribution. Mature males were collected in September, and females with egg sacs (Fig. 19E) were found in February. Burrows, not selfdug, were found under piles of coconut husks (Fig. 19B, G) piles and crevices of metamorphosed limestones (marble) at the roadside embankments (Fig. 19C–F). They are also found in the beach forest of Bon Bon Beach in the northwestern coast of Romblon Island. The spiders were found on the burrow entrance at night, waiting for prey to ambush. This species is known only to occur in Romblon Island, Philippines (Fig. 22).

Etymology. The specific epithet is a masculine adjective derived from the combined names of the collaborating academic institutions and an organization of the project in which this study is involved, namely, the University of Santo Tomas (UST), Romblon State University (RSU), Mindanao State University-Iligan State University (MSU-IIT), University of the Philippines-Diliman (UPD), and the Philippine Arachnological Society, Inc. (PASI), attached with the Latin suffix *-us*.



Figure 19. Selenobrachys ustromsupasius sp. nov. **A**–**H** habitat and burrows on metamorphosed limestone (marble) piles and crevices and piles of coconut husks **E** female with egg sac **E**–**H** paratype females, in situ.

Discussion

Morphology and gene sequences are the two most important data that can be used in taxonomy and systematics. Taxonomic information acquired from the morphological analysis is, in many cases, being questioned and subjected to debate among taxonomists due to their differences in interpretations and opinions (Sites and Marshall 2004; Wiens 2004; Will and Rubinoff 2004; Dayrat 2005; Vences et al. 2013). The gene sequencing method appears to be ideal as it is very specific, but this method still needs to be supported with a physically observable characteristic to affirm its validity. The review of the taxa in this study is based on the combined information of morphology and DNA sequences with insights into their biogeography.

Morphological basis on the validity of *Selenobrachys* stat. rev. and *Chilocosmia*. stat. rev.

Selenobrachys and Chilocosmia were considered junior synonyms of Orphnaecus by West et al. (2012) based on the characters used in their cladistic analysis. They considered the oval proximally truncated lyrate patch of Selenobrachys an autapomorphy in the *Orphnaecus* clade, but we consider this synapomorphy within *Selenobrachys* with the discovery of additional *Selenobrachys* species (*S. ustromsupasius* sp. nov.) and five other potential undescribed species from other islands of Romblon PAIC and West Visayas PAIC known to the authors, which share the same lyrate morphology and spermathecal morphology. Additionally, the maxillary lyra of *Selenobrachys* is differentiated from *Orphnaecus* by its short bacilliform rods forming a dense ovoid patch that is truncated proximally and mildly tapering distally, and the rows of paddle-shaped lyrate bacillae at the lowest rows have distinctly thicker shafts prolaterally whereas the largest ones in the lowest row have a more pointed tip in prolateral view (Figs 7G, 13F, 17F, 20B, E). The short bacilliform rods on the lyra of *Orphnaecus* form a reniform patch (West et al. 2012), less dense, with rows of clavate bacillae that have elongated paddles and longer and stouter shafts and the largest have a rounded tip (Fig. 20A, D).

The secondary rows of cheliceral strikers of *Selenobrachys philippinus* comb. rest. were scored similarly (lanceolate in shape) with *Orphnaecus* specimens in the same cladistic study (West et al. 2012). However, our analysis shows that all *Selenobrachys* materials we examined have secondary rows of cheliceral strikers that are shorter, slightly curved, and spiniform in shape (Figs 8C, 13C, D, 17C, D), while *Orphnaecus* has straighter and longer lanceolate secondary strikers (West et al. 2012). West et al. (2012) also noted < 50 strikers in *Orphnaecus* and *Phlogiellus*, as one of the synapomorphies of the tribe Yamiini Kishida, 1920, but we recorded > 150 strikers (excluding tertiary strikers) on our *Selenobrachys* specimens. We also noticed that the very short needle-form tertiary rows of cheliceral strikers in *Selenobrachys* are greater in number (100–200) (Figs 8C, 13C, D, 17C, D) which corresponds with the very dense lyrate patch of the genus.

West et al. (2012) mentioned the similarity in spermathecal morphology (tombstone-shaped) of *S. philippinus* comb. rest. to their *Orphnaecus* specimens (QM S83782, S83783) from Masbate Island. With the absence of an illustration of the spermathecae of their *Orphnaecus* species, we can assume that it is just a resemblance, considering that the spermathecal morphology of *O. kwebaburdeos* (Fig. 18C) is almost in a tombstone-shape but still fits our description of *Orphnaecus*, in having longer spermathecal lobes with more concave prolateral margins which slightly pointing inward apically, creating asymmetry to the lobe (Fig. 18C, D). All *Selenobrachys* specimens we examined have broad and short spermathecal lobes, in a tombstone shape, almost symmetrical, with lateral margins almost parallel and very mildly or not concave prolaterally, not converging distally, and the apically rounded ends not pointing inward (Fig. 18A, B).

The male of *S. philippinus* comb. rest. has been unknown since the original description of the species. Fortunately, we were able to collect new specimens from the type locality in Mambucal, Negros Island, which included a single adult male. Additional *S. philippinus* male specimens were examined from SMNS. The palpal tibiae of male *Selenobrachys* are distinctly longer and cylindrical (Figs 9A–D, 15A, B), while in the sister genera, the palpal tibiae of males are proximally incrassate and tapering distally. Additionally, *Orphnaecus* males have a distinct, very dense, very long dorsal brush on the palpal patellae (Fig. 15A; West et al. 2012) which are also present on the palpal tibiae in some species.


Figure 20. Largest stridulating setae on maxillary lyra A–C dorsal view D–F prolateral view A, D Orphnaecus sp. B, E Selenobrachys philippinus comb. rest C, F Chilocosmia dichromata comb. rest G Orphnaecus pellitus, syntype Q, lyra on prolateral maxillary surface.

West et al. (2012) mentioned the synapomorphy of Yamiini of having a single retrolateral keel on the embolus of males. Based on our analyses of the palpal organ morphology of male *Orphnaecus* and *Selenobrachys* specimens, the retrolateral keel is actually an extremely long prolateral superior keel, following the

work of Bertani (2000), for which we adopted the structures on the embolus of males based on the position of the keels. The retrolateral keel emerges retrolaterally from the apex of the embolus to the rear (Bertani 2000), which is not the case in *Orphnaecus* and *Selenobrachys*. However, further studies on homologizing the structures of male palpal organs of Selenocosmiinae are necessary.

Selenobrachys species have relatively stouter legs dorsally (except for *O. pellitus* which also has stout legs caused by troglomorphism). We also explored the setation field on the prolateral surface of femur I, which we herein call femoral setation (FS): we found that *Selenobrachys* species have a field of short sword-shaped setae with a narrow base (Fig. 21E–H), while *Orphnaecus* have longer needle-form setae (Fig. 21A–D). However, it is suggested to further study the ultramorphology of the femoral setation under a scanning electron microscope.

Chilocosmia stat. rev. was synonymized based on the synapomorphic characters of Orphnaecus (West et al. 2012). Schmidt and von Wirth (1992) described the lyra of this genus as an arcuate strip of short rods consisting of an arcuate row of clavate bacillae. The largest bacillae are stronger (Fig. 21C, F) and the short rods are less dense (< 200) than in Orphnaecus and Selenobrachys stat rev. (> 300; if not absent or not rudimentary which are consequences of phenotypic plasticity). In addition, the morphology of the embolus with its strongly twisted tegulum and its missing BL and the missing long PS keel of the male of C. dichromata comb. rest. are not found in any described species of Orphnaecus and Selenobrachys stat. rev. (Fig. 5A-E). The dense long palpal brush on the dorsal male palpal patellae is also missing in C. dichromata comb. rest. (Fig. 5D). The female spermathecae of C. dichromata comb. rest. have reduced ends or are distally converging as in Orphnaecus, but not concave prolaterally and not pointing inwards (Schmidt and von Wirth 1992). The median carapace line in Orphnaecus is not unique to the genus but is also present in other Yamiini genera.

Genetic data sequencing and phylogenetic investigation

Most barcoding studies in animals utilize the cytochrome c oxidase subunit I (COI) gene due to its key characteristics such as universality and rapid substitution at the third codon position; the expansion of genetic databases has established a firm basis for utilizing this gene in the identification of specimens. However, the assessment of COI and 16S for DNA barcoding of farmland spiders from previous studies showed the potential efficiency of rRNA genes in identifying genetic species boundaries (Hamilton et al. 2011, 2016; Turner et al. 2018; Briggs et al. 2023) and their capability in identification during high-throughput experiments using various metabarcoding protocols (Wang et al. 2017; Vences et al. 2005). In this study, COI and rRNA genes have provided a preliminary understanding of the relationships between known Philippine tarantula species. Although the COI phylogenetic tree received very low bootstrap support, the assessment of genetic distances and support from morphology collectively contribute to a more robust depiction of the distinction between Orphnaecus, Selenobrachys stat. rev., and Chilocosmia stat. rev. This first molecular analysis for Philippine tarantula spiders is an important initiative for more molecular studies on these taxa.



Figure 21. Femoral Setation (FS) on left prolateral femur I A, B *Orphnaecus kwebaburdeos*, ♀, UST-ARC 0064 C, D *Orphnaecus* sp.' L3', ♀, UST-ARC 0134 E, F *Selenobrachys ustromsupasius* sp. nov., paratype ♀, UST-ARC 0005 G, H *Selenobrachys philippinus* comb. rest., ♀, UST-ARC 0112 I, J *Chilocosmia dichromata* comb. rest., ♂, SMNS Aran-004182 B, D, F, H, J magnified view of the left femur I median prolateral surface.

Significantly, the arrangement of species within clades on the phylogenetic trees (Fig. 1A, C) supports the morphological synapomorphies, particularly evident in instances like *S. philippinus* comb. rest. and the newly described species *S. ustromsupasius* sp. nov., across phylogenetic trees of both genes. The analysis of genetic distances offers quantitative insights into the extent of genetic similarity or divergence among different species that were initially delimited with morphological differences (Suppl. material 2). The observed pairwise distance values for both genes provide additional evidence of the relationships between genus and species. Earlier studies showed the differences in percentage values of intraspecific distance that was much lower than interspecific genetic distance (Powell and Caccone 1989; Cádiz et al. 2018; Ma et al. 2019, 2022). This supports the results of this study that the lower percentage differences between intraspecies and the higher percentage differences among the interspecies in the genetic distances of tarantula spiders indicate greater genetic similarity and divergence, respectively. The comparison of these values across species aligns well with both the morphological synapomorphies and the phylogenetic tree topology.

The Pleistocene Aggregate Island Complexes (PAICs) paradigm

Unlike most of the islands in Southeast Asia and Oceania, the Philippine Arc did not evolve from the Sahulian biogeographic realm nor the Eurasian plate (Queaño et al. 2020). The present islands of the Philippines, except Palawan, originated from the Philippine Islands arc system, which evolved as a result of subduction of the ocean floor along the trenches surrounding the Philippines during the Cretaceous period (Yumul et al. 2008; Aurelio et al. 2013). This paleogeographic history of the Philippines explains the existence of numerous flora and fauna unique to this country (Jones and Kennedy 2008).

Islands in the Philippine archipelago have been grouped and interconnected in the past due to Pleistocene sea level drops during the glacial periods (Heaney 1991; Heaney et al. 1998). These major island groups are known as Pleistocene Aggregate Island Complexes (PAICs) (Esselstyn and Brown 2009) and are based on the present 120 m isobath (Fig. 22). This formation has created the seven major biogeographical regions—namely, the Luzon PAIC (Greater Luzon), Mindanao PAIC (Greater Mindanao), West Visayan PAIC, Palawan PAIC, Sulu PAIC, Mindoro PAIC, and Romblon PAIC (Romblon Island Group; RIG) (Fig. 22). This concept may have facilitated the colonization and diversification of Filipino tarantulas. Tarantula spiders, like most mygalomorphs, have a limited mode of dispersion that is limited only to ground movement, making them less mobile than the araneomorph spiders. The archipelagic setting of the Philippines could have promoted rich diversification and unique evolutionary radiation for tarantula spiders, which is evident in the rich terrestrial fauna of the country (Heaney et al. 2016; Oaks et al. 2019; Pitchay and Torrentira 2022).

The results of our morphological and molecular analyses support the resurrection of the genus *Selenobrachys*, but we recognize its close relationship to *Orphnaecus* as a sister genus. Biogeographically, *Selenobrachys* might be limited to the Romblon Island Group + West Visayan PAIC. Citizen science sightings known by the first author provide the presence of the genus in other West Visayan + Romblon PAIC islands (except Masbate Island) which could still have five or more island-endemic new undescribed *Selenobrachys* species. Given its current distance from the rest of West Visayan PAIC islands, Masbate could have fragmented earlier from the rest of West Visayan PAIC islands before the diversification of the genus, hence explaining their potential absence on the island.



Figure 22. The seven major Philippine biogeographic regions based on the PAIC (Pleistocene Aggregate Island Complex) paradigm and the distribution map of valid species and published records of *Orphnaecus* (blue circles) and *Selenobrachys* (orange triangle). Inset: type localities of *Selenobrachys ustromsupasius* sp. nov.

We presume that the genus evolved from *Orphnaecus-Selenobrachys* ancestor from Luzon when West Visayan PAIC was still connected to Greater Luzon and became isolated upon its fragmentation. Molecular dating is needed to test this hypothesis. *Orphnaecus* is one of the most widespread theraphosid taxa in the Philippines, but they might not be able to colonize Mindoro, Romblon Island Group, Sulu Island Group, and the Palawan realm, owing to their different land mass origins. Proto-*Orphnaecus* may have ridden the proto-Luzon mass while rafting northward to its present position through the Philippine Arc System. The presence of *Orphnaecus* in West Visayan PAIC may be the result of recolonization after the first colonization and isolation of *Selenobrachys* when the islands reconnected through the oscillating water level during the Late Pleistocene, but during that time Romblon PAIC may not be reconnected to Panay thus explains the absence of *Orphnaecus* in the said island group, and Panay became their terminal expansion westward.

The placement of the Papuan species, *C. dichromata* comb. rest., in *Orphnae-cus* is biogeographically suspicious. Morphologically, this species is distinct from the Philippine *Orphnaecus* species for having an arcuate strip of short rods in lyra (Schmidt and von Wirth 1992), with slight differences in the structure of the spermathecae and a vastly different genital morphology of the males.

The Philippine Arc System and Papuan geographical disjunct is dramatic, although, the most recent possible land connection may have happened during the Late Oligocene to Late Miocene (~ 25–10 mya) when the Philippine Arc and Halmahera Arc may have aligned together creating a path for dispersal (Kalkman et al. 2018; Bocek and Bocak 2019). Genetic investigation by Autelin et al. (2021) on the freshwater gastropod subfamily, Miratestinae, reveals migration from Sahul to the Indo-Australian Archipelago, the Philippines, and the West Pacific Islands in the Early Miocene. It is interesting to test the same with Selenocosmiinae if they have utilized the same routes, which can only be done with the availability of more genetic data from the Sahulian and Sundaic taxa.

Conclusions

Evidence from morphological and molecular analyses as well as biogeographical insights revealed that the taxonomic status of the genus Selenobrachys is indeed valid. We therefore remove Selenobrachys Schmidt, 1999 from the synonymy of Orphnaecus Simon, 1892, and we restore the genus to its valid genus status, with its type species, Orphnaecus philippinus, original combination restored becoming Selenobrachys philippinus comb. rest., and we describe a second species for the genus, Selenobrachys ustromsupasius sp. nov., from Romblon Island. Furthermore, we recognize the close relationship of Selenobrachys and Orphnaecus based on the synapomorphic characters within the tribe Yamiini, thus placing Selenobrachys in this clade. As discussed above, we also restore the genus Chilocosmia Schmidt & von Wirth, 1992 stat. rev. and its type species Chilocosmia dichromata Schmidt & von Wirth, 1992, comb. rest. based on morphological, molecular, and biogeographic points. The number of genera within the subfamily Selenocosmiinae has now increased to 13, and the theraphosid fauna of the Philippines is now five genera and 14 species. Studying the biogeography of animals in the Philippines that are mostly limited to ground dispersal, like most mygalomorph spiders, may help us better understand the biogeographic history of the Philippines. Molecular analysis should always be supported by morphology (and vice versa) and other available evidence because delimitation based only on genetic divergence is subjective and has no universal standards and the variations in morphology do not always warrant species boundaries.

Acknowledgments

The authors would like to express their gratitude to the Philippines' Department of Science and Technology (DOST) and the National Research Council of the Philippines (NRCP) for the funding support provided to the GAGAMBA Research Program; to the Department of Environment and Natural Resources-Biodiversity Management Bureau (DENR-BMB) for granting our request for a gratuitous permit to collect samples and conduct research; to DENR- Region IV-B (MIMAROPA), Provincial Environment Natural Resources Office-Romblon (PENRO-Romblon), and Barangays Tambac and Guimpingan for providing the permits and clearances which allowed us to collect samples and conduct our research in Romblon Island; to the Community Environment and Natural Resources Office-Sipocot (CENRO-Sipocot), Protected Area Management

Bureau-Libmanan Caves National Park (PAMB-LCNP), PENRO-Camarines Sur for their hospitality and for allowing us to collect samples from LCNP; to the Provincial Government of Negros Occidental and the Northern Negros Natural Park-Protected Area Management Bureau (NNNP-PAMB) for generous assistance extended to us during collection and sampling in Negros Occidental; to CENRO-Real, Municipality of Real, and Barangay Aluyon, Burdeos for providing clearances during the sampling in Polillo Island and Real, Quezon Province; to the Municipality of Siniloan, Laguna and UP- Land Grant Management Office for providing clearances and assistance during the sampling in Siniloan and UPLG; to Dr. Henrik Krehenwinkel of Trier University (Germany) for conducting the COI sequencing of C. dichromata used in this study; to Dr. Christine Rolland (MHNP) and Boris Striffler for the opportunity to examine the syntypes of O. pellitus; to Dr. Peter Jaeger (SMF) for the opportunity to examine the holotype of S. philippinus and C. dichromata; to Dr. Joachim Holstein and Ingo Wendt (SMNS) for the opportunity to examine the specimens of C. dichromata and S. philippinus deposited in SMNS; to the University of Los Baños Museum of Natural History (UPLB-MNH) for the opportunity to examine the type specimens of O. kwebaburdeos; to Mr. Perry Buenavente of the Philippine National Museum (PNM) for the opportunity to examine the types of O. adamsoni and other spider specimens; to Mr. Frank Schneider for the natural history data and corresponding images of C. dichromata stat.rev.; to Mr. Erl Pfian Maglangit (MSU-IIT) for assisting in editing the images; to John Denver Fornillos of Laguna Polytechnic State University (Sta. Cruz), Lucky Jay Villaflor (Zambales), and Romel Enguito (Negros Occidental) for assistance in field sampling; and to the anonymous reviewers for their very detailed comments which greatly improved this paper.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by Department of Science and Technology - Philippines.

Author contributions

DCA is involved in the field sampling, conducted morphological and molecular phylogenetic analyses, examination of type specimens in the Philippines, and is the main person involved in writing the morphological and molecular analysis part of the manuscript; VVW examined the type specimens and other Selenocosmiinae specimens deposited in European collections and natural history museums and is also involved in writing the morphological analysis part of the manuscript. MKUD, MCR, and LPR performed DNA extraction and amplification, and were involved in writing the molecular analysis part; CNCN and ABRM participated in field sampling and preparation of the manuscript; GAF and MJAF assisted in sampling and preliminary morphological analysis; MRSB and LAG are involved in the conceptualization of the research, proposal writing and grants acquisition, preparation of the manuscript, and review, revision, and finalization of the manuscript.

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Data availability

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

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Supplementary material 1

List of all new genetic sequences deposited in GenBank

Authors: Darrell C. Acuña, Myla R. Santiago-Bautista, Leonardo A. Guevarra Jr Data type: pdf

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Supplementary material 2

Percent pairwise distances of all sequences of CO1 and 12S-tRNA-Val-16S

Authors: Darrell C. Acuña, Myla R. Santiago-Bautista, Leonardo A. Guevarra Jr Data type: pdf

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Short Communication

Redescription of *Tachyura* (s. str.) *ferrugata* (Reitter, 1895) (Coleoptera, Carabidae), with the comments on the availability of varieties described by Johan Reinhold Sahlberg in Tachyina

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Abstract

Tachyura (s. str.) *ferrugata* (Reitter, 1895) is redescribed and figured in detail, including male and female genitalia, and a lectotype is designated. Photographs of the type specimens, including labels, are presented. The female gonocoxites are figured for the first time. A review of varieties described by Johan Reinhold Sahlberg in Tachyina is presented, with comments on their availability. The following varieties are treated as unavailable infrasubspecific names: *Tachys* (*Tachyura*) *quadrisignatus* var. *caramanicus* J.R. Sahlberg, 1913, *Tachys* (*Tachyura*) *sexstriatus* var. *brunneicollis* J.R. Sahlberg, 1913, and *Tachys scutellaris* var. *obscurus* J.R. Sahlberg, 1913.

Key words: Bembidiini, museum collections, Palaearctic Region, Tachyina, taxonomy

Introduction

Reitter (1895) described *Tachys ferrugatus* Reitter, 1895 from Akbes (= Akbez municipality), which was at that time a Syrian territory and is now part of Hassa District, Hatay Province, Turkey. In the short original description, the number of type specimens is not mentioned, although there is a note that the species was common at the locality. However, only one syntype, designated here as the lectotype, was found in Reitter's collection deposited in the Hungarian Natural History Museum, Budapest (Kopecký 2009).

Winkler (1924) classified *Tachys ferrugatus* in the subgenus *Tachyura* Motschulsky, 1862. However, Csiki (1928) and Lorenz (1998) did not assign it to a subgenus. In the Catalogue of Palaearctic Coleoptera, Kopecký (2003) included this species in the nominal subgenus of *Tachyura* based on examination of the type specimen (although this was not stated) and based on following characters: body arched, the elytra sloping towards the apex from the posterior third at an angle of $40-50^{\circ}$ (in lateral view), the shape of male genitalia resembling "inverted knife blade" with a sclerotized lower part and a distinctly notched hook-like sclerotized tip, the first three humeral punctures are close to each other, the fourth puncture is clearly distant from them, the average ratio of the elytral length



Academic editor: Borislav Guéorguiev Received: 29 December 2024 Accepted: 5 March 2025 Published: 31 March 2025

ZooBank: https://zoobank. org/5835266C-11BA-494D-8B16-58750897A18E

Citation: Kopecký T, Bezděk J, Mattila J (2025) Redescription of *Tachyura* (s. str.) *ferrugata* (Reitter, 1895) (Coleoptera, Carabidae), with the comments on the availability of varieties described by Johan Reinhold Sahlberg in Tachyina. ZooKeys 1233: 195–206. https://doi.org/10.3897/ zookeys.1233.145545

Copyright: © Tomáš Kopecký et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). and width between 1.4–1.7. Finally, Kopecký (2009) published new distributional data and synonymized *Tachys schuberti* Jedlička, 1968 with *T. ferrugata*.

The original goals of this work were to present a detailed redescription of *T. ferrugata* and to synonymize *Tachys* (*Tachyura*) *quadrisignatus* var. *caramanicus* J.R. Sahlberg, 1913 with *T. ferrugata*. However, during the finalization of the manuscript, we discovered that var. *caramanicus* is an unavailable infrasubspecific name, which led us to check the availability of all the varieties described by J.R. Sahlberg in Tachyina. For the first time, *T. ferrugata* is redescribed and depicted using a vector graphic editor, including line drawings of the aedeagus and female gonocoxites.

Material and methods

This article is based on the study of extensive material deposited in the following collections: Hungarian Natural History Museum, Budapest, Hungary (HNHM); collection of Kamil Orszulik, Frýdek-Místek, Czech Republic (KOCF); collection of Michal Grycz, České Budějovice, Czech Republic (MGCC); Finnish Museum of Natural History, Helsinki, Finland (MZH); Natural History Museum, Milan, Italy (NHMM); National Museum, Prague, Czech Republic (NMPC); Naturhistorisches Museum, Wien, Austria (NMW); collection of Pavel Vonička, Liberec, Czech Republic (PVCL); collection of Tomáš Kopecký, Jablonné nad Orlicí, Czech Republic (TKCJ); Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia (ZIN); Museum für Naturkunde, Berlin, Germany (ZMHB).

Male and female genitalia were dissected, soaked in potassium hydroxide (KOH) then stained with chorazol black and glued using hydration resin onto glass mounted on a card under the specimens. Adult specimens were photographed using a FinePix S5600, Xiaomi 22101316G, Novex and Eakins binocular microscopes. Aedeagi and gonocoxites were photographed using a Xiaomi 22101316G camera on a VEVOR XSP-36TV microscope. Based on the photographs, precise anatomical line drawings were created with the Inkscape vector graphics editor. Exact label data are cited for all type specimens; backslash (\) separates data on different labels.

A lectotype was designated for *Tachys ferrugatus* in accordance with Articles 74 and 76 of the International Code of Zoological Nomenclature (International Commission of Zoological Nomenclature 1999) to preserve stability of nomenclature and fix unique bearer of the name of that taxon.

Comparative material

Tachys sinaiticus Schatzmayr, 1936

EGYPT • Holotype; "Sinai Wadi Ysla 27.2.35 W. Wittmer [white label] \ Typus [red label] \ T. sinaiticus Schatzm. [white label] \ HOLOTYPUS [red label]"; NHMM.

Tachys emeritus Péringuey, 1898

REPUBLIC OF SOUTH AFRICA • 1 paratype; "Graphicus barus [white label] \ Paratypus [red label] \ emeritus Pér. [white label]"; ZMHB.

Tachys thoracicus Kolenati, 1845

UNKNOWN COUNTRY • 1 syntype; "Transcauc. [red label] \ var. thoracica Kolent. [white label] \ Lectotypus Tachyura thoracica Kol. Kryzhanovskij det. [red label] \ ZOOLOGICAL INSTITUTE RAS ST. PETERSBURG [white label]"; ZIN.

Results and discussion

Tachyura (s. str.) ferrugata (Reitter, 1895) Figs 1, 2

Tachys ferrugatus Reitter, 1895: 79 (original description).
Tachys (Tachyura) quadrisignatus var. caramanicus J.R. Sahlberg, 1913b: 18 (unavailable infrasubspecific name).
Tachys schuberti Jedlička, 1968: 289 (original description).

Material examined. *Type material*: TURKEY • Lectotype (Fig. 1A, B); "Siria, Akbes [white label] \ coll. Reitter [white label] \ T. ferrugatus m. Akbes [white label] \ Holotypus 1895 Tachys ferrugatus Reitter [white label with red frame] \ Tachyura ferrugata Rtt. Det. K. Kult, 1955 [white label]"; HNHM.

Note on the type material: Reitter (1895) used the word "häufig" in the description of *Tachyura ferrugata*, meaning that the species was abundant in the type locality, without specifying the number of specimens examined. We found only one syntype in the Reitter collection deposited in the Hungarian Natural History Museum in Budapest (Fig. 1A, B). The syntype has a holotype label added by the curator Zoltán Kaszab in the 1960s without any justification. We cannot rule out that additional syntypes exist in other institutions. This is why we find it appropriate to follow Recommendation 73F of the Code for avoidance of assumption of holotype (ICZN 1999) and design the specimen in question as lectotype.

Jedlička (1968) described *Tachys schuberti* from Turkish localities "Selifke" (holotype) and "Namrum" (3 paratypes in coll. Schubert in NMW). The holotype (Fig. 1C, D) and 1 paratype are deposited in NMPC. The collections of the father Franz Theodor Adolf Schubert and his son Franz Xaver Schubert are stored in the Natural History Museum Vienna (Groll 2017) and two paratypes were traced there.

In the original description of *Tachys* (*Tachyura*) *quadrisignatus* var. *caramanicus*, Sahlberg (1913b) mentioned "25 specimina pauca invenit filius Unio" [= 25 specimens collected by his son Unio Johanson Sahlberg] collected in the valley of the Bulghar Dagh mountain range. In MZH, one original specimen of var. *caramanicus* from Bulghar Dagh, with the collector label "U. Sahlb." is deposited (Fig. 1E, F). The specimen is labelled "var. *anatolicus* J. Sahlb.", which can either be a mistake, or the name was changed during publication process, as no taxon with such name was described by J.R. Sahlberg. This specimen is conspecific with *Tachyura ferrugata*.

Non-type material: SYRIA • 2 spec.; 35 km E Latakia, Slanfah; 1200–1400 m a.s.l.; 29 Apr. 2011; K. Orszulik leg.; KOCF • 14 spec.; Homs env., 10 km N of Crac des Chevaliers, Mashta Al Hilu; 24 Apr. 2011, K. Orszulik leg.; KOCF • 2 spec.; same data as for preceding; TKCJ.



Figure 1. Tachyura (s. str.) ferrugata (Reitter, 1895), type specimens and their labels. A Tachys ferrugatus Reitter, 1895, lectotype B T. ferrugatus Reitter, 1895, lectotype, labels C T. schuberti Jedlička, 1968, holotype, female D T. schuberti Jedlička, 1968, holotype, labels E T. (Tachyura) quadrisignatus var. caramanicus J.R. Sahlberg, 1913, original specimen F T. (Tachyura) quadrisignatus var. caramanicus J.R. Sahlberg, 1913, labels.

TURKEY • 1 ♀ (Fig. 1C, D), holotype of *Tachys schuberti* Jedlička, 1968; "Selifke, Anat.m. 7.–9.6.63 leg. F. Schubert [white label] \ Holotypus [red label] \ Tachys schuberti sp. n. det. Ing. Jedlička [red label]"; NMPC • 3 spec., paratypes of *Tachys schuberti* Jedlička, 1968; "Namrun, Anat.m. 10.5.–3.6.63 leg. F. Schubert [white label] \ Paratypus [red label] \ Tachys schuberti sp. n. det. Ing. Jedlička [red label]"; NMW, NMPC • 1 spec. (Fig. 1E, F) [original specimen of *Tachys* (*Tachyura*) *quadrisignatus* var. *caramanicus* J.R. Sahlberg, 1913]; Bulghar Dagh; U. Sahlberg leg.; MZH • 1 spec.; Hatay Prov., Amanos Mts., Aslanli Beli; 970 m a.s.l.; 1 Jun. 1991; S. Kadlec leg."; MGCC • 8 spec.; same data as for preceding; TKCJ • 1 spec.; Harbiya; 22 May 1993; J. Krátký leg.; TKCJ • 1 spec.; Samsun Prov., Yunddagi Mts.; Cakiralan env.; 800–1000 m a.s.l.; 16 Jun. 1998, P. Vonička leg.; PVCL.

Redescription. *Body* (Fig. 2A–C). In lateral view, elytra more strongly arched, in anterior third slightly lowered, highest in posterior third, from which they bow to apex at an angle of 40°. Body length 2.38–2.50 mm, body width 0.88–0.95 mm.



Figure 2. *Tachyura* (s. str.) *ferrugata* (Reitter, 1895). **A** dorsal view, male, Turkey: Harbiya **B** dorsal view, male, Turkey: Harbiya, photograph contoured by line drawing **C** dorsal view, male, Turkey: Harbiya, line drawing **D** ventral view, female, Turkey: Bahçe, line drawing **E** aedeagus, Turkey: Harbiya, line drawing **F** aedeagus, Turkey: Harbiya **G** gonocoxite, Turkey: Çevlik.

Coloration. Dorsal side rusty shiny, not iridescent. Each elytron with indistinct traces of apical and humeral pale rust spots. Antennae rusty, antennomeres I, II and half of III pale rust. Legs pale rusty. Ventral part of head, prothorax, mesothorax and metathorax rusty, epipleuron of pronotum and elytra pale rusty, maxillary palpomeres I and II pale rusty, III pale, labial palpomeres I and II pale rusty, III pale.

Head. One-third narrower than pronotum, one quarter wider than long, eyes slightly prominent; labrum with convex anterior margin and with six short setae; clypeus with two punctures on anterior margin each one bearing long seta; frontal furrows double; external frontal furrows start near frontoclypeal suture, then sinuous to anterior setiferous punctures near eyes; internal frontal furrows broad, short and punctate, starting from clypeus and ending internally to anterior setiferous punctures; anterior setiferous punctures located on internal edges at anterior third of eyes, posterior setiferous punctures located on internal edges in posterior quarter of eyes; mandibles sharp, with one little tooth on internal edge; maxillae small, sharp, with two teeth and number of setae; antennomeres I–IV more than twice as long as wide, V–VI more than 1.5 times as long as wide, following antennomeres as long as wide, antennomere I with two setae, antennomeres II with four setae, rest of antennomeres with many setae.

Pronotum. 1.4 times as wide as long, widest before middle, lateral margins widely bent, except basal fifth subparallel; anterior margin nearly straight; posterior margin nearly straight, lateral parts of posterior margin slightly sinuate; anterior angles rounded; posterior angles sharp, each with posterolateral setiferous puncture bearing long seta; lateral margins bordered, each before middle with anterolateral setiferous puncture bearing long seta; basal humps indistinct; basal impressions acutely depressed, leading obliquely from posterior angle towards centre, each impression with ten punctures; area between basal impressions with deep median puncture; median line fine, impressed, beginning at anterior pronotal quarter and ending in median puncture of basal impression; surface without visible microsculpture at 50× magnification.

Elytra. Oval, 1.4 times as long as wide, with broad margin, without humeral teeth, widest at middle; each elytron with eight striae and without scutellar strioles, striae I–V and VIII well visible, with deep punctures, striae VI and VII punctiform (formed only by punctures), stria I begins next to scutellum and passes along suture to apex where it is connected with similarly prolonged stria VIII, which sinuate subapically, striae II–VII begin in anterior tenth and end in apical third of elytra; apical striola long, bent and directed towards stria III; surface without visible microsculpture at 50× magnification; humeral edge of the elytron passes humerus in form of short backward stria; each elytron with following setiferous punctures bearing long setae: large parascutellar puncture, four posthumeral umbilicate setiferous punctures, the first three at distance of their width, the fourth 1.5 times its diameter distant from third; one umbilicate puncture before apical third of elytral margin, two umbilicate punctures on elytral margin before apex; apical pore before half-length of apical striola at level of stria IV, two discal pores on inner edge of stria III, first in anterior third, second in posterior quarter.

Legs. Profemora with four setae on dorsal side, two long setae on anterior margin of ventral side and two long setae in middle of ventral side; outer edges of frontal parts of protibiae obliquely arcuately cut, with three spines, one spine on anterior margin interiorly next to protarsomere I, one spine on anterior margin exteriorly next to protarsomere I and one bigger spine on interior margin of ventral side; protibiae on exterior margin of dorsal side and in ventral side with row of setae; first two protarsomeres in male slightly serrated and extended to sides, each protarsomere on dorsal ventral sides with two setae, protarsomere I 1.5 times as long as wide, protarsomeres II–IV as long as wide, protarsomere V 3.0 times as long as wide, claw falcate. Mesofemora with some setae on anteri-

or margins of dorsal and ventral sides; mesotibiae with rows of setae on dorsal and ventral sides; mesotibiae at apical margin with three spines, two spines interiorly next to mesotarsomere, first in dorsal side and second on ventral side, one spine exteriorly next to mesotarsomere in dorsal side; mesotarsomeres I and V 3.0 times as long as wide, mesotarsomere II 1.5 times as long as wide, mesotarsomeres with two setae on dorsal and ventral sides, claw falcate; metafemora with some setae on anterior margins of dorsal and ventral sides; metatibiae at apical margin with three spines, two spines interiorly next to metatarsomere, first in dorsal side and second on ventral side, one spine exteriorly next to metatarsomere in dorsal side and second on ventral side, one spine exteriorly next to metatarsomere in dorsal side and second on ventral side, one spine exteriorly next to metatarsomere II-III 1.5 times as long as wide, metatarsomere IV as long as wide and metatarsomere V 3.0 times as long as wide, all metatarsomere W as long as wide and metatarsomere V 3.0 times as long as wide, all metatarsomeres with two setae on dorsal and ventral sides and metatarsomere V 3.0 times as long as wide, all metatarsomeres with two setae on dorsal and ventral sides.

Ventral surface (Fig. 2D). Mentum with deep incision on front edge, anterior margin on each side next to incision pointed, in centre of incision with small tooth, surface below incision on each side with one setiferous puncture; maxillary palpomeres I and II 2.5 times as long as wide, with tiny setae, maxillary palpomere III small and narrow, twice longer as wide and without setae; labial palpomere I narrow, 4.0 times as long as wide, without setae, labial palpomere II 1.5 times as long as wide, kidney shaped, with setae, labial palpomere III small and narrow, 3.0 times as long as wide and without setae; anterior part of gula laterally widened; anterior part of prosternum wide, tapering posteriorly, in middle with pore, prosternal projection rounded axe-shaped; procoxa rounded; procoxal trochanter 1.5 times as long as wide; mesosternum triangular; mesepisternum 1.5 times as long as wide; mesepimeron very narrow, attached laterally to mesepisternum, almost indistinct; mesocoxa rounded; mesotrochanter 2.0 times as long as wide, pointed at end; metepisternum wide, elongate triangular; metepimeron narrow, almost indistinct; metasternum diamond-shaped, in middle with two pores in fine grove; metacoxa horn-shaped; metatrochanter elongate rounded, 2.5 times as long as wide; abdomen with six visible ventrites, ventrite I narrow, widened from centre to sides, ventrites II-III fused, laterally with indistinct sutures, ventrites III-V with two setiferous punctures in middle, ventrite VI with four setiferous punctures in middle.

Aedeagus (Fig. 2E, F). Length 0.396 mm, evenly curved from base to apex in lateral view; superior margin with three shallow depressions and strong incision before apex; apex rounded and strongly sclerotized on underside; inferior margin slightly rounded, sclerotized along its entire length, internal structure not sclerotized; length of right paramere 0.223 mm, more sclerotized at base, with two long setae at apex.

Female. Length of gonocoxite 0.204 mm, from base to middle of amphora shape, basal gonocoxite on ventral side with two long apical setae, and one medial seta, apical gonocoxite separates by abdominal collar from basal one, formed on ventral side with tooth-shaped, the anterior part sickle-shaped with three pores (Fig. 2G).

Bionomy. The species lives on sandy gravel banks of streams, from lowlands to mountains.

Differential diagnosis. In the East Mediterranean and the Near East, *Tachyura* (s. str.) *ferrugata* can be mixed with *T*. (s. str.) *confusa* Coulon & Felix, 2011, *T*. (s. str.) *emerita* (Péringuey, 1898), *T*. (s. str.) *sinaitica* (Schatzmayr, 1936), and

Species (<i>n</i> = 10)	Body length (mm)	Elytra length / width (mean)	Dorsal striae	Pronotum width / length (mean)	Pronotum shape	Eye length / width (mean)
T. ferrugata	2.38-2.50	1.55	Eight strongly punctured dorsal striae, striae I–V and VIII well visible, with deep punctures, striae VI and VII formed only by punctures	1.56	Narrowed to apex, lateral parts of posterior margin slightly sinuate, posterior angles, sharp	1.67
T. emerita	1.83-2.08	1.51	Eight punctured dorsal striae, striae I–IV, with punctures, striae V– VIII formed only by punctures	1.94	Slightly narrowed to apex, lateral parts of posterior margin little sinuate, posterior angles obtuse	1.55
T. thoracica	2.00-2.45	1.57	Five finely punctured dorsal striae	1.37	Narrowed to apex, posterior margin little sinuate, posterior angles obtuse	1.43
T. sinaitica	2.00-2.23	1.65	Five finely punctured dorsal striae	1.33	Narrowed to apex, lateral parts of posterior margin slightly sinuate, posterior angles, sharp	2.00
T. confusa	1.88-2.05	1.59	Four dorsal striae, striae III in middle well visible and finely punctate, striae IV formed only by fine punctures	1.40	Narrowed to apex, posterior margin not sinuate, posterior angles obtuse	1.67

Table '	1. Differential	characters	for a	species	similar to	Tachvura	ferrugata.
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T. (s. str.) *thoracica* (Kolenati, 1845). Therefore, we list differential characters that help to distinguish the above five species (Table 1).

Distribution. Syria (Kopecký 2009; present paper), Turkey (Reitter 1895; Sahlberg 1913b; Jedlička 1968; Kopecký 2009; present paper), Yemen (Socotra Island) (Kopecký 2009). The occurrence in Greece, reported in the first edition of Catalogue of Palaearctic Coleoptera (Kopecký 2003), was based on erroneously determined specimens of *Tachyura thoracica* (Kopecký 2009). However, the occurrence in Greece was erroneously repeated in the second edition (Kopecký 2017).

Key to the identification of the East Mediterranean and the Near East species related to *Tachyura ferrugata*

- 3(6) Each elytron with five striae......4
- 5(4) Eyes more prominent, mean ratio of eye length / width 1.43. Body more robust than in *T. sinaitica*. Posterior pronotal angles obtuse. Coloration of dorsal side very variable, usually brown or dark brown, in dark specimens each elytron usually with apical and humeral rusty spots, in lighter specimens with distinct extensive light rusty apical and humeral spots, sometimes light rusty spots fused into longitudinal band (number of dark specimens in population increases towards north and to higher altitudes),

antennae dark rusty or brown, antennomeres I, II and half of III dark yellow or pale rusty, legs dark yellow or pale rusty. Body length 2.00–2.45 mm ... *T. thoracica* (Kolenati, 1845)

The varieties described by Johan Reinhold Sahlberg in Tachyina

Johan Reinhold Sahlberg (1903, 1913a, 1913b) described altogether six varieties in Tachyina. During a careful reading of Sahlberg's papers we found that four of these varieties represent unavailable infrasubspecific names since a subspecies concept was used in the same works, and according to available information, these names were not validated prior to 1985 (International Commission on Zoological Nomenclature 1999, Article 45.6.4.1). The type specimens and one specimen of each species from the former Johan Reinhold Sahlberg collection are deposited in the Finnish Museum of Natural History (MZH) and the rest of his collection is deposited in the Zoological Museum of the University of Turku (Groll 2017). However, from the original material of the varieties described in Tachyina, we found only one specimen of *Tachys* (*Tachyura*) quadrisignatus var. caramanicus in MZH.

Tachys scutellaris var. flavicollis J.R. Sahlberg, 1903: 5

An available name. A junior primary homonym of *Tachys flavicollis* Motschulsky, 1862. In catalogues it is usually listed as synonym of *Tachys (Tachys) dimediatus dimediatus* Motschulsky, 1849 (Kopecký 2003, 2017).

Tachys (Tachyta) parvulus var. coarctatus J.R. Sahlberg, 1903: 5

An available name. Sahlberg was evidently considering whether to describe this taxon as a separate species ("An species distincta?") but ultimately chose to describe it as a new variety. In catalogues it is usually listed as synonym of *Tachyura* (*Tachyura*) parvula (Dejean, 1831) (Csiki 1928; Kopecký 2003, 2017).

Tachys (Tachyura) sexstriatus var. brunneicollis J.R. Sahlberg, 1913b:19

An unavailable infrasubspecific name since a subspecies concept was used in the same work and the name was not validated prior to 1985 (International Commission on Zoological Nomenclature 1999, Article 45.6.4.1). It was previously listed as synonym of *Tachys euphratica* Reitter, 1885 (Kopecký 2017).

Tachys (Tachyura) quadrisignatus var. caramanicus J.R. Sahlberg, 1913b:18

An unavailable infrasubspecific name since a subspecies concept was used in the same work and the name was not validated prior to 1985 (International Commission on Zoological Nomenclature 1999, Article 45.6.4.1). It was listed by Kopecký (2017) as a subspecies of *Tachyura (Tachyura) quadrisignata* (Duftschmid, 1812).

Tachys scutellaris var. obscurus J.R. Sahlberg, 1913a:7 Tachys scutellaris var. obscurus J.R. Sahlberg, 1913b:19

Sahlberg (1913a, 1913b) proposed the name *obscurus* twice for the specimens from different localities. If they were available names, they would be homonyms. Because the subspecies concept was used in both works and the names were not validated prior to 1985 (International Commission on Zoological Nomenclature 1999, Article 45.6.4.1), they are infrasubspecific and unavailable. The first name was previously listed as a synonym of *Tachys centromaculatus* Wollaston, 1864, and the second name as a homonym of *T. scutellaris* var. *obscurus* J.R. Sahlberg, 1913a (Kopecký 2017).

Discussion

In Tachyina, the shape of gonocoxites is a neglected character which has been described in only a few species of the genus *Tachyura* (Baehr 2016; Kopecký and Bezděk 2023). The preliminary results of the comparison of gonocoxites drawings of several dozen species in Tachyina show this character is useful to distinguish the species, and it is possible to trace common characteristics in genera and subgenera.

Checking the availability of the names of varieties described by J.R. Sahlberg for the present paper points to a wider problem that apparently has never been comprehensively resolved within Carabidae (at least not in Tachyina). Not all variety and form names meet the conditions of Articles 45.5 and 45.6 of the Code (International Commission on Zoological Nomenclature 1999). For example, when a fourth name follows a trinomen, that name is automatically infrasubspecific. Likewise, the name is deemed to be infrasubspecific if it was first published after 1960 and the author expressly used one of the terms variety or form. In many cases it is not easy to determine whether the name of the variety or form is of subspecific or infrasubspecific rank. A dichotomous key for the determination of rank was published by Lingafelter and Nearns (2013). A thorough evaluation of the validity of names can significantly clean the system from unavailable names, as has recently happened for example in the Palaearctic Chrysomelidae (Bezděk and Sekerka 2024).

Acknowledgements

We would like to thank all colleagues who allowed us access to comparative material, to late Heinrich Schönmann (NMW) and Győző Szél (HNHM) for their help with study of the type material. We also thank Maxwell Barclay for checking the English language.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The research by Tomáš Kopecký in Naturhistorisches Museum, Wien was supported by Synthesys project No. AT-TAF-4893. The research by Jan Bezděk in the Finnish Museum of Natural History, Helsinki (FI-TAF-TA4-003) received support from the SYNTHESYS+ project: http://www.synthesys.info/, which is financed by the European Community Research Infrastructure Action under the H2020 Integrating Activities Programme, project number 823827.

Author contributions

Conceptualization: JB, TK. Data curation: TK. Formal analysis: TK. Investigation: TK. Methodology: TK. Resources: JM. Supervision: JB. Validation: JM. Visualization: JB. Writing – original draft: TK. Writing – review and editing: JB.

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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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Checklist

Revisiting Bacan Island in the footsteps of Alfred Russel Wallace: unveiling the diversity of land snails (Mollusca, Gastropoda)

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Academic editor: Frank Köhler Received: 4 December 2024 Accepted: 31 January 2025 Published: 31 March 2025

ZooBank: https://zoobank. org/9698E4DD-5CFE-4FCD-89C4-572E88C4623A

Citation: Nurinsiyah AS, Azizah SS, Prasetia AG, Mujiono N, Laitupa IW, Heryanto (2025) Revisiting Bacan Island in the footsteps of Alfred Russel Wallace: unveiling the diversity of land snails (Mollusca, Gastropoda). ZooKeys 1233: 207–243. https://doi. org/10.3897/zookeys.1233.143563

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Abstract

There are a total of 47 species from ten families of Gastropoda recorded on Bacan Island from 1861 to 1963 by 15 studies. In 2022, the island was revisited to investigate its current land snail diversity. Our survey yielded 555 individuals, which were identified and classified into 27 species from eleven families. Among these, nine species were newly recorded on Bacan Island, bringing the total number of known land snail species to 56. These new records include a new species, *Diancta batubacan* Nurinsiyah, Prasetia, Mujiono & Heryanto, **sp. nov**. The most abundant species collected was *Trochomorpha ternatana* (family Trochomorphidae). Differences in sampling locations and the extent of forest habitats in the surveyed areas may account for the different number of recorded species from previous and recent studies. Comprehensive systematic and standardised surveys are crucial for ensuring sampling completeness to further assess species endemism and biogeographic patterns. Furthermore, cataloguing all known species and resolving land snail's systematics with integrative approach are important to understand the true diversity of land snail in this region.

Key words: Biodiversity, Gastropoda, Indonesia, limestone, Maluku

Introduction

Bacan Island is one of the major islands in the North Moluccas archipelago, Indonesia. It is part of the Wallacea region, which also includes the Moluccan islands, Nusa Tenggara, and Sulawesi. Administratively, Bacan Island belongs to the South Halmahera Regency in the Province of North Moluccas, with Labuha serving as its capital. The island spans an area of 2,792.85 km² and has a population of 115,612 people (Badan Pusat Statistik Kabupaten Halmahera Selatan 2023). Geographically, Bacan is situated at the convergence of the Eurasian, Philippine Sea, and Australian tectonic plates, with Mount Batusibela (2,111 m a.s.l.) as its highest peak. The island's oldest rocks, part of the Sibela Continental Suite, are believed to date back to the Precambrian era (Malaihollo and Hall 1996). The island can be divided into two primary ecological zones: (1) forested areas with emerging karst, and (2) non-karst areas such as forest fringes, the banana field, and the cocoa garden both with shrubs (Fig. 1).

Research on the biodiversity of Bacan Island has a long history, with one of the earliest studies conducted by Alfred Russel Wallace. From October 1858 to April 1859, Wallace stayed at Bacan Island, where he collected a large number of animal specimens (Wallace 1869). Several land snail specimens were also collected. Many of his collections were sent to collectors and museums in Europe. Hugh Cuming received the land snail specimens from Bacan Island collected by Wallace, which were later studied and published by Pfeiffer (1861). Pfeiffer described numerous species, including *Helix ignescens* (= *Nanina ignescens*), *Helix batchianensis* (= *Trochomorpha ternatana*), *Helix nodifera* (= *Papuina nodifera*), *Helix atrofusca* (= *Planispira atrofusca*), amongst others. Wallace's land snail collections were also preserved in the Melvill-Tomlin Collection. Notably, among these collections was the genus *Pyrochilus*, which is endemic to the islands of Gilolo, Ternate, and Bacan (Rowson and Wood 2015).

Edgar A. Smith (1896) published a list of land snails from the Moluccas, which included specimens from Bacan Island provided by Bruno Strubell. In total, Smith (1896) listed 43 species, among which 29 were recorded from Bacan Island. He described one new species from Bacan, *Planispira (Cristigibba) lacteocincta*. Later, Sykes (1904) described two additional new species from Bacan Island: *Obba subgranulata* and *Papuina ecolorata*. The latter was synonymised with *Papuina vitrea* by van Benthem Jutting (1959). Sykes' specimens were obtained from



Figure 1. A map of Bacan Island and sampling locations of this study **B** map of Indonesia showing the location of Bacan Island (red square) **C** map of the North Moluccas Province showing the location of Bacan Island (red square).

various sources, primarily through collections made by Fruhstorfer. Also in the same year, Gude (1904) provided a list of land snail species from Bacan Island which included 35 species. However, four species were later synonymised or excluded in the list of van Benthem Jutting (1959) for several reasons. The species *Albersia zonulata* Férussac 1821 and *Papuina gaberti* Lesson 1831 were previously recorded by Wallace (1865) as native to Papua, while their presence in the Moluccas remained uncertain (van Benthem Jutting 1959). The species *Planispira* (*Cristigibba*) anozona was synonymised with *Planispira expansa* (= *Cristigibba*) expansa (Pfeiffer, 1861)) and *Planispira buelowi*, Rolle 1903 was synonymised with *Planispira lacteocincta* Smith, 1896 by van Benthem Jutting (1959).

A comprehensive record of land snails from Bacan Island, especially from Labuha, Babang, and Wajaua, was compiled by van Benthem Jutting (1959) documenting 52 species including six variations i.e., *Sulfurina parva* fa. *electrina* (Pfeiffer, 1859), *Papuina pileolus* var. *convexa* (Férussac, 1821), *Papuina pileolus* var. *parabolica* (Férussac, 1821), *Papuina pileolus* var. *furnita* (Férussac, 1821), *Planispira* (*Vulnus*) *endoptycha* var. *depressa* Sykes, 1904, and *Planispira loxotropis* var. *angusticlavia* (Martens, 1867). Additionally, two species were synonymised (van Benthem Jutting 1959): *Cyclotus bernsteini* von Martens, 1863 with *Cyclotus batchianensis* Pfeiffer, 1861 and *Cyclotus codonostoma* Möllendorff, 1902 with *Cyclotus guttatus* (Pfeiffer, 1853). In 1963, Loosjes added *Euphaedusa cumingiana moluccensis* in the species list from Bacan Island based on the collection of A.M.R. Wegner stored in the Zoologisches Museum of Amsterdam. In total, 47 land snail species are currently recognised from Bacan Island.

In 2022, we conducted a land snail expedition in Bacan Island. During this mission, we identified nine new records of land snails for the island. Among the new records we also discovered a new species from the island: *Diancta batubacan* sp. nov. from the family Diplommatinidae. With the addition of the new records, including the new species, the total number of land snail species currently recorded on Bacan Island has increased to 56.

Materials and methods

Study sites

The study was conducted in five locations in Bacan Island (Fig. 1), divided into karst and non-karst areas. The non-karst areas included (M1) a banana plantation with shrubs, a flat environment in Marabose Village, 0°38.97'S, 127°31.91'E of 128 m a.s.l.; (M2) a cocoa garden with shrubs, another flat environment in Sawadai Village, 0°45.18'S, 127°27.12'E of 84 m a.s.l.; (M3) a forest edge not far from a small stream, slope in Babang Village, 0°39.09'S, 127°32.39'E of 45 m a.s.l. The karst areas included (M4) a forest on karst at the small hill of Patinti Strait, Babang Village, 0°37.80'S, 127°37.71'E of 46 m a.s.l.; and (M5) a forest on karst, slope in Sumae Village, 0°35.11'S, 127°24.19'E of 54 m a.s.l.

Sample collection

Field work was conducted from May to June 2022. Both live snails and empty shells were carefully searched for by three persons for approximately five hours per sampling site, among leaf litter, rocks, wood debris, and plant stems. Live

snails were preserved in 70% alcohol, while empty shells were stored in labelled plastic bags. All specimens are deposited in the Museum Zoologicum Bogoriense (**MZB**), National Research and Innovation Agency in Cibinong, West Java.

Sample determination

Sample preparation was conducted at the Research Center for Biosystematics and Evolution, Soekarno Science and Technology Area. Species identification was conducted by referring to Martens (1867), Fulton (1899), Kobelt (1902), Neubert and Bouchet (2015), and Greķe (2017). Species systematics followed Molluscabase.org (2025). All collected species were photographed using a Nikon d3200 camera for macro snail (D > 5mm) and DMC5400 camera with L.A.S V4.13.0 software adapted to a Z6 APO (Leica Microsystems, Heerbrugg, Switzerland) for micro snail (D < 5mm). Shell characteristics for each species were described and measured with the measuring program in Leica M60 and a vernier caliper to the nearest 0.1 mm. The shell microsculpture of the new species was documented using a JEOL JSM-IT200 scanning electron microscope (SEM). The following abbreviations are used throughout the text:

D	Shell diameter/width
н	Shell height
ha	height of aperture
da	diameter/width of aperture
MZB	Museum Zoologicum Bogoriense
W	number of whorls

Results and discussion

A total of 555 individuals were collected from Bacan Island, representing 27 species across 11 families. Of these, nine species were recorded for the first time on the island including one species, *Diancta batubacan* sp. nov. which was identified as new to science. Combined with previous records from the literature, a total of 56 land snail species from 13 families are now known from Bacan Island (Table 1) with 13 species so far only recorded from the island.

The family Cyclophoridae exhibited the greatest species richness, comprising six species. However, Diplommatinidae was the most abundant family, accounting for 127 individuals despite being represented by only three species, followed by Trochomorphidae accounting for 111 individuals from two species. Notably, *Trochomorpha ternatana* (Trochomorphidae) was the most abundant species found in Bacan Island (18.2%). More than 50% of the collected species belong to the Caenogastropoda, a group of land snails often utilised as ecological indicators (Nurinsiyah et al. 2016).

A large difference in the number of specimens collected was observed across the five sampling locations and may be attributed to the land use (forested areas) in sites M3, M4, and M5 (Table 2). Specimens collected from M1 and M2 were fewer compared to those from M3, M4, and M5. The lowest abundance and species richness were observed at the cocoa garden sampling site (M2). However, the highest abundance was recorded in the forest non-karst area (M3) and the highest richness was recorded in the forest karst area (M5). Our findings revealed that

		0		This study		
NO.	Family	Species	Literature	Number of individuals	Relative abundance (%)	
1	Helicinidae	Sulfurina parva (Sowerby II, 1842)	√3,4,5,6,7,14	22	4.0	
2	Cyclophoridae	Cyclotus batchianensis Pfeiffer, 1861	√1,3,4,7,14	11	2.0	
3		Cyclotus guttatus (Pfeiffer, 1853)	√1,3,4,5,6,7,8,14	54	9.7	
4	-	Leptopoma decipiens Pfeiffer, 1861	√1,14	×	×	
5	-	Leptopoma globulosum Pfeiffer, 1861*	√ ^{1,7,14}	7	1.3	
6	-	Leptopoma halmahericum Strubell, 1892	×	8	1.4	
7	-	Leptopoma leucorhaphe von Martens, 1863	√4,7,14	12	2.2	
8	-	Leptopoma massena (Lesson, 1831)	√9,14	×	×	
9	-	Leptopoma papuanum Dohrn, 1862	√3,5,14	×	×	
10	-	Leptopoma pulicarium Pfeiffer, 1861*	√1,14	×	×	
11	-	Platvraphe plicosa (von Martens, 1863)	√4,7,14	5	0.9	
12	Diplommatinidae	Diancta batubacan sp. nov.*	×	16	2.9	
13	Dipioninatinado	Diancta torta Boettger 1891*	√6,7,14	45	81	
14	-	Diplommatina radiiformis Preston 1913	×	66	11.9	
15	Puninidae	Moulinsia cylindrica (Fulton, 1899)		16	2.9	
16	1 upinidue	Moulinsia solitaria von Martens, 1863	×	45	81	
17	_	Tylotoechus nfeifferianus (Adams 1869)*	/3,7,14	×	¥	
19	Veronicelloidae	Laevicaulia alte (Eérussac 1822)	v _/13	×	×	
10	Charapidaa	Dhilolopko kuoono (Aldrich 1990)	v	0	10	
20	Chronidae	Kolialla acandona (Cox 1972)	~	9	0.2	
20	Clausiliidee	Deceduce cumingiane melucenencia (ucn Martene 1964)	/15	1	0.2	
21	Trachamarphidae	Trachemarpha fraggetti (Iradala 1041)	V	3	0.5	
22	riochornorphidae	Treshana ar be terrestere (Le Quilley, 1941)	V-,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	10	1.0	
23	Muoroovotidoo	Lamprocycetia embanica Boettaar 1901	V 1,3 5,11,14	101	18.2	
24	wycrocystidae	Lamprocystis ambonica Boetiger, 1891	V ^{0,11}	8	1.4	
25	Autouloudidou	Lamprocystis Bacan 1 ^	×	10	2.9	
20	Anophantidae	Nanina Ignescens (Piellier, 1861)	V 1,0,5,1,1,14	×	×	
27	-	Nanina luctuosa Beck, 1837	√ ^{3,3,11,14}	×	×	
28		Nanina sulfurata von Martens, 1864	√ ^{2,4,7,11,14}	×	×	
29	Helicidae	Xesta citrina (Linnaeus, 1758)	X (24781114	6	1.1	
30	Camaenidae	Cochiostyla publicepa von Martens, 1864	V2,4,7,0,11,14	13	2.3	
31	_	Cristigibba corniculum (Hombron & Jacquinot, 1847)	√ ^{3,3,7,11,14}	×	×	
32	_	Cristigibba expansa (Pfeiffer, 1861)*	√ ^{1-4,0-9,11,14}	29	5.2	
33	_	Landouria winteriana (Pfeiffer, 1842)	V 0,7,11,14	×	×	
34	_	Obba subgranulata Sykes, 1904*	√10,14	×	×	
35	_	Papulna lanceolata (Pfeiffer, 1862)	√ ¹ *	X 1	×	
30	_	Papulna nodilera (Pleifier, 1861)^	V 3,7,11,14	I	0.2	
37	_	Papulna onlendorfil Kobelt, 1897*	√0,11,14	×	X	
38	_	Papulna pileolus (Ferussac, 1821)	V 3,4,7,0,9,11,14	17	1.3	
39	_	Papulna mynchostoma (Pfeiffer, 1861)*	√1,3,4,7,11,14 (510.14	17	3.1	
40	_	Papuna viirea (Ferussac, 1821)	V 0,10,14	×	×	
41	-	Planispira atrolusca (Pleiller, 1861)	V 1,3,4,7,0,11,14	×	×	
42	-	Planispira biconvexa (von Martens, 1884)	/34781114	×	×	
43	_	Planispira exceptioncula (Ferussac, 1823)	V 371114	×	×	
44	_	Planispira kurri (Pfeiffer, 1848)	V 3,7,11,14	×	×	
45	-	Planispira lacteocincia Smith, 1896°	V	×	×	
40	-	Planispira duadrifacciata (La Cuillau 1942)	V 757,11,199	× 0	×	
4/	-	Planiapira thatia (Deaiffor 1951)	×	Z	0.4	
48	-	Planiapira zapalia (Eárusasa 1931)	V 7,11,17	×	×	
49	_		V ····*	×	X	
50	-	Providence public contraction (Limitateus, 1707)	V	×	×	
51	_	Preseutopapulna scheepmakeri (Pteltter, 1850)	V 5,0,11,14	×	×	
52	_	Pyrochilus pyrostoma (Ferussac, 1821)	V ','',''	×	×	
53	-	Pyrochilus suicocinctus (von Martens, 1865)	√**,7,0,11,14 /473134	×	×	
54		Pyrochilus xanthostoma (von Martens, 1867)*	√ ^{4,7,11,14}	×	×	

Table 1. Comparative species list for Bacan Island, North Moluccas, Indonesia. Symbols – √: found, ×: not listed, *: so far only recorded in Bacan Island.

¹Pfeiffer (1861), ²von Martens (1864), ³Wallace and Adams (1865), ⁴von Martens (1867), ⁵Tapparone Canefri (1883), ⁶Boettger (1891), ⁷Smith (1896), ⁸Kobelt (1897), ⁹Fulton (1899), ¹⁰Sykes (1904), ¹¹Gude (1904), ¹²Boettger (1914), ¹³Grimpe and Hoffmann (1925), ¹⁴van Benthem Jutting (1959), ¹⁵Loosjes (1963).

√^{8,11,12,14}

√^{2,3,4,7,9,11,14}

×

25

Sulcobasis concisa rubra (Albers, 1857)

Vulnus endoptycha (von Martens, 1864)

55

56

×

4.5

	M1	M2	М3	M4	M5		
	N	lon-karst area	Karst		t area		
	Agriculture	/plantation	Forest				
Number of species abundance							
Abundance of operculate species	1	1	38	74	108		
Abundance of pulmonate species	27	0	147	86	73		
Total species abundance	28	1	185	160	181		
Number of species richness							
Richness of operculate species	1	0	5	4	7		
Richness of pulmonate species	3	1	4	6	10		
Total species richness	4	1	9	10	17		

Table 2. Number of species abundance and richness in each sampling site.

forest habitats had more individuals and species (526 specimens and 27 species) than agricultural habitats (29 specimens and 5 species). The species composition (operculate and pulmonate species) was also higher in the forest area compared to agricultural areas both in richness and abundance. This suggests that forest ecosystems have higher population density and species richness than agricultural habitats. This result aligns with previous studies: Raheem et al. (2008) recorded 46 land snail species in forest area and 28 species in home gardens in Sri Lanka and indicated that habitat types have significant effects on land snail composition. Higher numbers of species richness in forest areas compare to agricultural areas were also demonstrated in studies in Java (Nurinsiyah et al. 2016; Nurhayati et al. 2021).

Among the 27 species recorded on Bacan Island, the majority were distributed in humid areas containing karst formations. This pattern aligns with findings by Hausdorf (2019), who noted that land snails are more commonly found in volcanic soils and karst regions across various altitudes. This observation corresponds to the environmental characteristics of Bacan Island, which features extensive volcanic mountain ranges and karst forest areas. Our study reveals that the species abundance and species richness did not differ greatly between karst and non-karst areas. However, when considering habitat type, forest in karst area has the highest species richness. Previous studies indicated that the species richness and abundance in limestone areas were higher compared to non-limestone areas (Valdez et al. 2021; Boonmachai et al. 2024). These applied both for operculate and pulmonate species.

Differences in the richness of land snail species on Bacan Island can be attributed to several factors, with habitat alteration by humans being a critical driver. In particular, changes in forest cover on Bacan Island appears to have impact on land snail populations. However, even in the absence of direct human impact, the land snail biodiversity varies depending on various habitat characteristics, for instance soil moisture, soil pH, temperature, depth of leaf litter, canopy coverage, presence of deadwood, and the presence of herbaceous layers (Heryanto 2012; Douglas et al. 2013; Nurinsiyah et al. 2016; Rosales et al. 2020).

Land snails are known for their restricted geographic ranges and high sensitivity to habitat disturbances, including human activities, making them particularly vulnerable to local extinction (Nurinsiyah et al. 2016; Nurhayati et al. 2021; Boonmachai et al. 2024). The decline of land snail populations is often linked to their inability to adapt to extreme habitat changes. A notable indicator of habitat and community changes is the presence of micro-sized land snails. These species play a vital ecological role in the decomposition processes by facilitating fungal movement and contributing to nutrient cycling (Caldwell 1993; Pearce 2008). Micro-sized land snails are especially susceptible to environmental changes, particularly those caused by human disturbances (Douglas et al. 2013; Boonmachai et al. 2024).

The time elapsed since the earlier expeditions, such as those conducted in 1865, 1959, and 2017, highlights the need to compare the community structures of land snails in the recent and past forest conditions. The recorded diversity of land snails on Bacan Island likely represents only a small fraction of the biodiversity in Indonesia, particularly in the North Moluccas. Conducting more extensive research is essential to provide a more accurate estimation of species diversity and to identify the various environmental factors influencing biodiversity in the region. Furthermore, cataloguing all known species, resolving taxonomic ambiguities through integrative methods (e.g., molecular analysis, shell morphometrics, and anatomical studies), and stabilising species nomenclature will establish a robust foundation for understanding the true diversity of land snails on Bacan Island and the Moluccas Archipelago. Additionally, systematic and standardised surveys are essential to achieving sampling completeness. Such efforts are crucial for assessing species richness, endemism, and biogeographic patterns in the region.

Systematics

Class Gastropoda Cuvier, 1795 Subclass Caenogastropoda Family Diplommatinidae L. Pfeiffer, 1857 Genus *Diancta* E. von Martens, 1864

Diancta batubacan Nurinsiyah, Prasetia, Mujiono & Heryanto, sp. nov. https://zoobank.org/2AFDFC5E-4A8F-4BA7-B184-2C966DB3C03A Fig. 2A-D

Type material. *Holotype.* INDONESIA • Shell H = 5.2 mm; D = 2.8 mm; ha = 2.1 mm; da = 2.1 mm; W = 7.5; North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 23.855. *Paratype.* INDONESIA • same locality as holotype; MZB Gst. 23.856/15. Both holotype and paratypes were deposited in the Museum Zoologicum Bogoriense (MZB).

Diagnosis. Shell with bulbous penultimate whorl and a distinct constriction on the dorsal part of the penultimate whorl. Radial ribs are distinct before and after the constriction, but on the penultimate whorl the ribs are less distinct and almost smooth with no spiral striae. One outer parietalis, three parallel palatalis, and three perpendicular palatalis are present beside the constriction inside the penultimate whorl. Colour almost white.

Description. The shell is small, elongated, and sinistral with a pointed apex, whitish cream in colour. The shell has 7–7.5 whorls that increase in size, with the body whorl being narrower than the penultimate whorl. There is a rapid increase in shell whorl size from the beginning of the teleoconch until the 5th whorl, followed by a drastic constriction at the whorl 5.5–6, reducing the whorl size to that of the third whorl. The penultimate whorl (before constriction) is bulbous. The whorl size then increases again after the constriction for 1.5 whorls until



Figure 2. Shells of *Diancta batubacan* sp. nov. **A** holotype (MZB Gst.23.855) **B, C** paratypes MZB Gst.23.856 **D** scanning Electron Microscope image apical view of paratype MZB Gst. 23.856. Scale bars: 1 mm (**A**–**C**); 0.4 mm (**D**).

the aperture. The umbilicus is closed. The protoconch is smooth, lacking ribs on the first whorl. Radial ribs are not sinuous but rather distinct, low, thin, and densely placed. The spacing between ribs increases from the beginning of the teleoconch until the 5th or 6th whorl, where the whorl size increases rapidly, causing the ribs to become less distinct and almost smooth. After the constriction, the ribs become more widely spaced before transitioning to a tighter spacing towards the aperture. Spiral striae are absent. A constriction is present on the penultimate whorl, with one outer parietalis, three parallel palatalis, and three perpendicular palatalis beside the constriction. The aperture is slightly oval and nearly rounded. Peristome simple because the outer and inner peristome fused. Operculum multispiral. Shell dimensions of the specimens (n = 16): H = 4.9– 5.7 mm (mean 5.4 mm); D = 2.8–3.3 mm (mean 3.1 mm); ha = 2.0–2.4 mm (mean 2.2 mm); da = 1.8–2.3 mm (mean 2.1 mm).

Geographic distribution and habitat. The species has so far been recorded on Bacan Island, making it possibly endemic to this region. Specimens were collected from the karst forest habitat.

Remarks. Only one species of *Diancta* was previously recorded from Bacan Island, *Diancta torta* Boettger, 1891 (Fig. 4D). The species differs from *D. batubacan* sp. nov. by having prominent and more radial ribs both in the penultimate and body whorl; the shell of *D. batubacan* sp. nov. has a smooth penultimate whorl (almost without ribs). The aperture of *D. torta* is tilted and oval, while the aperture in *D. batubacan* sp. nov. is more rounded. The shell of *D. batubacan* sp. nov. is similar to *D. halmaherica* Greķe, 2017 which is currently known only from Halmahera Island, and *D. constricta* which so far only found in Ternate and Tidore islands. Compared to *D. halmaherica, D. batubacan* sp. nov. has a more bulbous penultimate whorl, and the ribs on the penultimate whorl are less distinct. Additionally, the aperture of *D. batubacan* sp. nov. is not tilted. The constriction on the penultimate whorl of *D. halmaherica* is more pronounced. The shell of *D. batubacan* sp. nov. also differs with *D. constricta* in having less distinct ribs, no spiral striae, and has three parallel palatalis and three perpendicular palatalis inside the penultimate whorl.

Etymology. The species name "batu bacan" refers to the famous Chrysocolla mineral/stone found on Bacan Island. Known for its striking beauty, the mineral shares a similar beauty to that of *Diancta batubacan* sp. nov.

Checklist

Class Gastropoda Cuvier, 1795 Subclass Caenogastropoda Cox, 1960 Family Helicinidae Férussac, 1822 Genus *Sulfurina* Möllendorff, 1893

Sulfurina parva (Sowerby II, 1842) Fig. 3A

Type locality. Philippine Islands.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°37.80'S, 127°37.71'E; alt. 46 m (M4); 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.22.926/22.



Figure 3. Shells of land snail species from Bacan Island **A** *Sulfurina parva* (Sowerby II, 1842) MZB Gst. 22.926 **B** *Cyclotus guttatus* (Pfeiffer, 1853) MZB Gst. 22.914 **C** *Cyclotus batchianensis* Pfeiffer, 1861 MZB Gst. 22.915 **D** *Leptopoma halmahericum* Strubell, 1892 MZB Gst. 23.941 **E** *Leptopoma leucorhaphe* von Martens, 1863 MZB Gst. 22.927. Scale bars: 1 mm (**A**); 5 mm (**B**–**E**).
Geographic distribution and habitat. *Sulfurina parva parva* (Sowerby II, 1842) and *Sulfurina parva* forma *electrina* (Pfeiffer, 1859) were both recorded on Bacan and Halmahera islands. In addition, the former species was also recorded on Obi Island (van Benthem Jutting 1959). In this study, the species was found in karst forest.

Description. (n = 10) Shell with moderate size with H = 3.8–7.6 mm (mean 4.9 mm), D = 4.9–7.0 mm (mean 5.8 mm), ha = 2.1–3.0 mm (mean 2.3 mm), da = 1.3–2.5 mm (mean 1.9 mm), and whorl 4–5. Rounded - conical shell, yellowish white colour with a smooth and shiny surface. Umbilicus narrow, aperture quadrangular, peristome not continuous.

Family Cyclophoridae Gray, 1847 Genus *Cyclotus* Swainson, 1840

Cyclotus batchianensis Pfeiffer, 1861 Fig. 3C

Type locality. Bacan Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°39.09'S, 127°32.39'E; alt. 45 m (M3); 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.915/2• North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.929/9.

Geographic distribution and habitat. The species was so far only recorded on Bacan and Obi islands. In this study, the species was found in forest and karst area.

Description. (n = 10) Shell moderate size with H = 10.4–12.7 mm (mean 11.4 mm); D = 19.7–23.9 mm (mean 21.6 mm); ha = 5.9–7.8 mm (mean 6.5 mm); da = 5.3–6.9 mm (mean 6.2 mm); and whorl 4–4.5. Flat shell with bulging aperture, mottled brown colour, 4–4.5 whorls, last whorl large and rounded. Umbilicus open, aperture oblique, peristome continuous.

Remarks. The species was first described by Pfeiffer (1861: pl. III, fig. 1) as *Cyclotus batchianensis*. *Pterocyclos batchianensis* Pfeiffer, 1861 was later recorded on Bacan Island and placed in a different genus by Wallace (Wallace and Adams 1865), although no detailed description was provided. Möllendorff (1902) and van Benthem Jutting (1959) subsequently documented *Cyclotus batchianensis bernsteini* von Martens, 1863 from Bacan and Obi islands. The shells of *C. batchianensis* and *C. pruinosus* von Martens, 1863 from Ternate, Tidore, Moti, and Halmahera islands are similar. However, resolving their systematics will require molecular phylogenetics and integrative taxonomy approach which is beyond the scope of this study.

Cyclotus guttatus (Pfeiffer, 1851)

Fig. 3B

Type locality. Unknown.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°39.09'S, 127°32.39'E alt. 45 m (M3); 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.914/32• North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.930/22. **Geographic distribution and habitat.** The species was recorded in Bacan Is., Ternate Is., and Halmahera Is. (van Benthem Jutting 1959). The species is distributed in the Wallacean region including North Moluccas (van Benthem Jutting 1959), Aru Island (Schepman 1919), and the northern part of Sulawesi (von Martens 1873). In this study, the species was found in the karst forest areas.

Remarks. Pfeiffer described the species in 1851 as *Cyclostoma guttatum* from the collection of Hugh Cuming. However, the type locality of this species was not mentioned. Later, Pfeiffer (1961) described *Cyclotus subflammulatus* from the collection of Alfred Russel Wallace on Bacan Island. Van Benthem Jutting (1959) synonymised the species to *Cyclotus guttatus*. Furthermore, *Cyclotus guttatus* forma *codonostoma* Möllendorff, 1902 was recorded in Bacan Island (van Benthem Jutting 1959). The subspecies has a more yellow-brown shell colour, the aperture double and slightly larger than *C. guttatus* with H = 14 mm and D = 22 mm (Möllendorff 1902).

Description. (n = 15) Shell large with H = 8.0–13.7 mm (mean 11.1 mm); D = 13.0–19.5 mm (mean 15.9 mm); ha = 5.0–7.7 mm (mean 6.5 mm); da = 5.0– 7.8 mm (mean 6.3 mm); 4–4.5 whorls. The shell has a pyramidal shape, brown with yellowish white tones, last whorl large and rounded. Surface of shell textured. Umbilicus open, aperture oblique, peristome continuous.

Genus Leptopoma Pfeiffer, 1847

Leptopoma decipiens Pfeiffer, 1861

Type locality. Bacan Island.

Remarks. Not found in this study. This species was described from Bacan Island as *Leptopoma decipiens* and figured in Pfeiffer (1861: pl. III, fig. 10).

Leptopoma globulosum Pfeiffer, 1861

Fig. 4A, B

Type locality. Bacan Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°37.80'S, 127°37.71'E; alt. 46 m (M4); 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.925/4 • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 23.942/8, 23.943/3.

Geographic distribution and habitat. So far, this species has only been found on Bacan Island. In this study, it was found at the forest edge in karst areas.

Description. (n = 11) Shell moderate size, H = 10.7–13.5 mm (mean 12.2 mm); D = 9.0–12.7 mm (mean 10.9 mm); ha = 5.3–7.7 mm (mean 6.3 mm); da = 5.0–6.7 mm (mean 5.9 mm); whorl 5–6.5. Conical shell with a pointed apex, transparent purplish-white in colour, sometimes with white base and brown stripes, last whorl large and convex. Umbilicus slightly open, aperture semicircle-oblique, peristome continuous. This species is said to be the same as *Leptopoma vitreum*, but the difference between the two is the variation of shell colour and size (von Martens 1867).



Figure 4. Shells of land snail species from Bacan Island **A**, **B** *Leptopoma globulosum* Pfeiffer, 1861 MZB Gst. 23.942 **C** *Platyraphe plicosa* (von Martens, 1863) MZB Gst. 22.930 **D** *Diancta torta* Boettger, 1891 MZB Gst. 23.860 **E** *Diplommatina radiiformis* Preston, 1913 MZB Gst. 23.861. Scale bars: 5 mm (**A**–**C**); 1 mm (**D**, **E**).

Remarks. *Leptopoma vitreum* von Martens (1867) was described from the Moluccas region, in several forms, including those with a uniform colouration and those with brown spiral bands. In Bacan Island, most recorded specimens exhibited a uniformly white coloration, although individuals with brown spiral bands were also noted (von Martens 1867). However, a similar shell form from Bacan Island was previously described, *L. globulosum* Pfeiffer, 1861. The *Leptopoma* species show intra/interspecies variability and sexual dimorphism in shell shape and colour banding patterns (Phung et al. 2017, 2022). Geographical variation also influences shell characters in the *Leptopoma* (Phung et al. 2017). Based on this, we classify the *Leptopoma* species found in this study to *L. globulosum* and not *L. vitreum*. Phylogenetic analysis is required to confirm the taxonomic relationships of the two species and determine whether these forms represent intraspecific variation or distinct species.

Leptopoma halmahericum Strubell, 1892

Fig. 3D

Type locality. Halmahera Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°39.09'S, 127°32.39'E alt. 45 m (M3); 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.913/2 • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.MZB Gst. 23.941/6.

Geographic distribution and habitat. New records for Bacan Island. The species was recorded on the Halmahera Is. (Greķe 2012). In this study, the species was found at the forest edge of the karst areas.

Description. (n = 8) Shell moderate size with H = 11.0-14.9 mm (mean 13.1 mm); D = 9.4-14.8 mm (mean 12.9 mm); ha = 5.2-7.8 mm (mean 6.5 mm); da = 4.3-7.8 mm (mean 6.3 mm); whorl 5-5.5. Conical shell with a pointed apex, white colour with brownish spotted pattern, and the last whorl large. Umbilicus closed, aperture oblique and looks like semicircle, peristome continuous.

Leptopoma leucorhaphe von Martens, 1863

Fig. 3E

Type locality. Halmahera Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.927/12.

Geographic distribution and habitat. This species has been found in Halmahera and Kajoa islands (von Martens 1863) as well as South Halmahera and Bacan islands (van Benthem-Jutting 1959). In this study, the species was found in the karst forest areas.

Description. (n = 10) Shell large with H = 10.5–13.0 mm (mean 12.1 mm); D = 9.0–13.9 mm (mean 11.4 mm); ha = 4.0–7.7 mm (mean 5.4 mm); da = 4.1– 6.6 mm (mean 5.1 mm); whorl 5–5.5. Conical shell with a pointed apex, brown with yellowish on the last whorl, 5–5.5 whorls, last whorl large and convex. Umbilicus slightly open, aperture oblique and semicircular, peristome continuous.

Leptopoma massena (Lesson, 1831)

Type locality. New Guinea.

Remarks. Not found in this study. This species was described as *Cyclostoma massena* from New Guinea. Subsequently van Benthem Jutting (1959) recorded the species from Bacan Island and placed it in the genus *Leptopoma*.

Leptopoma papuanum Dohrn, 1862

Type locality. New Guinea.

Remarks. Not found in this study. This species was described from New Guinea. Tapparone Canefri (1886) recorded the species from the Wallace collection on Bacan Island.

Leptopoma pulicarium Pfeiffer, 1861

Type locality. Bacan Island.

Remarks. Not found in this study. This species was described from Bacan Island as *Leptopoma pulicarium* and illustrated in Pfeiffer (1861: pl. 3, fig. 7).

Genus Platyrhaphe Möllendorff, 1890

Platyraphe plicosa (von Martens, 1863) Fig. 4C

Type locality. Halmahera Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 24.186/5.

Description. (n = 5) Shell small with H = 4.7–9.9 mm (mean 6.8 mm); D = 7.6– 15.9 mm (mean 11.3 mm); ha = 3.6–6.0 mm (mean 4.8 mm); da = 3.1–5.7 mm (mean 4.5 mm); whorl 5–5.5. Flat shell with prominent apex, whitish colour, brown on apex, the shell has roughly textured lines. Umbilicus open, perpendicular aperture, peristome continuous.

Remarks. New record for Bacan Island. This species was described from Halmahera Island as *Cyclotus plicosus* and figured in von Martens (1867: pl. 2, figs 13, 14). In this study, it was collected in karst forest.

Family Diplommatinidae Pfeiffer, 1857 Genus *Diancta* von Martens, 1864

Diancta torta Boettger, 1891 Fig. 4D

Type locality. Bacan Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°37.80'S, 127°37.71'E; alt. 46 m (M4); 31 May 2022; Heryanto, N.

Mujiono, I.W. Laitupa leg.; MZB Gst.23.858/13, MZB Gst.23.859/19, MZB Gst.23.860/13.

Geographic distribution and habitat. The species is so far only recorded on Bacan Island (van Benthem Jutting 1959; Greķe 2017). Thus, the species is possibly endemic to the island. In this study, the species was found in the karst forest.

Description. (n = 38) Shell small with H = 3.6-4.4 mm (mean 3.9 mm); D = 2.0-2.8 mm (mean 2.5 mm); ha = 1.1-1.8 mm (mean 1.6 mm); da = 1.1-1.8 mm (mean 1.6 mm); whorl = 6-7. Elongate and sinistral shell with pointed apex, cream whitish colour. Protoconch smooth without ribs for the 1.5 whorl. Teleoconch with dense distinct curved ribs. The ribs became distant on the body whorl towards the aperture. There is a constriction on the penultimate whorl. There are 6-6.5 whorls increasing in size, with body whorl narrower than penultimate whorl. Umbilicus closed, aperture oval to the left side, peristome expanded, thickened but not doubled.

Genus Diplommatina Benson, 1849

Diplommatina radiiformis Preston, 1913 Fig. 4E

Type locality. Belang-belang Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°39.09'S, 127°32.39'E alt. 45 m (M3); 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 23.861/66.

Geographic distribution and habitat. New record to Bacan Island. The species were recorded in the Moluccas archipelago i.e., Belang-belang Is. (Beilan-beilan Is.) to Obi Island (Preston 1913), Tidore Is., Halmahera Is. (Greķe 2017), and Moti Is. (Heryanto et al. 2023). In this study, the species was found at the edge of the forest and karst forest.

Description. (n = 10) Shell small size with H = 2.2–2.4 mm (mean 2.3 mm); D = 0.95–1.3 mm (mean 1.1 mm); ha = 0.6–0.7 mm (mean 0.7 mm); da = 0.7–0.9 mm (mean 0.8 mm); whorl = 7.5–8. Dextral shell and spindle shape with conical apex, whitish or corneous colour. Protoconch smooth, teleoconch with oblique ribs (penultimate and body whorl almost have same number of ribs) with spiral striae between ribs. The shell has 7.5–8 whorls increasing in size. Body whorl ventrally with constriction, on the inside with two spiral palatal folds close to suture. Umbilicus closed. Almost rounded aperture with distinct columellar lamella. Peristome expanded, thickened, and doubled.

Family Pupinidae Pfeiffer, 1853 Genus *Moulinsia* Grateloup, 1840

Moulinsia cylindrica (Fulton, 1899) Fig. 5A

Type locality. Dodinga, Gilolo Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Marabose Village; 0°38.97'S, 127°31.91'E; alt. 128 m (M1); 29 May 2022; Heryanto, N. Mujiono,



Figure 5. Shells of land snail species from Bacan Island **A** *Moulinsia cylindrica* (Fulton, 1899) MZB Gst. 22.909 **B, C** *Moulinsia solitaria* (von Martens, 1863) MZB Gst. 22.916, 23.853 **D** *Philalanka kusana* (Aldrich, 1889) MZB Gst. 23.862 **E** *Kaliella scandens* (Cox, 1872) MZB Gst. 23.478. Scale bars: 1 mm.

I.W. Laitupa leg.; MZB Gst. 22.909/1• North Moluccas, Bacan Is., Babang Village; 0°37.80'S, 127°37.71'E E; alt. 46 m (M4); 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 24.600/15.

Geographic distribution and habitat. The species was recorded in Gilolo Island (now Halmahera Island) and Bacan Island (van Benthem Jutting 1959). In this study, the species was collected in banana field.

Description. (*n* = 5) The species was described by Fulton (1899) as *Pupina* (*Moulinsia*) cylindrica. Shell dextral, medium-sized for the genus with size H = 4.8-5.8 mm (mean 5.4 mm); D = 2.6-3.3 mm (mean 3.0 mm); ha = 1.9-2.1 mm (mean 2.0 mm); da = 1.7-2.2 mm (mean 1.9 mm) and whorl 5.5. The shell is brown and glossy, covers with minute nodules. The apex is obtuse rather than pointed. The last whorl constitutes ~ 3/5 of the total shell height. The umbilicus is closed, and the aperture is rounded-oblique. Aperture lip is thickened, and the peristome is not continuous. Parietal tooth and parietal callus are absent. A small and vivid perpendicular columellar tooth present, creating a little anterior canal. Posterior canal is absent.

Moulinsia solitaria (von Martens, 1863)

Fig. 5B, C

Type locality. Makian and Moti islands.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°39.09'S, 127°32.39'E; alt. 45 m; 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.916/27, MZB Gst. 23.853/1, MZB Gst. 23.854/17.

Geographic distribution and habitat. New record for Bacan Island. The species was recorded on the islands of Makian and Moti (von Martens 1863) and Halmahera Island (Fulton 1899). In this study, the species was found in the forest edge.

Description. (n = 11) Shell moderate size with H = 8.1–9.8 mm (mean 8.8 mm); D = 5.5–6.8 mm (mean 5.9 mm); ha = 2.9–3.8 mm (mean 3.3 mm); da = 3.0–3.6 mm (mean 3.3 mm). Oval shell and slightly convex shell, yellow-ish, shiny, 4.5–5.5 whorls, the last whorl large and convex. Umbilicus closed, aperture highly rounded-oblique, peristome continuous. Parietal tooth pointed and receding. Sometimes a well-developed, vertically oriented tooth is present on the parietal wall, partially concealing the posterior slit that separates peristome. Parietal callus is present connecting parietal tooth and columellar tooth. Columellar tooth thickened and rectangular. Anterior and posterior canals are present.

Genus Tylotoechus Kobelt & Möllendorff, 1897

Tylotoechus pfeifferianus (H. Adams, 1869)

Type locality. Bacan Island.

Geographic distribution and habitat. Not found in this study, but this species was only recorded on Bacan Island by van Benthem Jutting (1959).

Remarks. The species was described by Adams (1865) in Wallace and Adams (1865) as *Pupina pfeifferi* based on the collection of Sounders from Bacan Island. In 1869, Adams corrected the species name to *Pupina pfeifferiana* because the previous name was pre-occupied by *Signepupina pfeifferi* (Dohrn, 1862). MolluscaBase (2024) has updated the name to *Tylotoechus pfeifferianus*.

Subclass Heterobranchia Family Veronicellidae Genus *Laevicaulis* Simroth, 1913

Laevicaulis alte (Férussac, 1822)

Type locality. Pondicherry.

Geographic distribution. Not found in this study. This species was described as *Vaginulus alte* from Pondicherry or Puducherry, India. van Benthem Jutting (1959) recorded the species from Bacan Island.

Family Charopidae Hutton, 1884 Genus Philalanka Godwin-Austen, 1898

Philalanka kusana (Aldrich, 1889)

Fig. 5D

Type locality. Southeastern Borneo.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village; 0°39.09'S, 127°32.39'E; alt. 45 m; 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 23.862/1.

Geographic distribution and habitat. New record for Bacan Island and the first record of a Charopidae in Bacan Island. The species is widely distributed from the eastern part of Indonesia (West Papua, Moluccas) to the western part of Indonesia (Sumatra), Singapore, and Malaysia (Vermeulen and Liew 2022).

Description. (n = 1) Shell very small with H = 2.1 mm, D = 2.7 mm, ha = 1.5 mm, da = 1.9 mm, and W 4.5. The shell is whitish, conical in shape, and ornamented by two spiral ridges on the second and third whorl and three apparent spiral ridges on the body whorl.

Family Clausiliidae Gray, 1855 Genus *Phaedusa* Adams & Adams, 1855

Phaedusa cumingiana moluccensis (von Martens, 1864)

Fig. 6A

Type locality. Halmahera Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.933/1, MZB Gst. 23.469/2.

Geographic distribution and habitat. The species is distributed in Bacan Island (Loosjes 1963) and the Moluccas archipelago i.e., Halmahera Island,



Figure 6. Shells of land snail species from Bacan Island **A** *Phaedusa cumingiana moluccensis* (von Martens, 1864) MZB Gst. 23.469 **B** *Trochomorpha froggatti* (Iredale, 1941) MZB Gst. 22.198 **C** *Trochomorpha ternatana* (Le Guillou, 1842) MZB Gst. 22.923. Scale bars: 5 mm.

Ternate Island (van Benthem Jutting 1959). In this study, the species was found in karst forest areas.

Description. (n = 2) The species was described from Djilolo, Halmahera Island as *Clausilia moluccensis* with H = 17 mm, D = 3 mm, ha, 3 mm, da 2.3 mm (von Martens 1864). The current expedition recorded the species with shell H = 16.9–17.5 mm (mean 17.2 mm); D = 3.5–3.6 mm (mean 3.5 mm); ha = 3.3 mm; da = 2.5–2.6 mm (mean 2.5 mm); whorls 9–11 increasing in size. Hight conical shell, brownish purple. Umbilicus closed, aperture oblique, peristome continuous and has three palatal plicae.

Family Trochomorphidae Möllendorff, 1890 Genus Trochomorpha Albers, 1850

Trochomorpha froggatti (Iredale, 1941) Fig. 6B

Type locality. Western New Guinea.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village, 0°39'5.61"S, 127°32'23.17"E; alt. 45 m; 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.918/4; North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.932/6

Geographic distribution and habitat. The species is widely distributed in Indonesia. In this study, the species was found on the karst forest edge.

Description. (n = 10) Shell moderate size with H = 2.2–5.7 mm (mean 4.7 mm); D = 7.8–13.9 mm (mean 10.8 mm); ha = 1.9–4.8 mm (mean 2.8 mm); da = 2.1–5.5 mm (mean 4.0 mm); whorl 4.5–5. Conical shell, gold in colour and shiny shell, 4.5–5 Whorls increasing in size. Umbilicus open, aperture semicircle-oblique, peristome not continuous.

Remarks. The species was recorded on Bacan Island by Wallace (1865) as *Trochomorpha planorbis* Lesson, 1831. However, the name *Helix planorbis* Lesson was invalid as it was preoccupied by Linnaeus and Iredale (1941) revised the Papuan species to *Necvidena*, which was later reclassified to *Trochomorpha* (MolluscaBase 2024).

Trochomorpha ternatana (Le Guillou, 1842) Fig. 6C

Type locality. Ternate.

Material examined. INDONESIA • North Moluccas, Bacan Is., Marabose Village; 0°38.97'S, 127°31.91'E; alt. 128 m (M1); 29 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.22.910/20; North Moluccas, Bacan Is., Babang Village, 0°39.09'S, 127°32.39'E; alt. 45 m; 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.22.917/22; North Moluccas, Bacan Is., Babang Village 0°37.80'S, 127°37.71'E; alt. 46 m; 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.22.923/36; North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.22.931/23.

Geographic distribution and habitat. The species was recorded in Bacan Island by van Benthem Jutting (1959). It was also recorded in the surrounding islands such as Ternate Is. (Le Guillou 1842) and Halmahera and Obi islands (van Benthem Jutting 1959). In this study, the species was collected in forest with karst and non-karst area, in the banana field, cocoa garden, and on the forest edge.

Description. (n = 40) Shell with moderate size H = 5.4–13.9 mm (mean 9.6 mm); D = 11.2–19.3 mm (mean 15.1 mm); ha = 3.1–6.8 mm (mean 4.9 mm); da = 3.8–8.8 mm (mean 6.9 mm); whorls 6–7 increasing in size. Conical shell, brown and shiny. Umbilicus open, aperture sickle-shaped, peristome not continuous.

Remarks. Pfeiffer (1861) described *Helix batchianensis* from Bacan Island. Later in 1865, Wallace mentioned the species but classified it in the genus *Tro-chomorpha* and van Benthem Jutting (1959) synonymised the species with *Tro-chomorpha ternatana*.

Family Chronidae Thiele, 1931 Genus *Kaliella* W.T. Blanford, 1863

Kaliella scandens (Cox, 1872) Fig. 5E

Type locality. Port Macquarie, Australia.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village 0°37.80'S, 127°37.71'E; alt. 46 m; 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 23.478/1.

Geographic distribution and habitat. New record for Bacan Island. In the Moluccas, the species was known from Ema, South Ambon, and other larger surrounding islands such as Hitulama, North Ambon, and Sirisori in Saparua (Boettger 1891). The species is widely distributed in Indonesia. In this study, the species was found in karst forest.

Description. (n = 1) Shell small size with H = 2.2 mm; D = 2.8 mm; ha = 0.5 mm; da = 0.8 mm; whorl 4. Hight conical shell, brown transparent shell. Umbilicus closed, aperture sickle-shaped, peristome not continuous.

Remarks. Boettger (1891) described this species as *Kaliella indifferens* from Ema, South Ambon. Based on Vermeulen et al (2015) the species name was synonymised to *Kaliella scandens*.

Family Microcystidae Thiele, 1931 Genus Lamprocystis Pfeiffer, 1883

Lamprocystis ambonica Boettger, 1891 Fig. 7A

Type locality. Ema, Ambon.



Figure 7. Shells of land snail species from Bacan Island A *Lamprocystis ambonica* Boettger, 1891 MZB Gst. 23.476 B *Lamprocystis* 'Bacan 1' MZB Gst.23.474 C *Xesta citrina* (Linnaeus, 1758) MZB Gst. 22.939 D *Cochlostyla pubicepa* von Martens, 1864 MZB Gst. 22.907 E *Cristigibba expansa* (Pfeiffer, 1861) MZB Gst. 22.937. Scale bars: 1 mm (A, B); 5 mm (C, E); 10 mm (D).

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village 0°37.80'S, 127°37.71'E; alt. 46 m; 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 23.476/ 8.

Geographic distribution and habitat. The species was recorded in Bacan Is. (Boettger 1891). It was also recorded from the surrounding islands such as Ema (Ambon), Banda Neira, and Haruku Island (Boettger 1891), and Halmahera Island (van Benthem Jutting 1959). In this study, the species was found in karst forest.

Description. (n = 8) Shell small size with H = 2.3–2.8 mm (mean 2.5 mm); D = 3.7–4.4 mm (mean 4.2 mm); ha = 1.5–1.9 mm (mean 1.7 mm); da = 1.2– 1.9 mm (mean 1.5 mm). Shell conical, yellowish golden in colour, shiny, transparent; 4–4.5 whorls increasing in size. Umbilicus closed, aperture sickle-shaped, peristome not continuous.

Lamprocystis 'Bacan 1'

Fig. 7B

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village 0°37.80'S, 127°37.71'E; alt. 46 m; 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.23.474/2, MZB Gst.23.477/3; North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.23.475/11.

Geographic distribution and habitat. The species was recorded in karst forest. **Description.** (*n* = 16) Shell small size with H = 3.3–5.3 mm (mean 4.3 mm); D = 3.9–7.1 mm (mean 5.3 mm); ha = 1.1–2.5 mm (mean 1.5 mm); da = 1.6– 2.7 mm (mean 2.2 mm). High conical shell, shiny shell with white colour and transparent, 4.5–5 whorls increasing in size and convex. Umbilicus close, aperture sickle-shaped, peristome not continuous.

Remarks. The species is similar to *Lamprocystis subangulata* Boettger, 1891 but differs in having a more convex whorl and rounded penultimate whorl. The species *L. subangulata* was recorded in Ema (South Moluccas) and in Carbau Mountain and Waai (Boettger 1891).

Family Ariophantidae Godwin-Austen, 1888

Nanina ignescens (Pfeiffer, 1861)

Type locality. Bacan Island.

Remarks. Not found in this study. This species was described from Bacan Island as *Helix ignescens* and figured (Pfeiffer 1861: pl. 2, fig. 1).

Nanina luctuosa Beck, 1837

Type locality. Indo-Australia archipelago.

Remarks. Not found in this study. This species was described by Beck (1937) and figured by Tryon (1886: pl. 19, fig. 85).

Nanina sulfurata von Martens, 1864

Type locality. Bacan and Halmahera islands.

Remarks. Not found in this study. This species was described from Bacan and Halmahera islands and figured in von Martens (1867: pl. 8, figs 1, 1b).

Genus Xesta Albers, 1850

Xesta citrina (Linnaeus, 1758) Fig. 7C

Type locality. Jamaica.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst.22.939/6.

Geographic distribution and habitat. New record for Bacan Island. The species was recorded in the Moluccan region including Ambon, Seram, and Buru islands (von Martens 1867), and Halmahera and Ternate islands (van Benthem Jutting 1959). The species can also be found in the Cendrawasih Bay on Biak Island (Tapparone-Canefri 1886). In this study, the species was found in karst forest.

Description. (n = 5) Shell large with H = 16.3-17.0 mm (mean 16.7 mm); D = 23.3-25.8 mm (mean 24.4 mm); ha = 10.0-12.6 mm (mean 10.7 mm); da = 11.4-14.9 mm (mean 12.6 mm). Rounded shell, dominant white colour with brown and yellow stripes from apex until body whorl, 4-5 whorls increasing in size. Umbilicus closed, aperture semicircle-oblique, peristome not continuous.

Remarks. The species was described by Linnaeus (1758) in *Helix*. The shell of *X. citrina*, *N. ignescens*, *N. luctuosa*, and *N. sulfurata* are very similar. Systematic revision with integrative approaches (phylogenetic, morphology, and anatomy) of this group is necessary to assess whether the colour patterns are useful for species diagnosis, as well as to clarify their taxonomy.

Family Camaenidae Pilsbry, 1895 Genus Cochlostyla A. Férussac, 1821

Cochlostyla pubicepa von Martens, 1864 Fig. 7D

Type locality. Halmahera and Bacan islands.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.934/9; North Moluccas, Bacan Is., Marabose Village; 0°38.97'S, 127°31.91'E; alt. 128 m (M1); 29 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.907/4. **Geographic distribution and habitat.** The species was recorded in Halmahera and Bacan islands (von Martens 1864), as well as on Obi Island (van Benthem Jutting 1959). In this study, the species was collected in the banana field and karst forest.

Description. (n = 11) Shell large with H = 14.6–24.0 mm (mean 18.5 mm); D = 17.3–31.8 mm (mean 23.0 mm); ha = 10.2–16.9 mm (mean 13.8 mm); da = 8.8–15.0 mm (mean 11.6 mm); whorl 5–5.5. Shell yellowish brown with dark brown spiral band on the body whorl.

Genus Cristigibba Tapparone Canefri, 1883

Cristigibba corniculum (Hombron & Jacquinot, 1847)

Type locality. New Guinea.

Remarks. Not found in this study. This species was originally described in *Helix*. It was recorded from Bacan Island by Wallace and Adams (1865).

Cristigibba expansa (Pfeiffer, 1861)

Fig. 7E

Type locality. Bacan Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.937/29.

Geographic distribution and habitat. The species is so far only recorded in Bacan Island, in karst forest.

Description. (n = 5) The species was described by Pfeiffer (1861) as *Helix* with H = 10 mm and D = 17.5–22 mm. The current expedition recorded shells with smaller sizes: H = 6.7–10.4 mm (mean 8.8 mm), D = 10.1–18.8 mm (mean 13.8 mm), ha = 5.4–8.0 mm (mean 6.8 mm), da = 4.5–7.8 mm (mean 6.2 mm) and whorl 4–4.5.

Genus Landouria Godwin-Austen, 1918

Landouria winteriana (Pfeiffer, 1842)

Type locality. Java.

Remarks. Not found in this study. Based on the recent systematic revision of *Landouria* in Java, the species of *L. winteriana* may have distributed in Java and Sumatera islands of Indonesia but the dispersal to the eastern part of Indonesia is unlikely (Nurinsiyah et al. 2019). The species in *Landouria* are often misidentified due to the similarities of shell morphology. Thus, the species in Bacan recorded by Boettger (1891) as *Helix (Plectotropis) winteriana* and Smith (1896) as *Eulota (Plectotropis) winteriana* might belong to different species; further collections are necessary.



Figure 8. Shells of land snail species from Bacan Island **A** *Papuina nodifera* (Pfeiffer, 1861) MZB Gst. 24.187 **B** *Papuina pileolus* (Férussac, 1821) MZB Gst. 22.921 **C** *Papuina rhynchostoma* (Pfeiffer, 1861) MZB Gst. 22.938 **D** *Planispira quadrifasciata* (Le Guillou, 1842) MZB Gst. 23.944 **E** *Vulnus endoptycha* (von Martens, 1864) MZB Gst. 22.920. Scale bars: 5 mm.

Genus Obba H. Beck, 1837

Obba subgranulata Sykes, 1904

Type locality. Bacan Island.

Remarks. Not found in this study. This species was described from Bacan Island as *Obba subgranulata* and figured by Sykes (1904: pl. 9, figs 5, 6).

Genus Papuina E. von Martens, 1860

Papuina nodifera (Pfeiffer, 1861)

Fig. 8A

Type locality. Bacan Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sawadai Village; 0°45.18'S, 127°27.12'E; alt. 84 m; 29 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 24.187/1.

Geographic distribution and habitat. So far, this species has only been found on Bacan Island (van Benthem Jutting 1959). In this study, the species was found in the cocoa garden.

Remarks. The species was described by Pfeiffer (1861) from the collection of Alfred Russel Wallace as *Helix nodifera* with the size H = 18 mm and D = 24-30 mm. The current expedition collected only one specimen of *P. nodifera* with a smaller shell, H = 14.3 mm, D = 18.3 mm, ha = 6.5 mm, da = 11.9 mm, and with 4.5 whorls.

Papuina ohlendorfii Kobelt, 1897

Type locality. Bacan.

Remarks. Not found in this study. It was recorded in Bacan Island and described and illustrated by Kobelt (1897: pl. 8, figs 6, 7).

Papuina pileolus (Férussac, 1821)

Fig. 8B

Type locality. Unknown.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village 0°37.80'S, 127°37.71'E; alt. 46 m (M4); 31 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.921/4; North Moluccas, Bacan Is., Marabose Village; 0°38.97'S, 127°31.91'E; alt. 128 m (M1); 29 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.908/3.

Geographic distribution and habitat. The species was recorded in southern Halmahera, Bacan Island, and near Telaga Manga Joang (van Benthem Jutting 1959). In this study, the species was found in the banana plantation in a non-karst area and in the karst forest.

Description. (n = 7) The species was described by Férussac (1821) as *Helix pileolus*. Shell large with: H = 17.1–20.5 mm (mean 18.6 mm); D = 22.0–25.9 mm (mean 23.9 mm); ha = 8.0–9.8 mm (mean 8.6 mm);

da = 8.1-11.5 mm (mean 10.1 mm). High conical shell, yellowish brown colour with white abstract pattern, 5-5.5 whorls increasing in size. Umbilicus closed, aperture oblique outer lips slightly thickened, peristome not continuous.

Papuina rhynchostoma (Pfeiffer, 1861)

Fig. 8C

Type locality. Bacan Island.

Material examined. INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.938/17.

Geographic distribution and habitat. So far, this species has only been found on Bacan Island (van Benthem Jutting 1959). In this study, the species was found in the karst forest.

Remarks. (n = 5) Shell large with H = 17.3–20.8 mm (mean 19.7 mm); D = 19.9–24.8 mm (mean 23.2 mm); ha = 7.0–10.0 mm (mean 8.6 mm); da = 9.2–14.2 mm (mean 10.8 mm). High conical shell, brownish white with yellowish strips, 5–5.5 rounded whorls increasing in size. Umbilicus closed, aperture oblique, and outer lips slightly thickened, peristome not continuous.

Papuina vitrea (Férussac, 1821)

Type locality. Unknown.

Remarks. Not found in this study. This species was described as *Helix vitrea*. Tapparone Canefri (1883) recorded the species from Bacan Island.

Genus Planispira H. Beck, 1837

Planispira atrofusca (Pfeiffer, 1861)

Type locality. Bacan Island.

Remarks. Not found in this study. This species was described from Bacan Island as *Helix atrofusca* and figured in Pfieffer (1861: pl. 3, fig. 3).

Planispira biconvexa (von Martens, 1864)

Type locality. Little Tawalli Island.

Remarks. Not found in this study. This species was described from Tawali Kecil Island near Bacan Island as *Helix biconvexa*. The species was figured later in von Martens (1867: pl. 16, fig. 13).

Planispira exceptiuncula (Férussac, 1823)

Type locality. Australia.

Remarks. Not found in this study. This species was described from Australia *Helix exceptiuncula* and figured in Pilsbry (1890: pl. 45, figs 50–53; pl. 65, figs 84–87). van Benthem Jutting (1959) recorded the species from Bacan Island.

Planispira kurri (Pfeiffer, 1848)

Type locality. Unknown.

Remarks. Not found in this study. This species was described as *Helix kurri*. It was recorded from Bacan Island by Wallace and Adams (1865) and figured by Pilsbry (1890: pl. 45, figs 21–23).

Planispira lacteocincta Smith, 1896

Type locality. Bacan Island.

Remarks. Not found in this study. This species was described from Bacan Island as *Planispira* (*Cristigibba*) *lacteocincta* and figured by Smith (1896: figs 3, 4).

Planispirra loxotropis (Pfeiffer, 1850)

Type locality. Moluccas.

Remarks. Not found in this study. This species was described as *Helix loxotropis* and figured by Pilsbry (1890: pl. 46, figs 60–64, 68). van Benthem Jutting (1959) recorded the species from Bacan Island.

Planispira quadrifasciata (Le Guillou, 1842)

Fig. 8D

Type locality. Ternate.

Material examined. INDONESIA INDONESIA • North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 23.944/2.

Geographic distribution and habitat. New record for Bacan Island. The species was recorded in Ternate (Le Guillou 1842), Halmahera, and Obi islands (van Benthem Jutting 1959). In this study, the species was found in karst forest.

Description. (*n* = 5) Shell large with H = 7.4-9.8 mm (mean 8.8 mm); D = 11.9-19.6 mm (mean 16.2 mm); ha = 6.0-8.6 mm (mean 6.9 mm); da = 4.7-7.0 mm (mean 6.3 mm). Rounded shell, white base colour with brown strip, 4-4.5 whorl increasing in size, last whorl has a wave that is close to the aperture. Umbilicus open, aperture oblique, peristome not continuous.

Planispira thetis (Pfeiffer, 1851)

Type locality. Unknown.

Remarks. Not found in this study. This species was described as *Helix thetis* and figured by Pilsbry (1890: pl. 56, figs 74–76). Van Benthem Jutting (1959) recorded the species from Bacan Island.

Planispira zonalis (Férussac, 1821)

Type locality. Moluccas.

Remarks. Not found in this study. This species was described *Helix zonalis* from Moluccan islands without mentioning a specific island or place. The species was figured by Pilsbry (1890: pl. 45, figs 24, 25, 29, 30). Van Benthem Jutting (1959) recorded the species from Bacan Island.

Planispira zonaria (Linnaeus, 1767)

Type locality. Southern Europe.

Remarks. Not found in this study. This species was described as *Helix zonaria*. von Martens (1867: pl. 16, figs 6–11; pl. 19, fig. 6) recorded the species from the Moluccan islands such as Ambon, Seram, Buru and Banda islands. Van Benthem Jutting (1959) recorded the species from Bacan Island.

Genus Pseudopapuina F. Haas, 1934

Pseudopapuina scheepmakeri (Pfeiffer, 1850)

Type locality. Moluccas.

Remarks. Not found in this study. This species was described as *Helix scheep-makeri* and figured by Pilsbry (1890: pl. 55, figs 40, 48, 49). van Benthem Jutting (1959) recorded the species from Bacan Island. Based on the figures from Pilsbry's plate, this species is very similar to *P. biconvexa* and they are possibly synonymous.

Genus Pyrochilus Pilsbry, 1893

Pyrochilus pyrostoma (Férussac, 1821)

Type locality. East Indies.

Remarks. Not found in this study. This species was described as *Helix pyrostoma* and figured by Pilsbry (1890: pl. 20, fig. 42). van Benthem Jutting (1959) recorded the species from Bacan Island.

Pyrochilus sulcocinctus (von Martens, 1864)

Type locality. Halmahera.

Remarks. Not found in this study. This species was described as *Cochlastyla sulcocincta* and figured by Pilsbry (1890: pl. 59, figs 39–41) as *Helix sulcocincta*. van Benthem Jutting (1959) reassigned it to the genus *Pyrochilus* and recorded the species from Bacan Island.

Pyrochilus xanthostoma (von Martens, 1867)

Type locality. Bacan Island.

Remarks. Not found in this study. This species was described as *Helix xanthostoma* Herklots; however, we cannot find any further description. The species described by von Martens (1867) was from Bacan Island.

Genus Sulcobasis Tapparone Canefri, 1883

Sulcobasis concisa rubra (Albers, 1857)

Type locality. Misool.

Remarks. Not found in this study. This species was described as *Helix rubra* from Aru Island. However, based on the examination of Boettger (1914), the species which Albers (1857) described referred to other subspecies *Sulcobasis concisa cumingi* Gude, 1906. True species of *Sulcobasis concisa rubra* were the ones recorded from the Moluccas and Bacan Island (Boettger 1914).

Genus Vulnus Sykes, 1904

Vulnus endoptycha (von Martens, 1864) Fig. 8E

Type locality. Ternate and Bacan islands.

Material examined. INDONESIA • North Moluccas, Bacan Is., Babang Village, 0°39'5.61"S, 127°32'23.17"E; alt. 45 m; 30 May 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.919/3; North Moluccas, Bacan Is., Babang Village 0°37.80'S, 127°37.71'E; alt. 46 m; 31 May 2022; HER, NM, ILW leg.; MZB Gst. 22.920/20; North Moluccas, Bacan Is., Sumae Village; 0°35.11'S, 127°24.19'E; alt. 54 m (M5); 1 June 2022; Heryanto, N. Mujiono, I.W. Laitupa leg.; MZB Gst. 22.936/2.

Geographic distribution and habitat. The species was recorded in Bacan Is. and Ternate Is. (von Martens 1864). In this study, the species was collected in the banana field and karst forest.

Description. (n = 14) Shell moderate in size with H = 5.3–7.9 mm (mean 6.8); D = 11.0–14.9 mm (mean 13.2 mm); ha = 4.0–5.9 mm (mean 5.2 mm); da = 2.9–3.9 mm (mean 3.5 mm). Flat shell, brown and shiny, 3.5–4 whorls increasing in size, last whorl rounded and large. Presence of a basal tooth, and a groove perpendicular to the coiling axis at the periphery of the body whorl ~ 1/4 of a whorl before the peristomal thickening. Umbilicus open, aperture oblique, peristome not continuous.

Acknowledgements

We are grateful to L. Muhdar, M. Djafar, U. Tukan, J. Sibua, MY. Alhaddad, S. Deky, K. Bungan, Firmansyah, M. Ilham, JK. Bunga, HH. Syarif for the help during field work. We also thank Junn Kitt Foon, Thor-Seng Liew, and Chih-Wei Huang for their valuable comments and suggestions for the improvement of this manuscript. We also thank the Nature Conservation Agency Maluku Province (BKSDA Maluku) for the permit.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The study was supported by Pendanaan Ekspedisi dan Eksplorasi Gelombang I 2022 (SK Deputi FRI No. 373/II/FR/3/2022) and Rumah Program Pengungkapan dan Pemanfaatan Biodiversitas Nusantara (SK Kepala Organisasi Riset Hayati dan Lingkungan 9/ III.5/HK/2023). The study is part of the research of "Revisi Biosistematika Beberapa Genera Keong Darat (Gastropoda: Cyclophoroidea: Cyclotus, Diplommatina, dan Papuina) dari Maluku Utara". The article processing charges is funded by RIIM Award 2024 Expedition Category (BRIN-LPDP).

Author contributions

ASN is the principal investigator, validate the species and wrote the manuscript; NM and HER conducted the field work and formulate the manuscript; IWL conducted the field work; SSA and AGP conducted the laboratory work and visualization.

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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

Survey of the Notonectidae (Insecta, Hemiptera, Heteroptera, Nepomorpha) from northeastern Brazil

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Abstract

A four-year survey for Notonectidae (Insecta: Hemiptera: Heteroptera: Nepomorpha) was conducted in eleven conservation units and adjacent areas distributed over six states in northeastern Brazil. Nearly 1400 specimens of the following 18 species, four genera, and two subfamilies have been collected: *Buenoa amnigenus* (White, 1879), *B. femoralis* (Fieber, 1851), *B. fuscipennis* (Berg, 1879), *B. koina* Nieser & Pelli, 1994, *B. konta* Nieser & Pelli, 1994, *B. mutabilis* Truxal, 1953, *B. pallipes* (Fabricius, 1803), *B. platycnemis* (Fieber, 1851), *B. pseudomutabilis* Barbosa, Ribeiro & Nessimian, 2010, *B. salutis* Kirkaldy, 1904, *B. tarsalis* Truxal, 1953, *B. unguis* Truxal, 1953 (Anisopinae); *Enitharoides brasiliensis* (Spinola, 1837), *E. tricomerus* Barbosa, Ribeiro & Nessimian, 2017, *Martarega bentoi* Truxal, 1949, *M. brasiliensis* Truxal, 1949, *M. membranacea* White, 1879, and Notonecta *disturbata* Hungerford, 1926 (Notonectinae). Altogether, they represent 30 new records from the states of Alagoas, Bahia, Ceará, Pernambuco, Piauí, and Sergipe, and seven new records from northeastern Brazil, increasing the number of species recorded from the region from 11 to 18. A key to these species is provided, as well as illustrations, diagnoses, taxonomic notes, and a summary of their known geographic distributions.

Key words: Aquatic insects, Atlantic Forest, backswimmers, biodiversity, Caatinga

Introduction

Notonectidae (Insecta: Hemiptera: Heteroptera: Nepomorpha) is a cosmopolitan family and second in richness only to Corixidae sensu lato among the aquatic bugs, with approximately 400 species and 11 genera (Polhemus and Polhemus 2008). Commonly known as backswimmers, because of the remarkable upside-down swimming method of all species in the family, they exhibit fusiform body up to 15 mm in length (Schuh and Slater 1995). They can be found in lotic and lentic habitats, like slow streams, artificial and natural ponds, and even small mud puddles, feeding on aquatic organisms such as mosquito larvae, crustaceans, and fish larvae (Papáček 2000). Notonectids go through four or five nymphal stages during their development and show wing polymorphism in some genera (Hungerford 1933). The family is currently divided in two subfamilies, Anisopinae and Notonectinae, both of which are present in Brazil.



Academic editor: Jader Oliveira Received: 9 February 2024 Accepted: 1 April 2024 Published: 1 April 2025

ZooBank: https://zoobank.org/ E95BDD70-F0F2-429D-9981-55AA5C47BBEA

Citation: Jordão R, Barbosa JF, Moreira FFF (2025) Survey of the Notonectidae (Insecta, Hemiptera, Heteroptera, Nepomorpha) from northeastern Brazil. ZooKeys 1233: 245–288. https://doi.org/10.3897/ zookeys.1233.120598

Copyright: © Rafael Jordão et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). The former is represented in the country by 31 species of the genus *Buenoa* Kirkaldy, 1904, and the latter by *Enitharoides* Brooks, 1953 (four species), *Notonecta* Fabricius, 1758 (eight species), and *Martarega* White, 1879 (fourteen species) (Moreira et al. 2011; Ribeiro et al. 2022).

Most of the data concerning backswimmers from Brazil are based on material collected in its southeastern portion (Moreira et al. 2011), with some recent advances in the northern, northeastern, and central-western regions (e.g., Barbosa et al. 2010a, b, 2012; Barbosa and Nessimian 2013a, b; Barbosa and Giehl 2014; Moreira et al. 2016; Takiya et al. 2016; Barbosa and Dias-Silva 2017). The Caatinga biome spreads through nearly 11% of the Brazilian territory, contained within nine states of the northeastern region and a small portion of northern Minas Gerais state (southeastern region). Surrounded by the Amazon, *Cerrado*, and Atlantic Forest biomes, it is a semiarid and dynamic environment.

The biodiversity of the Caatinga currently faces two large threats, namely the deforestation caused by cattle herding and climate change, both of which can interfere with the already erratic local rainfall regime and lead to a desertification process (Castelletti et al. 2004; Oyama and Nobre 2004). Moreover, recent observations indicate that conservation units in Brazil fail to effectively safeguard aquatic insects, as the protection they afford is comparable to that of randomly chosen areas. A primary contributing factor to this inefficiency is the haphazard selection of sites for development, often motivated by economic considerations or anecdotal evidence (Dias-Silva et al. 2021). Considering this scenario, we present here the partial results of a survey of the Nepomorpha from northeastern Brazil, focusing on the family Notonectidae and mainly on the Caatinga biome, but also including adjacent areas of Atlantic Forest.

Materials and methods

Specimens collected in Alagoas (AL) and Sergipe (SE) states were obtained in 2018 and 2019 as part of the project "Diversity and distribution of water bugs (Insecta: Heteroptera: Gerromorpha and Nepomorpha) of Alagoas and Sergipe, northeastern Brazil". Specimens collected in Bahia (BA), Ceará (CE), Pernambuco, (PE), and Piauí (PI) states are results of the project "Diversity and conservation of Hemiptera (Insecta) from the Caatinga", carried out from 2018 to 2021. The following eleven federal conservation units have been studied (Figs 11-20): Área de Proteção Ambiental da Costa dos Corais (APACC, AL), Área de Proteção Ambiental de Piaçabuçu (APAP, AL), Estação Ecológica de Murici (EEM, AL), Reserva Extrativista Marinha da Lagoa do Jeguiá (RESEX, AL), Reserva Biológica de Pedra Talhada (RBPT, AL/PE), Parque Nacional da Chapada Diamantina (PNCD, BA), Estação Ecológica de Aiuaba (EEA, CE), Parque Nacional do Catimbau (PNCA, PE), Parque Nacional da Serra das Confusões (PNSC, PI), Parque Nacional da Serra de Itabaiana (PNSI, SE), and Reserva Biológica de Santa Isabel (RBSI, SE). Brazilian states are abbreviated as follows: Acre (AC); Alagoas (AL); Amapá (AP); Amazonas (AM); Bahia (BA); Ceará (CE); Distrito Federal (DF); Espírito Santo (ES); Goiás (GO); Maranhão (MA); Mato Grosso (MT); Mato Grosso do Sul (MS); Minas Gerais (MG); Pará (PA); Paraíba (PB); Paraná (PR); Pernambuco (PE); Piauí (PI); Rio de Janeiro (RJ); Rio Grande do Norte (RN); Rio Grande do Sul (RS); Rondônia (RO); Roraima (RR); Santa Catarina (SC); São Paulo (SP); Sergipe (SE); Tocantins (TO).

The collecting methods employed were light traps (white sheet) and active sampling with sieves and aquatic nets in water bodies like swamps, puddles, streams, lakes, and small rivers. Specimen identification was based mostly on Truxal (1953), Barbosa and Nessimian (2013a, b), and Barbosa et al. (2017). Images of the specimens were acquired with a Leica M205C stereomicroscope at different focal distances and stacked using LAS CORE v. 4.6. Image enhancements were made using Adobe Photoshop CC 2015. Drawings and photographs were made based on male specimens. Material examined is deposited in the Coleção Entomológica do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (**CEIOC**), and in the Coleção Zoológica do Maranhão, Caxias, Brazil (**CZMA**).

In the distribution section of each species, all references that we are aware of are provided for the records from Brazilian states. For other countries or territories, only the first known record is provided. New state records are preceded by an exclamation mark "!" in the lists of material examined. Distribution maps were made with QGIS v. 3.16.3 (QGIS 2022). Truxal (1953) recorded *Buenoa antigone antigone* (Kirkaldy, 1899) from northeastern Brazil based on female specimens only, which is questionable. Because this is the only record of this species from the study area and it is absent from our samples, we decided to not include it in our results.

Results

Key to males of Notonectidae species found in northeastern Brazil

1	Labrum triangular (Fig. 1C, D); hemelytral commissure without hair-lined
	pit (Fig. 1A)[Notonectinae] 2
-	Labrum rounded (Fig. 1E); hemelytral commissure with hair-lined pit
	(Fig. 1B)[Anisopinae, Buenoa] 7
2	Middle femur with anteapical pointed spine (Fig. 2C, F) [Notonectini] 3
_	Middle femur without such structure[Nychiini, Martarega] 5
3	Anterolateral margins of pronotum straight, without foveae (Fig. 2D); male
	parameres symmetrical Notonecta disturbata Hungerford, 1926 (Fig. 10M)
-	Anterolateral margins of pronotum foveate (Fig. 2A); male parameres
	asymmetrical
4	Scutellum with wide, subrectangular, punctate area medially (Fig. 2B); mid-
	dle femur with short pubescence and poorly developed anteapical protuber-
	ance (Fig. 2C) Enitharoides brasiliensis (Spinola, 1837) (Fig. 10N)
-	Scutellum with widely distributed, deeply punctate area (Fig. 2E); middle fe-
	mur with long pubescence and robust anteapical protuberance (Fig. 2F)
	Enitharoides tricomerus Barbosa, Ribeiro & Nessimian, 2017 (Fig. 100)
5	Ocular commissure < 1/2 of eye width (Fig. 6A); ventral surface of middle
	trochanter with a small group of ensiform bristles centrally (Fig. 3A)
-	Ocular commissure 1/2 of eye width or more; ventral surface of middle
	trochanter with a patch of thin setae6
6	Ocular commissure half of eye width; third labial article pubescent; scutellum
	with hyaline apex (Fig. 3B) Martarega brasiliensis Truxal, 1949 (Fig. 10R)
_	Ocular commissure more than half of eye width; third labial article gla-
	brous; scutellum with posterior half of its length hyaline (Fig. 3C)
	Martarega membranacea White, 1879 (Fig. 10S)

7 Synthlipsis wide, half of anterior width of vertex or slightly	less (Fig. 6B) 8
- Synthlipsis narrow, less than half of anterior width of verte	ex 12
8 Labial prong longer than third labial article (Fig. 6C)	9
- Labial prong equal to or shorter than third labial article	11
9 Apex of fore femur narrowed (length > 3× the width at ape	ex, Fig. 6D); mid-
dle tarsus with first article deeply emarginated (Fig. 4A)	
Buenoa tarsalis Truxal,	1953 (Fig. 10K)
- Apex of fore femur slightly widened (length \leq 3× width a	at apex); middle
tarsus without such modification	10
10 Large species (7.8–8.6 mm); labial prong slightly longer tha	n third labial arti-
cle, nearly straight (Fig. 7A)Buenoa femoralis (Fieber,	1851) (Fig. 10B)
 Medium-sized species (5.5–6.2 mm); labial prong much l 	onger than third
labial article, arc-shaped (Fig. 6C)	~
Buenoa pallipes (Fabricius,	1803) (Fig. 10G)
11 Labial prong subequal to slightly longer than third labial ar	ticle length; fore
trochanter without conspicuous process pointing backw	ards; fore femur
widened at apex; middle tibia not widened at apex	
Buenoa koina Nieser & Pelli,	1994 (Fig. 10D)
- Labial prong distinctly shorter than third labial article (F	ig. 7D); fore tro-
chanter with a conspicuous process pointing backward	s (Fig. 4B); fore
femur not widened at apex; middle tibia widened at apex	
	1879) (Fig. 10C)
12 Fore femur with stridulatory area (Fig. 6D, small arrow)	
 Fore femur without stridulatory area 	15
13 Labial prong originating distally on third labial article; ap	ex of fore femur
widenedBuenoa platycnemis (Fieber,	1851) (Fig. 10H)
- Labial prong originating proximally or medially on third la	bial article; apex
of fore femur narrowed	
14 Tylus pubescent; stridulatory area of foretibia with 21-2	5 teeth; abdomi-
nal ventral laterotergite I with a dark nodule (Fig. 5B)	
Buenoa pseudomutabilis Barbosa, Ribeiro & Nessimian,	2010 (Fig. 10I)
- Tylus glabrous; stridulatory area of foretibia with 33-38 t	eeth; abdominal
ventral laterotergite I without dark nodule	
Buenoa mutabilis Truxal,	, 1953 (Fig. 10F)
15 Tylus flat; labial prong shorter than third labial article; fore ta	arsus with claws
orthogonal-angulated (Fig. 5A)Buenoa unguis Truxal	, 1953 (Fig. 10L)
- Tylus rounded; labial prong subequal to or longer than th	ird labial article;
fore tarsus with unmodified claws	16
16 Synthilipsis ~ 1/4 of vertex width; blackish brown hoof pri	nt-like pattern at
hemelytra in dorsal view; apex of fore femur widened	-
Buenoa konta Nieser & Pelli,	1994 (Fig. 10E)
 Synthilipsis < 1/4 of vertex width; hemelytra without such 	pattern; apex of
fore femur not widened	
17 Synthlipsis very narrow, < 1/10 of vertex width; frons narro	w; labrum sided
by pair of tufts of setae (Fig. 7B); labial prong slightly longer	r than third labial
article, with apex rounded Buenoa amnigenus (White,	1879) (Fig. 10A)
 Synthlipsis narrow, > 1/10 of vertex width; frons wide; labra 	um without tufts
of setae; labial prong subequal to slightly longer than th	ird labial article.
	,



Figure 1. Anisopinae and Notonectinae, examples of structures **A**, **B** scutellum and anterior part of hemelytra, dorsal view **A** Notonectinae **B** Anisopinae **C**–**E** head, anterior view, outline of labrum highlighted **C** Martarega **D** Enitharoides **E** Buenoa.



Figure 2. Notonectini, morphological characteristics **A** *Enitharoides*, foveate anterolateral portion of pronotum delimited by dotted line **B**, **C** *Enitharoides brasiliensis* **B** punctation of pronotum, dorsal view **C** middle femur, ventral view **D** *Notonecta disturbata*, unfoveate anterolateral portion of pronotum **E**, **F** *Enitharoides tricomerus* **E** punctation of pronotum, dorsal view **F** middle femur, ventral view.



Figure 3. *Martarega*, morphological characteristics **A** *Martarega bentoi*, middle trochanters, ventral view, small groups of ensiform bristles delimited by dotted line. Trochanters of right image without dots **B** *M*. *brasiliensis*, dorsal view of scute-llum, hyaline apex delimited by absence of dotted pattern **C** *M*. *membranacea*, dorsal view of scutellum.



Figure 4. *Buenoa*, structures of two species, highlighted with black dots in right illustrations **A** *B*. *tarsalis*, emarginated first article of middle tarsus, ventral view **B** *B*. *fuscipennis*, posteroventral portion of fore trochanters, ventral view. Right illustrations modified from Truxal 1953.



Figure 5. *Buenoa*, structures of two species **A***B*. *unguis*, male fore tarsus, ventral view **B***B*. *pseudomutabilis*, male abdominal ventral laterotergite I, ventral view.



Figure 6. *Martarega* and *Buenoa*, measurements of head and fore femur **A** *Martarega*, head, dorsal view **B**–**D** *Buenoa* **B** head, dorsal view **C** length of labial prong, lateral view **D** fore femur, ventral view, black arrow indicates stridulatory area. Abbreviations: ew = eye width, fl = femoral length, fw = femoral width, oc = ocular commissure, s = synthlipsis width, v = vertex width. Scale bars: 1 mm.



Figure 7. Buenoa, male head, lateral view **A** Buenoa femoralis **B** B. amnigenus **C** B. salutis **D** B. fuscipennis. Scale bars: 1 mm.



Figure 8. *Notonecta*, male genital capsule, lateral view, modified from Hungerford 1933 **A** *Notonecta disturbata* **B** *N*. *pulchra*. Abbreviations: cap = caudoventral protuberance, par = paramere.

New records

Subfamily Anisopinae Buenoa Kirkaldy, 1904

Buenoa amnigenus (White, 1879) Fig. 10A

Anisops amnigenus White, 1879: 271 [original description]. Buenoa amnigenus; Kirkaldy (1904): 120 [genus transfer].
Diagnosis. Small species, male length 4.8–5.4 mm. Synthlipsis very narrow, < 1/10 of vertex width. Labrum sided by pair of tufts of setae. Labial prong slightly longer than third labial article, originating proximally on article. Fore femur without stridulatory area.

Taxonomic notes. A member of the *salutis* group. It differs from the most similar species, *B. amnigenoidea* Nieser, 1970, by the chaetotaxy of the labrum and minor characteristics of the fore trochanter, femur, and tibia. Also, from *B. amnigenopsis* Nieser, 1975 by the presence of a notch on the apex of the fore femur of this species, which is absent in *B. amnigenus*. Finally, it can be distinguished from *B. salutis* based on the larger size, narrower synthlipsis and more pronounced tylus. More detailed comparisons between species of this group can be found in Barbosa and Nessimian (2013a).

Distribution. Argentina (Bachmann 1971). Bolivia (Truxal 1953). Brazil: AL [new record], AM (White 1879; Truxal 1953; Nieser 1975; Barbosa and Nessimian 2013a; Barbosa and Rodrigues 2013), BA [new record], CE (Truxal 1953; Nieser 1975; this work), GO (Truxal 1957), MG (Valbon et al. 2021), MS (Truxal 1953), MT (Heckman 1998), PA (Truxal 1953; Barbosa and Nessimian 2013a), PB (Truxal 1953; Nieser 1975), PE (Truxal 1953; Nieser 1975; this work), PI [new record], RN (Truxal 1953; Nieser 1975), SP (Castanhole et al. 2013; Pereira et al. 2015a), TO (Truxal 1957). Guyana (Truxal 1953). Paraguay (Truxal 1953). Peru (Truxal 1953). Suriname (Nieser 1975). Trinidad & Tobago (Nieser and Alkins-Koo 1991).

Material examined. BRAZIL, !AL, 2018: 1 3: Campo Grande municipality, açude, AL6 station, 07.VII.2018. -9.9436, -36.8275. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81609; 1 ♂, 1 2: Piaçabuçu municipality, Rio Piauí, 02.V.2018. -10.347, -36.4828. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81703. IBA, 2018: 1 d: Lençóis municipality, PNCD, Ribeirão do Meio, Terra 9 station, 341 m, 22.VIII.2018. -12.586, -41.3817. C.C. Gonçalves col., CEIOC 81907. CE, 2019: 2 d: Aiuaba municipality, EEA, casa da gameleira, Terra 1 station, light trap, 517 m, 09.IV.2019. -6.6976, -40.2812. J.M.S. Rodrigues col., CEIOC 81901; 1 중, 1 일: same data, except: Sítio Volta de Cima, sede do ICMBIO, Terra 4 station, light trap, 460 m, 10.IV.2019. -6.6025, -40.1286, CZMA; 1 🖧: same data, except: Sítio boa Vista, Terra 6 station, light trap, 583 m, -6.7256, -40.2226, CZMA; 1 3: same data, except: Terra 8 station, light trap, 650 m, 11.IV.2019. -6.7425, -40.3065. C.C. Gonçalves col., CZMA; 1 3, 1 2: same data, except: Sítio do Martins, Terra 10 station, light trap, 476 m, 12.IV.2019. -6.6667, -40.1750. J.S. Prando col., CEIOC 81899; 1 ♂: same data, except: C.C. Gonçalves col., CEIOC 81898; 1 ♂, 1 ♀: same data, except: J.M.S. Rodrigues col., CZMA; 1 ♂, 15 ♀: same data, except: casa da gameleira, Água 1 station, 532 m, 09.IV.2019. -6.6992, -40.2908, CEIOC 81900; 2 ♂, 3 ♀: same data, except: Açude do Letreiro, Água 12 station, 475 m, 13.IV.2019. -6.6163, -40.1545, CEIOC 81902; 2 ♂, 3 ♀: same data, except: CEIOC 81902. Same state, 2021: 6 강, 4 일: same data, except: estrada, Terra 3 station, light trap, 505 m, 08.VI.2021. -6.6974, -40.2819, CEIOC 81906; 7 ♂, 1 ♀: same data, except: C.C. Gonçalves col., CEIOC 82012; 2 ♂, 1 ♀: same data, except: Terra 5 station, light trap, 493 m, 03.VI.2021. -6.7003, -40.1919, CZMA; 6 ♂, 2 ♀: same data, except: estrada, Terra 11 station, light trap, 634 m, 05.VI.2021. -6.7259, -40.3065. C.C. Gonçalves col., CZMA; 2 3, 2 2: same data, except: estrada próxima ao riacho, Terra 16 station, light trap, 498 m, 06.VI.2021. -6.6872, -40.2683. CZMA; 1 \bigcirc , 4 \bigcirc : same data, except: Terra 19 station, light trap, 634 m, 07.VI.2021.

-6.7259, -40.3065, CZMA; 1 3: same data, except: C.C. Gonçalves col., CZMA; 1 3, 1 2: same data, except: Sítio Jatobá, açude, Água 1 station, 571 m, 06.VI.2021. -6.7351, -40.2424. J.M.S. Rodrigues & F.F.F. Moreira cols., CZMA; 3 3, 2 2: same data, except: Sítio Volta de Cima, lago, Água 4 station, 434 m, 05.VI.2021. -6.6019, -40.1246, CZMA; 16 3, 15 2, 4 nymphs: same data, except: Sítio Volta de Baixo, açude, Água 8 station, 431 m, 06.VI.2021. -6.6263, -40.1339, CEIOC 81905; 7 3, 7 2: same data, except: braço de rio, açude, Água 11 station, 513 m, 07.VI.2021. -6.6997, -40.1917, CZMA. PE, 2019: 2 3, 1 2: Tupanatinga municipality, PNCA, Fazenda Laranjeiras, Terra 18 station, light trap, 610 m, 20.III.2019. -8.4618, -37.3273. J.M.S. Rodrigues col., CZMA; 2 3, 3 2: same data, except: CEIOC 81904. !PI, 2020: 1 3, 1 2: Caracol municipality, PNSC, estrada próxima do Povoado Capim, Terra 15 station, light trap, 17.II.2020. -9.2149, -43.5034. A.A. Alves col., CEIOC 81903.

Buenoa femoralis (Fieber, 1851) Fig. 10B

Anisops femoralis Fieber, 1851: 483 [original description]. Buenoa femoralis; Kirkaldy (1904): 120 [genus transfer].

Diagnosis. Large species, male length 7.8–8.6 mm. Synthlipsis wide, nearly 1/2 of vertex width. Labial prong much longer than third labial article, originating slightly after half of article. Fore femur slightly widened at apex, with ~ 17 ridges in a triangular stridulatory area.

Taxonomic notes. Buenoa femoralis is one of the largest species of the genus in Brazil, together with *B. antigone antigone*, *B. crassipes* (Champion, 1901), and *B. oreia* Nieser, Melo, Pelli & Barbosa, 1997. It is usually larger than the first two species and smaller than the last one, and shows different shapes of the fore femur, stridulatory area, and labial prong. The closest species in northeastern Brazil is *B. pallipes*, which is a distinctly smaller species, with differently shaped labial prong.

Distribution. Brazil: AM (Barbosa and Nessimian 2013a), PI [new record], PR (Jaczewski 1928). Peru (Truxal 1953). Puerto Rico (Fieber 1851). U.S. Virgin Islands (Barber 1939).

Material examined. BRAZIL, !PI, 2018: 1 3, 2 9, 4 nymphs: Caracol municipality, PNSC, entre o Mirante "Janela do Sertão" e o cemitério, Água 2 station, 566 m, 10.XII.2018. -9.2188, -43.4902. J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 82007; 1 3: same data, except: Guaribas municipality, açude, Água 10 station, 640 m, 14.XII.2018. -9.3649, -43.7546, CEIOC 82006; 1 3, 2 9, 1 nymph: same data, except: Caracol municipality, Água 13 station, 526 m, 15.XII.2018. -9.2140, -43.4965, CZMA; 2 3: same data, except: proximo a estrada, Terra 6 station, 11.XII.2018. -9.2137, -43.4986. C.C. Gonçalves col., CEIOC 82080. Same state, 2020: 4 3, 2 9: same data, except: poças ao lado da estrada proximo ao cemitério, Água 4 station, 565 m, 13.II.2020. -9.2185, -43.4904. J.M.S. Rodrigues & I.R.S. Cordeiro cols., CEIOC 81910; 1 3: same data, except: Povoado Sobrado, alagado em área de pasto, Água 11 station, 499 m, 16.II.2020. -9.2111, -43.5202, CEIOC 81908; 1 3, 8 9: same data, except: Guaribas municipality, brejão, açude, Água 12 station, 640 m, 18.II.2020. -9.3650, -43.7544, CEIOC 81909.

Buenoa fuscipennis (Berg, 1879)

Fig. 10C

Anisops fuscipennis Berg, 1879: 198–199 [original description]. Anisops naias Kirkaldy, 1899e: 194 [synonym; Truxal (1953): 1460]. Buenoa fuscipennis; Kirkaldy (1904): 120 [genus transfer]. Buenoa dentipes Jaczewski, 1928: 127 [synonym; Truxal (1953): 1460].

Diagnosis. Medium to large species, male length 6.8–7.2 mm. Synthlipsis wide, slightly < 1/2 of vertex width. Labial prong shorter than third labial article, originating proximally on article. Fore trochanter with a conspicuous process pointing backwards. Fore femur without stridulatory area. Middle tibia widened at apex.

Distribution. Argentina (Berg 1879). Bolivia (Truxal 1953). Brazil: CE [new record], MT (Heckman 1998), PA (Barbosa and Nessimian 2013a; Nobre et al. 2019), PE [new record], PR (Jaczewski 1928), SC (Truxal 1953). Chile (Kirkaldy 1899a). Paraguay (Truxal 1953). The record from Venezuela (Kirkaldy 1904) is questionable.

Material examined. BRAZIL, !CE, 2019: 4 ♂, 2 ♀: Aiuaba municipality, EEA, Sítio Jatobá, açude alagado, Água 8 station, 572 m, 11.IV.2019. -6.7346, -40.2429. J.M.S. Rodrigues col., CEIOC 81922; 2 ♂, 1 ♀: same data, except: Sítio Volta de Baixo, Terra 12 station, light trap, 441 m, 14.IV.2019. -6.6254, -40.1336, CZMA. !PE, 2019: 4 ♂: Tupanatinga municipality, PNCA, Fazenda Brejo de São José, poça temporária, Água 8 station, 700 m, 16.III.2019. -8.506, -37.2237. H. Rodrigues & J.M.S. Rodrigues cols., CEIOC 82004; 1 male, 3 ♀: same data, except: Fazenda Laranjeiras, poça temporária ao lado da estrada, Água 9 station, 325 m, 17.III.2019. -8.5002, -37.3111, CZMA; 10 🖑: same data, except: Chapadão, poça temporária, Água 18 station, 957 m, 20.III.2019. -8.5244, -37.2394, CEI-OC 82003; 1 ♂, 4 ♀: same data, except: Buíque municipality, trilha para Sítio Arqueológico Alcobaça, Terra 3 station, light trap, 658 m, 15.III.2019. -8.5274, -37.1945, CZMA; 1 🖧, same data, except: Fazenda do Brejo, Terra 7 station, light trap, 706 m, 16.III.2019. -8.5199, -37.2249, CZMA; 3 ♂, 1 ♀: same data, except: Tupanatinga municipality, estrada do gado, Terra 14 station, light trap, 19.III.2019. -8.4866, -37.3404, CEIOC 82005. Same state, 2021: 2 🖑 same data, except: Barro branco, Comunidade Muquém, açudes, Água 1 station, 824 m, 10.III.2021. -8.5001, -37.3113. J.M.S. Rodrigues & R. Jordão cols., CEIOC 81923.

Buenoa koina Nieser & Pelli, 1994 Fig. 10D

Buenoa koina Nieser & Pelli, 1994: 3, 4, figs 8-10 [original description].

Diagnosis. Medium-sized species, male length ~ 5.9 mm. Synthlipsis wide, slightly > 1/2 of vertex width. Labial prong subequal to slightly shorter than third labial article, originating proximally on article. Fore femur with weak stridulatory area containing 4-6 ridges.

Taxonomic notes. Buenoa koina is one of smallest species of the genus with wide synthlipsis. It can be distinguished from the most similar species, *B. pallens* (Champion, 1901) and *B. pallipes* (Fabricius, 1803), by the pronotum not tricarinate and the shorter length of the labial prongthan in the two aforementioned species.

Distribution. Brazil: BA [new record], MG (Nieser and Pelli 1994; Nieser and Melo 1997; Carrenho et al. 2020).

Material examined. BRAZIL, !BA, 2018: 4 3, 1 9: Mucugê municipality, PNCD, Água 3 station, 19.VIII.2018. -12.9908, -41.3507. J.M.S. Rodrigues col., CEIOC 81921. Same state, 2021: 1 3, 2 9: same data, except: Palmeiras municipality, Rio Preto, Água 1 station, 820 m, 05.V.2021. -12.6036, -41.5250. J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81920.

Buenoa konta Nieser & Pelli, 1994

Fig. 10E

Buenoa konta Nieser & Pelli, 1994: 1–3, figs 1–4 [original description].

Diagnosis. Very small species, male length 3.7–4.3 mm. Synthlipsis narrow, 1/4 of vertex width). Labial prong subequal in length to third labial article, originating proximally on article. Blackish brown hoof print-like pattern at hemelytra in dorsal view. Fore femur with wide apex, without stridulatory area.

Taxonomic notes. Buenoa konta and B. salutis are the two smallest species of Buenoa occurring in Brazil. The former has a conspicuous blackish brown hoof print-like pattern on the hemelytra in dorsal view. This mark, with other morphological features like the short labial prong and the wide apex of the fore femur, ensures an easy identification.

Distribution. Brazil: AL [new record], BA [new record], CE [new record], GO (Barbosa and Dias-Silva 2017), MG (Nieser and Pelli 1994; Nieser and Melo 1997; Melo and Nieser 2004; Barbosa and Rodrigues 2013; Carrenho et al. 2020), MS (Barbosa et al. 2010c), PA (Barbosa et al. 2010a; Barbosa and Nessimian 2013a), RJ (Ribeiro et al. 2010), SE [new record].

Material examined. BRAZIL, !AL, 2018: 1 3: Maragogi municipality, APACC, APACC2 station, 29.IV.2018. -8.922, -35.1796. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81700; 1 3, 2 nymphs: same data, except: Murici municipality, EEM, EEM5 station, 28.IV.2018. -9.2540, -35.8010, CEIOC 81698. !BA, 2021: 2 3, 6 \bigcirc , 1 nymph: Andaraí municipality, PNCD, Rio Garapa, Água 5 station, 08.V.2021. -12.7456, -41.3453. J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81915. !CE, 2021: 2 3: Aiuaba municipality, EEA, sítio Volta de Cima, Iago, Água 4 station, 434 m, 05.VI.2021. -6.6019, -40.1246. J.M.S. Rodrigues col., CEIOC 81916. !SE, 2018: 4 3, 8 \bigcirc , 5 nymphs: Areia Branca municipality, PNSI, Riacho Negro, PNSI2 station, 08.VII.2018. -10.7475, -37.3402. C.F.B. Floriano, J.F. Barbosa & J.M.S. Rodrigues cols., CEIOC 81621.

Buenoa mutabilis Truxal, 1953

Fig. 10F

Buenoa mutabilis Truxal, 1953: 1432-1435, fig. 61 [original description].

Diagnosis. Medium-sized species, male length 5.2-6.0 mm. Synthlipsis narrow, ~ 1/3 of vertex width. Labial prong with variable shape, longer than third

labial article, originating proximally on article. Fore femur not widened at apex; stridulatory area subtriangular, with 10–18 ridges.

Distribution. Brazil: CE [new record], GO (Truxal 1957), MG (Truxal 1957; Nieser and Melo 1997; Vianna and Melo 2003; Melo and Nieser 2004), PI (Takiya et al. 2016; this work), SE [new record]. Guyana (Truxal 1953). Haiti (Truxal 1953). Paraguay (Truxal 1953). Peru (Truxal 1953). U.S. Virgin Islands (Rogers and Cruz-Rivera 2021). Venezuela (Truxal 1953).

Material examined. BRAZIL, !CE, 2021: 3 3, 2 2: Aiuaba municipality, EEA, estrada, Terra 3 station, light trap, 505 m, 08.VI.2021. -6.6974, -40.2819. J.M.S. Rodrigues col., CEIOC 82001; 1 3, 2 2: same data, except: estrada próximo ao riacho, Terra 16 station, light trap, 498 m, 06.VI.2021. -6.6871, -40.2683, CZMA; 18 3, 11 2: same data, except: açude/riacho, Água 3 station, 507 m, 04.VI.2021. -6.6921,-40.1871. J.M.S. Rodrigues & C.C. Gonçalves cols., CEI-OC 81914. PI, 2018: 9 3, 12 2: Guaribas municipality, PNSC, poça próxima ao "Museu do Vaqueiro", 525 m, 11.XII.2018. -9.1379, -43.5972. J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 82002. !SE, 2018: 2 3, 1 2, 4 nymphs: Areia Branca municipality, Riacho Vermelho, PNSI1 station, 08.VII.2018. -10.7387, -37.33547. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81681.

Buenoa pallipes (Fabricius, 1803)

Fig. 10G

Notonecta pallipes Fabricius, 1803: 103 [original description]. Anisops pallipes; Stål (1868): 137 [genus transfer]. Buenoa pallipes; Kirkaldy (1904): 123 [genus transfer].

Diagnosis. Medium-sized species, male length 5.5–6.2 mm. Synthlipsis wide, slightly < 1/2 of vertex width. Labial prong arc-shaped, distinctly longer than third labial article, originating distally on article. Fore femur apex widened; stridulatory area subtriangular, with 17 ridges.

Taxonomic notes. Another medium-sized species, *B. pallipes* is similar to *B. mutabilis* and *B. platycnemis* (Fieber 1851). However, it has the apex of the fore femur wider and the labial prong longer than in the former species, and the labial prong more parallel to the axis of the frons than in the latter.

Distribution. Barbados (Nieser 1967). Bolivia (Kirkaldy 1899d). Brazil: AL [new record] AM (Nieser 1970), PA (Nieser 1970), PE [new record]. Colombia (Kirkaldy 1899c). Costa Rica (de la Torre-Bueno 1906). Cuba (Nieser 1967). Ecuador (Kirkaldy 1899c). Guadeloupe (Kirkaldy 1904). Hawaiian Islands (Kirkaldy 1910). Honduras (Truxal 1953). Jamaica (Kirkaldy 1900). Martinique (Kirkaldy 1904). Mexico (Champion 1901). Panama (Kirkaldy 1899b). Paraguay (Truxal 1953). Peru (Truxal 1953). Puerto Rico (Fieber 1851). St. Barthélemy (Lethierry 1881). St. Vincent & The Grenadines (Uhler 1893). U.S. Virgin Islands (Fieber 1851). Records from North America north of Mexico are erroneous.

Material examined. Brazil, !AL, 2018: 1 ♂, 1 ♀: Quebrângulo municipality, RBPT, Sítio Juliana, brejo, PPT4 station, 04.VII.2018. -9.2608, -36.4157. C.F.B. Floriano, J.M.S. Rodrigues and J.F. Barbosa cols., CEIOC 81705. !PE, 2019: 5 ♂, 11 ♀, 6 nymphs: Tupanatinga municipality, PNCA, poço, aproximadamente 400 m da

casa dos brigadistas, Água 1 station, 818 m, 14.III.2019. -8.5725, -37.2367. H. Rodrigues & J.M.S. Rodrigues col., CEIOC 82011. Same state, 2021: 2 \triangleleft , 2 \bigcirc : same data, except: Buíque municipality, olho d'água do pico, Água 10 station, 763 m, 15.III.2021. -8.5577, -37.1951. J.M.S. Rodrigues & R. Jordão cols., CEIOC 82079; 20 \triangleleft , 16 \bigcirc : same data, except: Tupanatinga municipality, nascente do ICMBIO, brejo, Água 11 station, 807 m, 16.III.2021. -8.5724, -37.2368, CEIOC 81918.

Buenoa platycnemis (Fieber, 1851)

Fig. 10H

Anisops platycnemis Fieber, 1851: 485 [original description]. Buenoa platycnemis; Kirkaldy (1904): 123 [genus transfer].

Diagnosis. Medium to large species, male length 4.6-5.4 mm. Synthlipsis narrow, slightly < 1/2 of vertex width. Labial prong straight, much longer than third labial article, originating distally on article. Fore femur with apex widened; stridulatory area subtriangular, with 11-14 ridges.

Taxonomic notes. Similar to other medium-sized species, like the aforementioned *B. mutabilis* and *B. pallipes*. A thorough examination of the morphological characteristics described for each of these species is necessary for their correct identification.

Distribution. Argentina (Kirkaldy 1904). Bonaire (Nieser 1967). Brazil: AM (Barbosa and Nessimian 2013a), GO (Truxal 1957), MA (Truxal 1953), MT (Nieser 1970), PA (Nieser 1970; Barbosa and Nessimian 2013a; Nobre et al. 2019), PE [new record], TO (Truxal 1957), RJ (Kirkaldy 1904; Ribeiro et al. 1998, 2010; Nessimian and Ribeiro 2000; Barbosa et al. 2010c), SE [new record]. Cayman Islands (Truxal 1953). Colombia (Truxal 1953). Costa Rica (Truxal 1953). Cuba (Uhler 1884). Curacao (Nieser 1967). Guadeloupe (Nieser 1967). Haiti (Truxal 1953). Jamaica (Truxal 1953). Martinique (Truxal 1953). Mexico (Uhler 1884). Nicaragua (López et al. 1998). Panama (Truxal 1953). Peru (Truxal 1953). Puerto Rico (Fieber 1851). St. Martin (Nieser 1967). Trinidad & Tobago (Nieser and Alkins-Koo 1991). United States (Uhler 1884). Venezuela (Truxal 1953). U.S. Virgin Islands (Fieber 1851).

Material examined. !PE, 2019: 1 ♂: Tupanatinga municipality, PNCA, Riacho de Leís, poças temporárias ao longo do leito, Água 14 station, 750 m, 18.III.2019. -8.5829, -37.2445. J.M.S. Rodrigues & H. Rodrigues cols., CEIOC 81919. !SE, 2018: 1 ♂: Estância municipality, Complexo de Dunas da Praia do Saco, REBIOSI17 station, 05.V.2018. -11.4190, -37.3223. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81624; 1 ♂: same data, except: Canhoba municipality, açude, SE1 station, 07.VII.2018. -10.1287, -36.9698. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81627.

Buenoa pseudomutabilis Barbosa, Ribeiro & Nessimian, 2010 Fig. 101

Buenoa pseudomutabilis Barbosa, Ribeiro & Nessimian, 2010: 561–564, figs 1, 10–14 [original description].

Diagnosis. Medium-sized species, male length 5.8–6.0 mm. Synthlipsis narrow, ~ 2/5 of vertex width. Tylus inflated and pubescent. Labial prong longer than third labial article, originating proximally on article. Fore femur narrowed at apex; stridulatory area subtriangular, with 14 ridges. Dark nodule on abdominal ventral laterotergite I.

Taxonomic notes. Buenoa pseudomutabilis greatly resembles *B. mutabilis*, hence the specific name. This species differs from *B. mutabilis* in the lower width of head/anterior width of vertex ratio (5.0–5.4 in *B. pseudomutabilis*, 6.5 in *B. mutabilis*) and the fewer teeth on the stridulatory comb of the fore tibia (21–25 teeth in *B. pseudomutabilis*, 33–38 in *B. mutabilis*).

Distribution. Brazil: BA [new record], GO (Barbosa and Dias-Silva 2017), PI (Takiya et al. 2016), RJ (Barbosa et al. 2010c).

Material examined. BRAZIL, !BA, 2018: 1 3: Mucugê municipality, PNCD, Água 11 station, 23.VIII.2018. -13.2932, -41.2414. J.M.S. Rodrigues & F.F.F Moreira cols., CEIOC 81917.

Buenoa salutis Kirkaldy, 1904

Fig. 10J

Buenoa salutis Kirkaldy, 1904: 124 [original description].

Buenoa mallochi Jaczewski, 1928: 129–130, figs 23–27 [synonym; Truxal (1953): 1469].

Diagnosis. Small species, male length 3.4–4.0 mm. Synthlipsis narrow, ~ 1/10 tenth of vertex width. Labial prong subequal to slightly longer than third labial article, originating medially on article. Fore femur narrowed at apex, without stridulatory area.

Distribution. Argentina (Bachmann 1962). Bolivia (Truxal 1953). Brazil: AL [new record], AM (Truxal 1953; Nieser 1970; Barbosa and Nessimian 2013a), BA [new record], CE (Truxal 1953; this work), MG (Nieser and Melo 1997; Melo and Nieser 2004; Barbosa and Rodrigues 2013), MS (Floriano et al. 2013), MT (Heckman 1998), PA (Truxal 1953; Nieser 1970; Barbosa and Nessimian 2013a), PB (Truxal 1953), PE (Truxal 1953), PI (Takiya et al. 2016), PR (Jaczewski 1928), RJ (Ribeiro et al. 1998, 2010), RR (Barbosa and Nessimian 2013a), RS (Truxal 1953; Kleerekoper 1955), SE [new record], SP (Truxal 1953), TO (Truxal 1957). Colombia (Roback and Nieser 1974). French Guiana (Kirkaldy 1904). Guyana (Truxal 1953). Paraguay (Truxal 1953). Suriname (Nieser 1968). Trinidad & Tobago (Nieser and Alkins-Koo 1991). Venezuela (Truxal 1953).

Material examined. BRAZIL, !AL, 2018: 3 3, 2 9, 3 nymphs: Maragogi municipality, APACC, APACC2 station, 29.IV.2018. -8.922, -35.1795. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81695; 1 3, 2 9: same data, except: Piaçabuçu municipality, APAP, Rio Piauí, 02.V.2018. -10.3470, -36.4828, CEIOC 81697; 1 3: same data, except: Feliz Deserto municipality, APAP, APAP3 station, -10.3252, -36.3506, CEIOC 81696. Same state, 2019: 1 3, 1 9: Marimbondo municipality, Flexeiras, açude, ALSE11 station, 122 m, 23.V.2019. -9.5074, -36.2340. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira cols., CEIOC 81955. !BA, 2018: 2 3, 1 9: Lençóis municipality, PNCD, Ribeirão do Meio, Terra 9 station, 341 m, 22.VIII.2018. -12.586, -41.3817. J.S.

Prando col., CEIOC 81912. CE, 2019: 2 3, 1 2: Aiuaba municipality, EEA, casa da gameleira, Terra 1 station, light trap, 517 m, 09.IV.2019. -6.6976, -40.2812. J.M.S. Rodrigues col., CEIOC 81913; 1 3: same data, except: Sítio boa Vista, Terra 6 station, light trap, 583 m, 10.IV.2019. -6.7256, -40.2226, CZMA; 1 3: same data, except: Sítio do Martins, Terra 10 station, light trap, 476 m, 12.IV.2019. -6.6667, -40.1750. J.S. Prando col., CZMA. Same state, 2021: 15 3, 12 2: same data, except: estrada, Terra 11 station, light trap, 634 m, 05.VI.2021. -6.7259, -40.3065. J.M.S. Rodrigues col., CEIOC 81911. ISE, 2018: 1 3, 2 2: Areia Branca Municipality, PNSI, Riacho Negro, PNSI2 station, 08.VII.2018. -10.7475, -37.34025. C.F.B. Floriano, J.F. Barbosa & J.M.S. Rodrigues cols., CEIOC 81610.

Buenoa tarsalis Truxal, 1953

Fig. 10K

Buenoa tarsalis Truxal, 1953: 1392-1395, fig. 49 [original description].

Diagnosis. Medium to large species, male length 6.2-7.2 mm. Synthlipsis wide, $\sim 1/2$ of vertex width. Labial prong longer than third labial article, originating proximally on article. Fore femur with apex narrowed; stridulatory area oblong, with 17-23 ridges. First article of middle tarsus deeply emarginated.

Taxonomic notes. Buenoa tarsalis resembles *B. femoralis* but has a very conspicuous emargination on the first article of the middle tarsus, which ensures its correct identification.

Note. The most abundant and widespread species in this survey, present in all studied states.

Distribution. Brazil: AL [new record], AM (Nieser 1970; Barbosa and Nessimian 2013a), BA [new record], CE (Truxal 1953; this work), GO (Barbosa and Dias-Silva 2017), MG (Nieser and Melo 1997; Melo and Nieser 2004; Gutiérrez et al. 2017a, b), PA (Truxal 1953; Barbosa and Nessimian 2013a), PB (Truxal 1953), PE (Truxal 1953; this work), PI (Takiya et al. 2016; this work), RJ (Truxal 1953; Barbosa et al. 2010c), RN (Truxal 1953), SE [new record].

Material examined. BRAZIL, !AL, 2018: 2 \Diamond , 1 \heartsuit : Campo Grande municipality, açude, AL6 station, 07.VII.2018. -9.9436, -36.8275. C.F.*B.* Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81613; 15 \Diamond , 14 \heartsuit : same data, except: rio, AL7 station, -9.9559, -36.8373, CEIOC 81611; 3 \Diamond , 3 \heartsuit , 1 nymph, same data, except: Coité do Noia municipality, Estrada AL-110, Rio Coruripe, AL5 station, 06.VII.2018. -9.6987, -36.5845, CEIOC 81684; 1 \Diamond : same data, except: Maragogi municipality, APACC, APACC2 station, 29.IV.2018. -8.922, -35.1795, C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81614; 9 \Diamond , 9 \heartsuit 19 nymphs: same data, except: Quebrângulo municipality, RBPT, sítio Juliana, brejo, PPT4 station, 04.VII.2018. -9.2608, -36.4157. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81683. Same state, 2019: 13 \Diamond , 12 \heartsuit , 9 nymphs: Marimbondo flexeiras municipality, açude, ALSE11 station, 122 m, -9.5074, -36.2340. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira cols., CEIOC 82066; 16 \Diamond , 14 \heartsuit , 3 nymphs: same data, except: Minador do Negrão municipality, açude, ALSE13 station, 253 m, 23.V.2019. -9.3679, -36.8425,

CEIOC 82073. IBA, 2018: 1 d: Lençóis municipality, PNCD, Ribeirão do Meio, Terra 9 station, 341 m, 22.VIII.2018. -12.586, -41.3817. J.S. Prando col., CEI-OC 82039. CE, 2019: 1 ♂, 9 nymphs: Aiuaba municipality, EEA, Sítio do Martins, casa da gameleira, Água 1, 532 m, 09.IV.2019. -6.6992, -40.2908. J.M.S. Rodrigues col., CZMA; 2 ♂, 3 ♀: same data, except: área alagada ao lado da estrada para a casa do cajueiro, Água 4 station, 508 m, 10.IV.2019. -6.6923, -40.1873, CEIOC 81996; 2 ♂, 5 ♀, 7 nymphs: same data, except: Sítio Boa Vista, área alagada, Água 7 station, 570 m, 11.IV.2019. -6.7255, -40.2226, CE-IOC 82075; 2 ♂, 8 ♀: same data, except: Sítio Jatobá, açude alagado, Água 8 station, 572 m, -6.7346, -40.2429, CZMA; 3 ♂, 8 ♀, 2 nymphs: same data, except: Vaginha da Gameleira, açude, Água 9 station, 510 m, 12.IV.2019. -6.6866, -40.2625, CZMA; 10 ♂, 6 ♀, 2 nymphs: same data, except: Açude do Letreiro, Água 12 station, 475 m, 13.IV.2019. -6.6163, -40.1545, CEIOC 82000; 3 👌, 14 , 5 nymphs: Sítio Volta de Baixo, açude cheio, Água 13 station, 431 m, -6.6263, -40.1339, CZMA; 1 👌: same data, except: Serra da Lagoa, Terra 8 station, light trap, 650 m, 11.IV.2019. V. Quintas col., CEIOC 82072; 3 ♂, 3 ♀: Sítio Volta de Baixo, Terra 12 station, light trap, 441 m, 14.IV.2019. -6.6254, -40.1336. J.M.S. Rodrigues col., CEIOC 81985. Same state, 2021: 1 ♂, 18 ♀, 1 nymph: same data, except: Sítio Jatobá, açude, Água 1 station, 571 m, 03. VI.2021. -6.7351, -40.2424. J.M.S. Rodrigues & F.F.F Moreira cols., CZMA; 10 ♂, 2 ♀: same data, except: brejo, Água 6 station, 570 m, 05.VI.2021. -6.7263, -40.2229, CEIOC 81997; 4 ♂, 31 ♀: same data, except: poça, Água 10 station, 507 m, -6.6866, -40.2625, CZMA; 8 ♂, 4 ♀, 10 nymphs: same data, except: braço de rio, açude, Água 11 station, 513 m, 07.VI.2021. -6.6997, -40.1917, CEIOC 81998; 4 ♂, 6 ♀: same data, except: brejo, Água 12 station, 584 m, -6.7384, -40.2475, CZMA; 7 🖧, 2 📮: same data, except: estrada, Terra 3 station, light trap, 505 m, 08.VI.2021. -6.6974, -40.2819. J.M.S. Rodrigues col., CZMA; 1 ♂: same data, except: C.C. Gonçalves col., CEIOC 82069; 4 ♂, 2 ♀; same data, except: estrada, Terra 5 station, light trap, 493 m, 03.VI.2021. -6.7003, -40.1919. J.M.S. Rodrigues col., CZMA; 2 ♂, 2 ♀: same data, except: estrada ao lado do brejo, Terra 8 station, light trap, 486 m, 04.VI.2021. -6.6920, -40.1870, CZMA; 2 🖧, 2 😳: same data, except: estrada, Terra 11 station, light trap, 634 m, 05.VI.2021. -6.7259, -40.3065, CZMA; 5 ♂, 4 ♀: same data, except: estrada prox. ao riacho, Terra 16 station, light trap, 498 m, 06.VI.2021. -6.6871, -40.2683, CEIOC 81999. PE, 2019: 3 🖧 8 🖓: Tupanatinga municipality, PNCA, caldeirões, aproximadamente 600 m da torre dos brigadistas, poças temporárias, Água 3 station, 783 m, 14.III.2019: -8.5642, -37.2389. H. Rodrigues & J.M.S. Rodrigues cols., CEIOC 82064; 11 ♂, 10 ♀: same data, except: poça temporária na trilha da casa da farinha, Água 4 station, 814 m, -8.5615, -37.2340, CEIOC 82070; 3 ♂, 6 ♀: same data, except: Barreiro do Pititi, poça temporária, Água 5 station, 832 m, -8.5599, -37.2375, CEIOC 82030; 4 ♂: same data, except: Buíque municipality, riacho salgado do início da trilha para o sítio arqueológico Alcobaça, poças temporárias, Água 6 station, 670 m, 15.III.2019. -8.5261, -37.1938. H. Rodrigues & J.M.S. Rodrigues cols., CEI-OC 82041; 4 ♂, 20 ♀: same data, except: brejo, perto da sede, poça temporária, Fazenda Brejo de São José, Água 7 station, 699 m, 16.III.2019. -8.5372, -37.2200, CEIOC 82035; 8 🖑: same data, except: poça temporária, Água 8 station, 700 m, -8.506, -37.2237, CEIOC 82034; 34 ♂, 22 ♀: same data, except:

Fazenda Laranjeiras, poça temporária ao lado da estrada, Água 9 station, 825 m, 17.III.2019. -8.5002, -37.3111, CEIOC 82031; 1 ♂, 2 ♀, 3 nymphs: same data, except: Fazenda Juá, perto da sede, poça temporária coberta de macrófitas, Água 11 station, 541 m, -8.4116, -37.3601, CEIOC 82029; 2 ♂, 1 ♀, 1 nymph: same data, except: riacho temporário, Água 12 station, 548 m, -8.4350, -37.3467, CEIOC 82027; 4 🖧 8 🖓: same data, except: Riacho Catimbau, poça temporária ao lado do leito, Água 13 station, 745 m, 18.III.2019. -8.5834, -37.2469, CZMA; 3 🖧, 3 🖓: same data, except: Riacho de Leís, poças temporárias ao longo do leito, Água 14 station, 750 m, -8.5829, -37.2445, CEIOC 82023; 1 ♂, 4 ♀, 8 nymphs: same data, except: Caldeirões da Igrejinha, poças temporárias, Água 17 station, 949 m, 20.111.2019. -8.5024, -37.2543, CEIOC 82022; 1 🖧: same data, except: Chapadão, poça temporária, Água 18 station, 957 m, -8.5244, -37.2394, CZMA; 3 ♂, 1 ♀: same data, except: terreno do ICM-Bio, Terra 2 station, light trap, 730 m, 17.III.2019. -8.5274, -37.1945. J.M.S. Rodrigues col., CEIOC 82025; 1 ♂, 2 ♀: same data, except: CZMA; 1 ♂: same data, except: trilha para Sítio Arqueológico do Alcobaça, Terra 3 station, light trap, 658 m, 15.III.2019. -8.5274, -37.1945. H. Rodrigues col., CEIOC 82024; 4 ♂, 9 ♀; same data, except: J.M.S. Rodrigues col., CEIOC 82036; 4 ♂: same data, except: Fazenda do brejo, Terra 7 station, light trap, 706 m, 16.III.2019. -8.5199, -37.2249. C.C. Gonçalves & D.M. Takiya cols., CZMA; 12 ♂, 13 ♀: same data, except: J.M.S. Rodrigues col., CEIOC 82042; 5 ♂, 7 ♀: same data, except: estrada do gado, Terra 14 station, light trap, 663 m, 19.III.2019. -8.4866, -37.3404. J.MS. Rodrigues col., CEIOC 82043; 1 ♂, 1 ♀: same data, except C.C. Gonçalves col., CZMA. Same state, 2021: 4 ♂, 6 ♀: Tupanatinga municipality, PNCA, Barro branco, Comunidade Muquém, açudes, Água 1 station, 824 m, 10.III.2021. -8.5001, -37.3113. J.M.S. Rodrigues & R. Jordão cols., CEIOC 81993; 2 🖏 5 😫 same data, except: poça ao lado da estrada, Água 2 station, 904 m, -8.4982, -37.2852, CEIOC 81982; 1 ♂, 4 ♀: same data, except: região dos Breús, trilha das umburanas, caldeirão, Água 4 station, 940 m, 13.III.2021. -8.4891, -37.2637, CEIOC 81983; 3 ♂, 1 ♀, 3 nymphs: same data, except: caldeirões em rocha, ao lado de estrada de chão perto da igrejinha, Água 6 station, -8.5024, -37.2543, CEIOC 81989; 1 ♂, 6 ♀, 7 nymphs, 1 adult: same data, except: serrinha, caldeirões em rocha, Água 7 station, 924 m, -8.5188, -37.2337, CEIOC 81991; 5 ♂, 4 ♀: same data, except: Buíque municipality, Serra do Catimbau, açudes, Água 9 station, 976 m, 14.III.2021. -8.5791, -37.2147, CEIOC 81990; 13 ♂, 15 ♀; same data, except: Buíque municipality, Fazenda Brejo de São José, brejo, bebedouro de cabras, Água 13 station, 673 m, -8.5247, -37.1968, CEIOC 82068. PI, 2018: 3 🖑 Caracol municipality, açude, Água 6 station, 512 m, 11.XII.2018. -9.2131, -43.4984. J.M.S. Rodrigues col., CEIOC 82037; 20 ♂, 24 ♀: Caracol municipality, PNSC, proximo a estrada, Terra 6 station, -9.2137, -43.4986. C.C. Gonçalves col., CEIOC 82065; 4 🖧 same data, except: J.M.S. Rodrigues col., CEIOC 82076; 4 3, 7 2: same data, except: próximo a estrada, Terra 8 station, light trap, 12.XII.2018. -9.2149, -43.5036. J.S. Prando col., CEIOC 82078; 2 ♂, 2 ♀: same data, except: CEIOC 82032; 3 ♂, same data, except: CEIOC 82038; 4 ♂, 11 ♀: same data, except: Jurema municipality, Sucumbido, Sede do Parque, Terra 11 station, 562 m, 13.XII.2018. -8.8597, -43.1817. J.M.S. Rodrigues col., CEIOC 82077. Same state, 2020: 1 ♂, 1 ♀: same data, except: Guaribas municipality, poças na estrada para Santa

Luz, Água 5 station, 685 m, 14.II.2020. -9.376, -43.7914, CEIOC 81995; 2 🖧 4 \mathbb{Q} : same data, except: Jurema municipality, Sucumbido, caldeirões, Água 7 station, 549 m, 15.II.2020. -8.8792, -43.1658. J.M.S. Rodrigues & I.R.S. Cordeiro cols., CEIOC 81986; 5 ♂; same data, except: área alagada perto da estrada, Água 8 station, 562 m, 15.II.2020. -8.8719, -43.1771, CEIOC 81984; 6 🖧 5 ♀: Caracol municipality, PNSC, Povoado Sobrado, alagado em área de pasto, Água 11 station, 499 m, 16.II.2020. -9.2111, -43.5202, CEIOC 81988; 3 🖧 7 🗜 same data, except: entrada da trilha Cores da Caatinga, Terra 3 station, light trap, 13.II.2020. -9.2135, -43.4665. J.M.S. Rodrigues col., CEIOC 81994; 1 👌 2 ♀: same data, except: cruzamento entre as estradas Santa Luz e Viana, Terra 8 station, light trap, 14.II.2020. -9.3845, -43.8064. J.S. Prando col., CEIOC 82028; 3 ♂, 2 ♀: same data, except: A.A. Alves col., CEIOC 82040; 2 ♂: same data, except: São Bráz municipality, estrada do Parque, Terra 11 station, light trap, 15.II.2020, -8.8451, -43.1847. J.M.S. Rodrigues col., CEIOC 81987; 1 3: same data, except: CEIOC 82026; 2 ♂, 1 ♀: same data, except: estrada do Parque, próximo a clareira de gado, Terra 14 station, light trap, 16.II.2020. -9.212, -43.5195, CEIOC 81992. !SE, 2018: 1 ♂, 11 ♀, 4 nymphs: Canhoba municipality, açude, SE1 station, 07.VII.2018. -10.1287, -36.9698. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81612; 3 3: same data, except: Estância municipality, RBSI, Complexo de Dunas da Praia do Saco, REBIOSI17 station, 05.V.2018. -11.4190, -37.3223. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81619; 3 ♂, 1 ♀: same data, except: CEIOC 81617. Same state, 2019: 7 ♂, 3 ♀: same data, except: Canindé de São Francisco municipality, açude, ALSE22 station, 301 m, 26.V.2019. -9.8549, -37.9391. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira cols., CEIOC 82074; 7 3, 5 2: same data, except: área alagada, Riacho do Boqueirão, ALSE23 station, 280 m, -9.9096, -37.8837, CEIOC 82067.

Buenoa unguis Truxal, 1953

Fig. 10L

Buenoa unguis Truxal, 1953: 1476-1479, fig. 78 [original description].

Diagnosis. Medium to large species, male length 5.9–7.1 mm. Synthlipsis narrow, 1/6 to 1/5 of vertex width. Tylus flat-sulcate. Labial prong distinctly shorter than third labial article, originating proximally on article. Fore femur apex narrowed, without stridulatory area. Fore tarsus robust and dilated, with orthogonal-angulated claws.

Distribution. Argentina (Truxal 1953). Bolivia (Truxal 1953). Brazil: AL [new record], AM (Rico et al. 2010; Barbosa and Nessimian 2013a), CE (Truxal 1953; Barbosa et al. 2010c; this work), MG (Truxal 1953; Nieser and Melo 1997; Melo and Nieser 2004; Barbosa et al. 2010c), PA (Truxal 1953; Andrade 1992; Barbosa and Nessimian 2013a), PB (Truxal 1953), PE (Truxal 1953; this work), PI (Takiya et al. 2016; this work), RJ (Truxal 1953; Barbosa et al. 2010c), RN (Truxal 1953), SE [new record], SP (Castanhole et al. 2013; Pereira et al. 2015a), TO (Truxal 1957). Paraguay (Truxal 1953). Peru (Truxal 1953). Venezuela (Herrera 2005).

Material examined. BRAZIL, !AL, 2018: 2 ♂, 2 ♀: Piaçabuçu municipality, APAP, APAP4 station, 02.V.2018. -10.3252, -36.3506. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81687. Same state, 2019: 4 3: same data, except: Marimbondo municipality, Flexeiras, açude, ALSE11 station, 122 m, -9.5074, -36.2340. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira col., CEIOC 82047. CE, 2019: 3 3: Aiuaba municipality, EEA, Sítio do Martins, casa da gameleira, Água 1 station, 532 m, 09.IV.2019. -6.6992, -40.2908. J.M.S. Rodrigues col., CEIOC 81945; 1 3: same data, except: Sítio Volta de Cima, Sede do ICM-BIO, poço, Água 6 station, 455 m, 10.IV.2019. -6.6019, -40.1248, CEIOC 82010; 9 \eth , 5 \bigcirc : same data, except: Vaginha da Gameleira, açude, Água 9 station, 510 m, 12.IV.2019, -6.6866, -40.2625, CEIOC 81953; 1 🖑: same data, except: açude do Letreiro, Água 12 station, 475 m, 13.IV.2019. -6.6163, -40.1545, CZMA; 1 3, 2 2: same data, except: Sítio Volta de Baixo, Terra 12 station, light trap, 441 m, 14.IV.2019. -6.6254, -40.1336, CEIOC 81950. Same state, 2021: 1 ♂, 7 ♀: same data, except: Sítio Jatobá, açude, Água 1 station, 571 m, 06.VI.2021. -6.7351, -40.2424. J.M.S. Rodrigues col., CZMA; 1 ♂, 7 ♀: same data, except: Sítio Volta de Cima, lago, Água 4 station, 434 m, 05.VI.2021. -6.6019, -40.1246, CZMA; 10 ♂, 4 ♀: same data, except: brejo, Água 6 station, 570 m, -6.7263, -40.2229. J.M.S. Rodrigues & F.F.F Moreira cols., CEIOC 81954; 8 ♂, 12 ♀: same data, except: braço de rio, açude, Água 11 station, 513 m, 07.VI.2021. -6.6997, -40.1917, CEIOC 81952; 2 🖧 1 🗜: same data, except: brejo, Água 12 station, 584 m, -6.7384, -40.2475, CZMA; 1 👌: same data, except: estrada, Terra 3 station, light trap, 505 m, -6.6974, -40.2819. C.C. Gonçalves col., CZMA; 1 🖑 same data, except: estrada, Terra 5 station, light trap, 493 m, 03.VI.2021. -6.7003, -40.1919. J.M.S. Rodrigues col., CZMA; 2 3, 4 2: same data, except: estrada prox. ao riacho, Terra 16 station, light trap, 498 m, 06.VI.2021. -6.6871, -40.2683, CEIOC 81951. PE, 2019: 3 🖑: Buíque municipality, PNCA, riacho salgado do início da trilha para o Sítio arqueológico Alcobaça, poças temporárias, Água 6 station, 670 m, 15.III.2019. -8.5261, -37.1938. H. Rodrigues & J.M.S. Rodrigues cols., CEIOC 82062; 1 ♂, 4 ♀: same data, except: Tupanatinga municipality, brejo, perto da sede, poça temporária, Fazenda Brejo de São José, Água 7 station, 699 m, 16.III.2019. -8.5372, -37.2200, CZMA; 3 🖑: same data, except: riacho temporário, Fazenda Juá, Água 12 station, 548 m, 17.III.2019. -8.4350, -37.3467, CEIOC 82055; 4 ♂, 2 ♀: same data, except: Igrejinha, Barragem de Valdira, 853 m, Água 16 station, -8.4902, -37.2465, CEIOC 82052; 1 🖧 same data, except: Chapadão, poça temporária, 957 m, Água 18 station, 20.III.2019. -8.5244, -37.2394, CZMA; 2 3: same data, except: J.M.S. Rodrigues col., CEIOC 82061; 2 ♂: same data, except: H. Rodrigues col., CEIOC 82060; 3 ♂, 8 ♀, 1 nymph: same data, except: Fazenda do brejo, Terra 7 station, light trap, 706 m, 16.III.2019. -8.5199, -37.2249, J.M.S. Rodrigues col., CEIOC 82053; 3 🖧 same data, except: C.C. Gonçalves col., CEIOC 82048; 4 ♂, 6 ♀: same data, except: H. Rodrigues col., CEIOC 82044; 2 🖧 3 🙄 same data, except: estrada do gado, Terra 14 station, light trap, 663 m, 19.III.2019. -8.4866, -37.3404. H. Rodrigues col., CZMA; 14 ♂, 32 ♀: same data, except: J.M.S. Rodrigues col., CEIOC 82056; 2 ♂: same data, except: Fazenda Laranjeiras, Terra 18 station, light trap, 610 m, 20.111.2019. -8.5244, -37.2394, CEIOC 82051. Same state, 2021: 2 ♂, 1 ♀: same data, except: Tupanatinga municipality, Barro branco, Comunidade Muquém, açudes, Água 1 station, 824 m, 10.III.2021. -8.5001, -37.3113. J.M.S. Rodrigues & R. Jordão

cols., CEIOC 81947; 2 🖧, 3 🖓: same data, except: poça ao lado da estrada, Água 2 station, 904 m, -8.4982, -37.2852, CEIOC 81948; 1 ♂, 1 ♀: same data, except: Chapadão, açude, Água 3 station, 940 m, 12.III.2021. -8.5244, -37.2394, CZMA; 2 d, 4 nymphs: same data, except: caldeirões em rocha, ao lado de estrada de chão perto da Igrejinha, Água 6 station, 13.III.2021. -8.5024, -37.2543, CZMA; 1 d: same data, except: Serrinha, caldeirões em rocha, Água 7 station, 924 m, -8.5188, -37.2337, CZMA; 5 🖧, 4 🗜: same data, except: Buíque municipality, Serra do Catimbau, açudes, Água 9 station, 976 m, 14.III.2021. -8.5791, -37.2147, CEIOC 81949; 3 🖧 3 🗣 same data, except: Fazenda Brejo São José, brejo, bebedouro de cabras, Água 13 station, 673 m, 16.III.2021. -8.5247, -37.1968, CZMA. PI, 2018: 3 d: PNSC, açude, Água 6 station, 512 m, 11.XII.2018. -9.213083, -43.498444, CEIOC 82063; 5 3, 7 nymphs: same data, except: Guaribas municipality, açude, Água 10 station, 640 m, 14.XII.2018. -9.364889, -43.754583, CEIOC 82049; 1 ♂, 2 ♀: same data, except: Caracol municipality, açude, Água 12 station, 498 m, 14.XII.2018. 9.2109, -43.5201, CEIOC 82050; 1 ♂, 1 ♀: Caracol municipality, PNSC, entre o mirante "Janela do Sertão" e o cemitério, Terra 2 station, light trap, 566 m, 10.XII.2018. -9.2188, -43.4902. J.S. Prando col., CEIOC 82046; 16 d: Caracol municipality, PNSC, proximo á estrada, Terra 6 station, light trap, 11.XII.2018. -9.2137, -43.4986. J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 82059; 1 ♂, 2 ♀: same data, except: J.S. Prando col., CZMA; 57 ♂, 110 ♀: same data, except: C.C. Gonçalves col., CEIOC 82045; 23 ♂: same data, except: próximo a estrada, Terra 8 station, light trap, 12.XII.2018. -9.2149, -43.5036. C.C. Gonçalves col., CEIOC 82058; 4 3: same data, except: J.M.S Rodrigues col., CZMA; 1 ♂: same data, except: Jurema municipality, casa do sucumbido, Sede do Parque, Terra 11 station, 562 m, 13.XII.2018. -8.8597, -43.1817, CEIOC 82054; 1 🖧 1 🖓: same data, except: ao lado da estrada, em rio seco, Terra 15 station, light trap, 15.II.2018. -9.2137, -43.5000. J.M.S. Rodrigues & O.M. Magalhães cols., CZMA. same state, 2020: 1 ♂, 1 ♀: Guaribas municipality, PNSC, poças na estrada para Santa Luz, Água 5 station, 685 m, 14.II.2020. -9.376, -43.7914. J.M.S. Rodrigues & I.R.S. Cordeiro cols., CZMA; 2 👌: same data, except: Jurema municipality, Sucumbido, caldeirões, Água 7 station, 549 m, 15.II.2020. -8.8792, -43.1658, CEIOC 82009; 3 ♂, 4 ♀: same data, except: área alagada perto da estrada, Água 8 station, 562 m, -8.8719, -43.1771, CEIOC 81946; 3 🖒, 16 🖓: same data, except Caracol municipality, povoado Sobrado, alagado em área de pasto, Água 11 station, 499 m, 16.II.2020. -9.2111, -43.5202, CZMA; 2 ♂, 7 ♀: same data, except: Guaribas municipality, Brejão, açude, Água 12 station, 640 m, 18.II.2020. -9.3650, -43.7544, CZMA; 12 🖧, 18 🗜: same data, except: Bom Jesus municipality, Viana, poças em estrada de terra, Água 13 station, 396 m, -9.4484, -43.0743, CEIOC 82008; 1 🖧 same data, except: São Bráz municipality, estrada do Parque, Terra 11 station, light trap, 15.II.2020. -8.8451, -43.1847. J.M.S. Rodrigues col., CZMA; 1 3, 1 2: same data, except: Caracol municipality, estrada do parque, próximo a clareira de gado, Terra 14 station, light trap, 16.II.2020. -9.212, -43.5195. J.S. Prando col., CZMA. ISE, 2018: 3 3, 20 2: Japaratuba municipality, Estrada BR-101, poça, REBIOSI8 station, 03.V.2018. -10.5476, -36.9698. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81623. Same state, 2019: 6 ♂, 13 2: Canindé de São Francisco municipality, açude, ALSE22 station, 301 m, 26.V.2019. -9.8549, -37.9391. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira cols., CEIOC 82057.

Subfamily Notonectinae Tribe Notonectini *Notonecta* Linnaeus, 1758

Notonecta (Paranecta) disturbata Hungerford, 1926 Fig. 10M

Notonecta disturbata Hungerford, 1926: 13, 14, fig. 2 [original description]. Notonecta (Paranecta) disturbata; Hutchinson (1929): 363 [subgenus placement].

Diagnosis. Medium-sized, ~ 8 mm long. Vertex/synthlipsis ratio ~ 2.7. Lateral margins of pronotum straight and divergent. Genital capsule with two caudoventral protuberances; apex of paramere not bifurcated (Fig. 8A).

Taxonomic notes. It is most similar to *N. pulchra* Hungerford, 1926, which has the same size. Examination of the male genital capsule is needed to ensure correct identification in most species of this genus, and *N. disturba-ta* is no exception. In this species, the genital capsule has two caudoventral protuberances, and dorsal portion is acute (Fig. 8A), opposing to *N. pulchra*, which has only one protuberance and the dorsal portion of the genital capsule rounded (Fig. 8B).

Distribution. Argentina (Bachmann 1963). Brazil: AL [new record], CE [new record], ES (Bachmann 1963), GO (Truxal 1957), MG (Melo and Nieser 2004; Barbosa and Nessimian 2013b), MT (Nieser 1970), PA (Barbosa and Nessimian 2013b; Nobre et al. 2019), PE [new record], PI (Barbosa and Nessimian 2013b; Takiya et al. 2016), RJ (Hungerford 1926, 1933; Ribeiro et al. 1998, 2010; Barbosa and Nessimian 2013b), SE [new record], SP (Barbosa and Nessimian 2013b), TO (Truxal 1957). Paraguay (Hungerford 1933).

Material examined. BRAZIL, !AL, 2018: 1 ♂, 1 ♀: Quebrângulo municipality, RBPT, Sítio Juliana, brejo, PPT4 station, 04.VII.2018. -9.2608, -36.4157. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81679. Same state, 2019: 1 3, 2 2: same data, except: Minador do Negrão municipality, açude, ALSE13 station, 253 m, 23.V.2019. -9.3679, -36.8425. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira cols., CEIOC 82013; 3 3, 2 adults: same data, except: Maravilha municipality, rodovia BR-316, açude, ALSE15, 294 m, 24.V.2019, CEIOC 82014. !CE, 2019: 2 🖑: Aiuaba municipality, EEA, área alagada ao lado da estrada para a casa do cajueiro, Água 4 station, 508 m, 10.IV.2019. -6.6923, -40.1873. J.M.S. Rodrigues col., CEIOC 81941; 1 d: same data, except: sítio Boa Vista, área alagada, Água 7 station, 570 m, 11.IV.2019. -6.7255, -40.2226, CEIOC 81943. Same state, 2021: 1 🖧 2 ♀: same data, except: brejo, Água 6 station, 570 m, 05.VI.2021. -6.7263, -40.2229. J.M.S. Rodrigues & F.F.F Moreira cols., CEIOC 81944. PE, 2019: 1 3: Tupanatinga municipality, PNCA, Fazenda Brejo de São José, poça temporária, Água 8 station, 700 m, 16.III.2019. -8.506, -37.2237. H. Rodrigues & J.M.S. Rodrigues cols., CEIOC 82015; 2 🖧 1 🗣 same data, except: Chapadão, poça temporária, Água 18 station, 957 m, 20.111.2019. -8.5244, -37.2394, CEIOC 81942. !SE, 2018: 1 🖑 Areia Branca municipality, PNSI, Riacho Negro, PNSI2 station, 08.VII.2018. -10.7475, -37.3402. C.F.B. Floriano, J.F. Barbosa J.M.S Rodrigues cols., CEIOC 81674; 2 🖧 2 , 1 nymph: Lagarto municipality, Estrada SE-170, açude, 09.VII.2018. [-10.91319, -37.67136]. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81661.

Same state, 2019: 5 ♂, 3 ♀: same data, except: Canindé de São Francisco municipality, açude, ALSE22 station, 301 m, 26.V.2019. -9.8549, -37.9391. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira cols., CEIOC 81940.

Enitharoides Brooks, 1953

Enitharoides brasiliensis (Spinola, 1837) Fig. 10N

Enithares brasiliensis Spinola, 1837: 60 [original description].

- Notonecta grandis Germar in Herrich-Schäffer, 1849: 42, fig. 901 [synonym; Kirkaldy (1904): 101].
- Bothronotus brasiliensis; Fieber (1852): 472 [unnecessary generic replacement name].
- Enithares (Enitharoides) brasiliensis; Brooks (1953): 74, 75, figs A, D [subgenus placement].
- Enitharoides brasiliensis; Štys and Říha (1975): 166 [genus transfer].

Diagnosis. Male length 15–16 mm. Wide rectangular punctuated area on scutellum. Middle femur with short and dark pubescence; anteapical protuberance weakly developed. Apex of metaxyphus variable, acute to spatulated.

Taxonomic notes. This species shows some variation in general and hemelytral color pattern, from light-yellow to dark brown, with deep punctures on the scutellum. The amount of these punctures seems to follow the overall color of the specimens. It resembles *E. lanemeloi* Barbosa, Ribeiro & Nessimian, 2017, as both species show middle femur with short setae and poorly developed apical spur, but can be distinguished from it by the coarser aspect and different pattern of the scutellar punctures, the different pattern of the punctuated area on the scutellum, and by differences in paramere shape. Differences between *E. brasiliensis* and the other species occurring in the study area, *E. tricomerus*, are provided in the key.

Distribution. Brazil: BA [new record], ES (Kirkaldy 1904), MG (Kirkaldy 1904; Nieser and Melo 1997; Goulart et al. 2002; Vianna and Melo 2003; Pelli et al. 2006; Souza et al. 2006; Barbosa et al. 2017), RJ (Henriques-Oliveira and Nessimian 2010; Ribeiro et al. 2010; Cordeiro and Moreira 2015; Barbosa et al. 2017), SP (Barbosa et al. 2017).

Material examined. BRAZIL, !BA, 2021: 1 ♂, 2 adults, 1 nymph: Mucugê municipality, PNCD, rio próximo ao Mirante do Cachoeirão, Água 10 station, 1191 m, 19.V.2021. -12.8110, -41.4429. J.M.S. Rodrigues col., CEIOC 81956.

Enitharoides tricomerus Barbosa, Ribeiro & Nessimian, 2017 Fig. 100

Enitharoides tricomerus Barbosa, Ribeiro & Nessimian, 2017: 480, 482, figs 49–61 [original description].

Diagnosis. Male length 15.3–16.8 mm. Deeply punctuated area widely distributed on scutellum. Middle femur with long pubescence and robust anteapical protuberance. Apex of metaxyphus triangular.

Taxonomic notes. This species shows conspicuous long brown setae and a well-developed anteapical spur on the middle femur. These features are present in another species, *E. lucasduquei* Barbosa, Ribeiro & Nessimian, 2017, but the latter has a triangular-shaped spur on the middle femur and a different pattern of scutellar punctures. Also, the middle femur of *E. lucasduquei* bears shorter setae restricted to the apical half of the segment, and shows a less dense pubescence aspect.

Distribution. Brazil: AL [new record], ES (Barbosa et al. 2017), MG (Barbosa et al. 2017).

Material examined. BRAZIL, !AL, 2018: 1 3: Murici municipality, EEM, EEM4 station, 28.IV.2018. -9.2540, -35.8010. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81659. Same state, 2019: 2 3, 1 2, 2 nymphs: same data, except: Vale do Socorro, riacho sem nome em área de dendê, ALSE6 station, 22.V.2019. -9.2386, -35.8649. J.M.S. Rodrigues, W. Souza & F.F.F. Moreira cols., CEIOC 82016; 4 3, 3 2, 1 nymph: same data, except: Fazenda Bananeira, riacho, ALSE9 station, -9.2193, -35.8789, CEIOC 82017.

Tribe Nychiini Martarega White, 1879

Martarega bentoi Truxal, 1949

Fig. 10P, Q

Martarega bentoi Truxal, 1949: 22, 23, figs 7, 8 [original description].

Diagnosis. Male length 5.2–5.3 mm. Ocular commissure > 1/3 of eye width. Scutellum with hyaline apex; ventral surface of middle trochanter with a group of ensiform bristles. Hemelytra of brachypterous specimens with medial longitudinal hyaline stripe wide, tapering from base of hemelytra up to coastal margin in membrane suture; wide range of hemelytral process stripe, 25% up to 40% of hemelytral length; hemelytral process shorter than membrane length (Fig. 9A).

Taxonomic notes. *Martarega bentoi* is a medium-sized species that is very common and widespread in Brazil. It is very similar to *M. uruguayensis* (Berg, 1883), but can be distinguished from it after examination of the hyaline band on the hemelytra of coleopteroid specimens (Fig. 9A), or the different position of the ensiform bristles on the middle trochanter. Also, it differs from *Martarega brasiliensis* and *M. membranacea*, the other two species found in this survey, by the combination of a scutellum with a hyaline apex and the middle trochanter with ensiform bristles.

Distribution. Argentina (Mazzucconi 2008). Brazil: AL [new record], BA (Moreira et al. 2016; this work), CE (Takiya et al. 2016; this work), GO (Barbosa and Dias-Silva 2017), MG (Barbosa and Rodrigues 2013; Gutiérrez et al. 2017b; Novais et al. 2017), MT (Barbosa and Giehl 2014), PE (Truxal 1949, this work), PI (Barbosa and Rodrigues 2013), RJ (Cordeiro and Moreira 2015), SE [new record].

Material examined. BRAZIL, !AL, 2018: 1 \Diamond : Murici municipality, EEM, EEM5 station 28.IV.2018. -9.2540, -35.8010. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81654; 1 \Diamond , 1 \heartsuit : Maragogi municipality, APACC, APACC2 station, 29.IV.2018. -8.9220, -35.1795, CEIOC 81656; 10 \Diamond , 4 \heartsuit , 5 nymphs: same data, except: APACC3 station, -8.9307, -35.2105, CEIOC 81678; 8 \Diamond , 8 \heartsuit , 5 nymphs: Coité do Nóia municipality, Taquarana, queda d'água, AL4

station, 06.VII.2018. -9.6723, -36.4987. C.F.B. Floriano, J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81657; 1 d: Campo Grande municipality, açude, AL7 station, 07.VII.2018. -9.9559, -36.8373, CEIOC 81668. Same state, 2019: 4 3, 4 2, 9 nymphs: same data, except: União dos Palmares, municipality, Rio Mundaú, ALSE1 station, 21.V.2019. -9.1342, -36.0809. J.M.S. Rodrigues, H. Rodrigues, W. Souza, F.F.F. Moreira cols., CEIOC 82083; 4 ♂, 4 ♀: same data, except: Santana do Mundaú municipality, rio na estrada AL-205, ALSE2 station, -9.1464, -36.2430, CEIOC 82081; 16 ♂, 6 ♀, 1 nymph: same data, except: Viçosa municipality, Fazenda da Baixa Funda, riacho, ALSE3 station, -9,3243, -36.2830, CEIOC 82085; 7 ♂, 4 ♀, 2 nymphs: same data, except: Murici municipality, EEM, Vale do Socorro, Cachoeira do Socorro, ALSE5 station, 361 m, 22.V.2019. -9.236, -35.8609, CEIOC 82084; BA, 2018: 4 🖧 3 nymphs: Mucugê municipality, PNCD, Água 2 station, 19.VIII.2018. -13.0004, -41.3982. J.M.S. Rodrigues & F.F.F Moreira cols., CZMA; 8 ♂, 2 ♀, 40 nymphs: same data, except: Água 3 station, -12.9908, -41.3507, CE-IOC 81931; 4 ♂, 8 ♀, 12 nymphs: same data, except: Andaraí municipality, Água 5 station, 20.VIII.2018. -12.9002, -41.3249, CZMA; 6 ♂, 2 ♀, 5 nymphs: same data, except: Água 6 station, -12.8401, -41.3235, CEIOC 81928; 1 ♀, 4 nymphs: same data, except: Mucugê municipality, Água 7 station, 21.VIII.2018. -13.0174, -41.3441, CZMA; 1 🖧 2 📮 4 nymphs: same data, except: Lençóis municipality, Água 9 station, 22.VIII.2018. -12.5865, -41.3822, CZMA; 1 ♂, 2 ♀: same data, except: Água 10 station, -12.6008, -41.3811, CZMA; 3 3, 4 nymphs: same data, except: Mucugê municipality, Água 11 station, 23.VIII.2018. -13.2932, -41.2414, CZMA; 1 ♂, 5 ♀, 1 nymph: same data, except: Água 12 station, -13.2886, -41.2646, CEIOC 81925. Same state, 2021: 6 ♂, 6 ♀, 1 nymph: same data, except: Palmeiras municipality, Rio Preto, Água 1 station, 820 m, 05.V.2021. -12.6036, -41.5250. J.M.S. Rodrigues & J.F. Barbosa cols., CEIOC 81927; 2 ♂, 3 ♀, 5 nymphs: same data, except: Mucugê municipality, Cachoeira Véu da Noiva, Água 2 station, 776 m, 06.V.2021. -13.2881, -41.2683, CZMA; 19 ♂, 6 ♀, 1 nymph: same data, except: Itaeté municipality, Rio Timbó, Água 3 station, 428 m, 07.V.2021. -12.6036, -41.5250, CEIOC 81930; 1 👌 (macropterous): same data, except: CEIOC 81926; 3 ♂, 4 ♀: same data, except: Rio Samina, Água 4 station, 413 m, -13.1105, -41.1587, CZMA; 2 🖧, 3 📮: same data, except: Andaraí municipality, Rio Garapa, Água 5 station, 330 m, 08.V.2021. -12.7456, -41.3454, CZMA; 6 ♂, 2 ♀, 2 nymphs: same data, except: Lençóis municipality, Rio Capivara, Água 8 station, 405 m, 09.V.2021. -12.6232, -41.3763, CZMA; 1 🖧 1 🖓: same data, except: Mucugê municipality, Rio Preto, Água 9 station, 1249 m, 10.V.2021. -12.7978, -41.4835, CZMA; 1 👌 (macropterous): same data, except: Cachoeira da Moça Loira, Água 11 station, 724 m, -12.7978, -41.4835, CEIOC 81929; 8 ♂, 3 ♀: same data, except: CEIOC 81924; 2 ♂, 3 2: same data, except: Mucugê, Rio Una, Água 12 station, 11.V.2021. -13.2921, -41.2524, CEIOC 82020. CE, 2021: 1 ♂, 1 ♀ (both macropterous), 1 nymph: Aiuaba municipality, EEA, brejo, Água 2 station, 489 m, 04.VI.2021. -6.6850, -40.1841, CEIOC 82018. PE, 2019: 15 ♂, 16 ♀: Tupanatinga municipality, PNCA, Riacho de Leís, poças temporárias ao longo do leito, Água 14 station, 750 m, 18.111.2019. -8.5829, -37.2445. J.M.S. Rodrigues & H. Rodrigues cols., CEIOC 82019. !SE, 2018: 12 🖧, 2 🖓: Areia Branca municipality, PNSI, Riacho Negro, PNSI2 station, 08.VII.2018. -10.7475, -37.3402, C.F.B. Floriano, J.F. Barbosa, J.M.S. Rodrigues cols., CEIOC 81655; 14 ♂, 4 ♀: same data, except: Riacho Vermelho, PNSI1 station, -10.7387, -37.33547, CEIOC 81675; 4 ♂, 5 ♀, 12 nymphs: Japoatã municipality, Estrada SE-335, RebioSI7 station, 03.V.2018. -10.3679, -36.8074. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81667; 3 $3, 2 \bigcirc$, 3 nymphs: São Cristóvão municipality, Rio Pitanga, RebioSI11 station, 04.V.2018. -10.9633, -37.1663, CEIOC 81669; 11 $3, 8 \bigcirc$, 7 nymphs: same data, except: Estrada Rita Cacete, RebioSI12 station, -10.9851, -37.2868, CEIOC 81677.

Martarega brasiliensis Truxal, 1949

Fig. 10R

Martarega brasiliensis Truxal, 1949: 16, figs 3, 4 [original description].

Diagnosis. Medium-sized species, male length 5.2–5.4 mm. Ocular commissure 1/2 of eye width. Ventral surface of third labial article pubescent. Scutellum with hyaline apex. Fore femur with a short spiniform seta. Ventral surface of middle trochanter with a patch of thin setae. Hemelytra of brachypterous specimens with medial longitudinal hyaline stripe narrow, slightly tapering from base of hemelytra up to membrane suture; hemelytral process stripe short, 20– 30% of hemelytral length; hemelytral process slightly shorter than membrane length (Fig. 9B).

Taxonomic notes. *Martarega brasiliensis* is slightly larger than *M. nessimiani* Barbosa & Rodrigues, 2013, a species with which it shares some similarities. *Martarega brasiliensis* has some distinctive features, like the pubescent labial surface and differences in the width of the hyaline band of the hemelytra.

Distribution. Brazil: AL [new record], CE (Truxal 1949; this work), GO (Barbosa and Dias-Silva 2017), MS (Floriano et al. 2013), MT (Barbosa and Giehl 2014), PA (Nieser 1970; Barbosa et al. 2010b, 2012), PE (Truxal 1949), RJ (Ribeiro et al. 2010), RR (Barbosa et al. 2012; Barbosa and Rodrigues 2013), SP (Castanhole et al. 2013; Pereira et al. 2015a, 2015b). Colombia (Padilla-Gil 2014). Peru (Truxal 1949). Suriname (Nieser 1968).

Material examined. BRAZIL, !AL, 2018: 2 3, 16 9, 4 nymphs: Jequiá da Praia municipality, RESEX, Mutuca, RJ3 station, 30.IV.2018. -9.9446, -36.0846. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81663; 2 3, 6 9: same data, except: RJ4 station, -9.9502, -36.0859, CEIOC 81660. CE, 2019: 1 3: Aiuaba municipality, EEA, Sítio Volta de Cima, sede do ICMBIO, poço, Água 6 station, 455 m, 10.IV.2019. -6.6019, -40.1248. J.M.S. Rodrigues col., CEIOC 81932; 1 3: same data, except: Sítio Volta de cima, sede do ICMBIO, Terra 4 station, light trap, 460 m, -6.6025, -40.1286. J.S. Prando col., CZMA; 1 3: same data, except: Sítio Boa Vista, Terra 6 station, Light trap, 583 m, 13.IV.2019. -6.6025, -40.1286. V. Quintas col., CEIOC 81957; 1 3: same data, except: J.S. Prando col., CEIOC 81933.

Martarega membranacea White, 1879

Fig. 10S

Martarega membranacea White 1879: 272 [original description].

Diagnosis. Small species, male length 4.1–4.2 mm. Ocular commissure longer than 1/2 of eye width. Scutellum with 1/2 of its length hyaline. Ventral surface of middle

trochanter smooth. Hemelytra of brachypterous specimens with claval commissure expanded, hyaline, reaching the anterior margin of membrane; medial longitudinal hyaline stripe very wide, sinuous, with same width from base of hemelytra up to coastal margin in membrane suture; hemelytral process stripe large, 20–30% of hemelytral length; hemelytral process slightly longer than membrane length (Fig. 9C)

Taxonomic notes. This species can be distinguished from *M. chinai* Hynes, 1948, the most similar congener, by the longer posterior hemelytral process and by the absence of a pubescent nodule on the middle trochanter.

Distribution. Argentina (López-Ruf et al. 2003). Bolivia (Truxal 1949). Brazil: AM (White 1879; Truxal 1949; Nieser 1970; Pereira and Melo 2007; Barbosa et al. 2012; Barbosa and Rodrigues 2013), BA [new record], GO (Nieser 1970; Barbosa and Dias-Silva 2017), MG (Melo and Nieser 2004; Barbosa and Rodrigues 2013), MS (Floriano et al. 2013), MT (Barbosa and Giehl 2014), PA (Truxal 1949; Nieser 1970; Andrade 1992; Barbosa et al. 2012), RJ (Ribeiro et al. 2010), RO (Truxal 1949), SE [new record], SP (Castanhole et al. 2013; Pereira et al. 2015a), TO (Truxal 1957). Colombia (Roback and Nieser 1974). Ecuador (Kirkaldy 1899c). Guyana (Truxal 1949). Suriname (Nieser 1968).

Material examined. BRAZIL, !BA, 2018: 2 3, 11 \bigcirc : Lençóis municipality, PNCD, Água 10 station, 22.VIII.2018. -12.6008, -41.3811, J.M.S. Rodrigues & F.F.F Moreira cols., CEIOC 81934. !SE, 2018: 3 3, 3 \bigcirc , 2 nymphs: Pirambu municipality, RBSI, RebioSI5, 01.V.2018. -10.6515, -36.7571. C.F.B. Floriano, J.M.S. Rodrigues & O.M. Magalhães cols., CEIOC 81662.



Figure 9. *Martarega*, aspect of brachypterous male hemelytra, lateral view; hyaline portions are represented as undotted, modified from Barbosa 2014 **A** *Martarega bentoi* **B** *M*. *brasiliensis* **C** *M*. *membranacea*. Abbreviations: cc = claval commissure, hp = hemelytral process, hs = hemelytral process stripe, ms = medial longitudinal stripe.



ZooKeys 1233: 245-288 (2025), DOI: 10.3897/zookeys.1233.120598



Figure 11. New records of Notonectidae species in Alagoas State, Brazil.



Figure 12. New records of Notonectidae species in Parque Nacional da Chapada Diamantina, Bahia State, Brazil.







Figure 14. New records of Notonectidae species in Parque Nacional do Catimbau, Pernambuco State, Brazil.







Figure 16. New records of Notonectidae species in Sergipe State, Brazil.



Figure 17. Aspects of some localities surveyed in Parque Nacional da Chapada Diamantina, Bahia State, Brazil, 2018 **A** Água 1 station **B** Água 2 station **C** Água 3 station **D** Água 8 station **E** Água 6 station **F** Água 4 station **G** Água 7 station **H** Água 5 station I Água 11 station **J** Água 9 station.



Figure 18. Aspects of some localities surveyed in Estação Ecológica de Aiuaba, Ceará State, Brazil, 2019 **A** Água 2 station **B** Água 3 station **C** Água 4 station **D** Água 5 station **E** Água 6 station **F** Água 7 station **G** Água 10 station **H** Água 13 station.



Figure 19. Aspects of some localities surveyed in Parque Nacional do Catimbau, Pernambuco State, Brazil, 2019 A Água 2 station B Água 4 station C Água 8 station D Água 6 station E Água 12 station F Água 11 station G Água 12 station H Água 13 station.



Figure 20. Aspects of some localities surveyed in Parque Nacional da Serra das Confusões, Piauí State, Brazil, 2018 A Água 4 station B Água 6 station C Água 3 station D Água 4 station E Água 13 station F Água 8 station G Água 12 station H Água 9 station I Água 7 station J Água 2 station K Água 5 station L Água 11 station.

Discussion

General distribution of represented species

In this survey, approximately 1400 specimens from six of the nine states in northeastern Brazil have been examined, representing 18 (ca 31%) of the 58 species of the Notonectidae recorded from Brazil (Moreira et al. 2011; Ribeiro et al. 2022). Among the studied species, six (35%) are endemic from the country and 15 (88%) are present only in South America (including Trinidad & Tobago), with the exceptions of *Buenoa mutabilis*, *B. platycnemis*, and *B. pallipes*. This last species has a wide distribution, from Mexico to Paraguay, but had not been found in Brazil since the second half of the 20th century (Barbosa and Nessimian 2013a). Among the backswimmer species occurring in Brazil, only 12 had been previously recorded from the northeastern region of the country, a number that here is elevated to 18. The species firstly recorded from the region are *Buenoa femoralis*, *B. fuscipennis*, *B. koina*, *B. konta*, *B. pallipes*, *Enitharoides brasiliensis*, *E. tricomerus*, and *Martarega membranacea*. Our data show that the distribution range of *Buenoa* and *Enitharoides* species are wider and much more environmentally variable than what had been reported in the literature.

Distribution of represented species in Brazilian biomes

Of all species collected, only *Buenoa salutis* is present in all Brazilian biomes. *Buenoa amnigenus*, *B. konta*, and *Notonecta disturbata* are second in numbers of biomes occupied, the first two lacking records from the Pampa, and the last one, from the Pantanal. Many species found in this survey (10 spp., 55%) are present in other three Brazilian biomes, as shown in Table 1. This reinforces the already observed similarity of the biota found in the Caatinga with that present in adjacent biomes (Santos et al. 2007; Takiya et al. 2016; Rodrigues et al. 2021).

Distribution of represented species in the conservation units studied

The richest conservation unit was EEA (CE), with records of three genera and ten species, representing more than half (56%) of the surveyed diversity despite being the fifth in total area (MMA 2022). The least species-rich unit was RESEX, with only M. brasiliensis. The most frequent and abundant species in our samples was B. tarsalis, found in eight out of the eleven conservation units studied. Six species were found in only one unit each, namely Buenoa femoralis, B. koina, B. platycnemis, B. pseudomutabilis, Enitharoides brasiliensis, and E. tricomerus (Table 2), three of which are found in PNCD. Nevertheless, some are relatively widespread in the country, showing that more surveys are needed in order to better understand the distribution of backswimmers in northeastern Brazil. Five species, B. amnigenus, B. platycnemis, B. tarsalis, Notonecta disturbata, and M. bentoi were found outside conservation units in Alagoas and Sergipe states. All of them were also found inside conservation units in these and/or other states as well. The results achieved in this work greatly improve the knowledge on the distribution of Notonectidae in Brazil, filling a gap of biodiversity information for the northeastern states of the country.

Biome State		Caatinga									Atlantic Forest										Pampas	
			DA	OF			DE	ы		ог		го	MC	MC	DE	00	ы	DC		OF	CD	DC
Subfam.	Species	AL	DA	UE	IVIA	РБ	PE	PI	RN	SE	AL	ES	IVIG	1012	PE	PR	ĸJ	кэ	30	SE	3P	ĸs
Anisopinae	B.amnigenus	Х	Х	XX	-	Х	Х	Х	Х	-	Х	-	Х	-	-	-	-	-	_	-	Х	-
	B. femoralis	-	-	-	-	-	-	Х	-	-	-	-	-	-	-	Х	-	-	_	-	-	-
	B. fuscipennis	-	-	Х	-	-	Х	-	-	-	-	-	-	-	-	Х	-	-	Х	-	-	-
	B. koina	-	Х	-	-	-	-	-	-	-	-	-	Х	-	-	-	-	-	_	-	-	-
	B. konta	-	Х	Х	-	-	-	-	-	-	Х	-	Х	-	-	-	Х	-	-	Х	-	-
	B. mutabilis	-	-	Х	-	-	-	Х	-	-	-	-	Х	-	-	-	-	-	_	Х	-	-
	B. pallipes	-	Х	-	-	-	Х	-	-	-	Х	-	-	-	-	-	-	-	-	-	-	-
	B. platycnemis	-	-	-	Х	-	Х	-	-	-	-	-	-	-	-	-	Х	-	-	Х	-	-
	B. pseudomutabilis	-	Х	-	-	-	-	Х	-	-	-	-	-	-	-	-	Х	-	_	-	-	-
	B. salutis	-	Х	XX	-	Х	-	Х	-	-	Х	-	Х	Х	-	Х	Х	-	-	Х	Х	Х
	B. tarsalis	Х	XX	XX	-	Х	XX	XX	Х	-	Х	-	Х	-	Х	-	Х	-	-	Х	-	-
	B. unguis	-	XX	XХ	-	Х	Х	X X	Х	-	Х	-	Х	-	Х	-	Х	-	-	Х	Х	_
	N. disturbata	-	-	Х	-	-	-	Х	-	Х	Х	-	Х	-	-	-	Х	-	_	Х	Х	Х
Notonectinae	E. brasiliensis	-	Х	-	-	-	-	-	-	-	-	-	Х	-	-	-	Х	-	-	-	Х	-
	E. tricomerus	-	-	-	-	-	-	-	-	-	Х	Х	Х	-	-	-	-	-	-	-	-	-
	M. bentoi	Х	Х	XХ	-	-	-	Х	-	-	Х	-	Х	-	Х	-	Х	Х	-	Х	-	-
	M. brasiliensis	-	-	XX	-	-	-	-	-	-	Х	-	-	Х	-	-	Х	-	-	-	Х	-
	M. membranacea	-	Х	-	-	-	-	-	-	-	-	-	-	Х	-	-	Х	-	-	Х	-	-
Biome		Amazon Forest								Cerrado Savannah							Pan	tanal				
State		AC	AP	AM	PA	МТ	RO	RR	DF	GO	MA	MG	MS	МТ	PI	SP	то	MS	MT			
Subfam.	Species																					
	B. amnigenus	-	-	Х	Х	-	-	-	-	Х	-	-	-	-	Х	Х	-	Х	Х			
	B. femoralis	-	-	Х	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	B. fuscipennis	-	-	-	Х	-	-	-	-	-	-	-	-	-	-	-	-	-	Х			
	B. koina	-	-	-	-	-	-	-	-	-	-	Х	-	-	-	-	-	-	_			
	B. konta	-	-	-	Х	-	-	-	-	Х	-	Х	-	-	-	-	-	Х	_			
Anisopinae	B. mutabilis	-	-	-	-	-	-	-	-	Х	-	Х	-	-	-	-	-	-	-			
	B. pallipes	-	-	Х	Х	-	-	-	-	-	-	-	-	-	-	-	-	-	_			
	B. platycnemis	-	-	Х	Х	-	-	-	-	Х	-	-	-	Х	-	-	Х	-	_			
	B. pseudomutabilis	-	-	-	-	-	-	-	-	Х	-	-	-	-	-	-	-	-	-			
	B. salutis	-	-	Х	Х	-	-	Х	-	-	-	Х	-	-	-	-	Х	Х	_			
	B. tarsalis	-	-	Х	Х	-	-	-	-	Х	-	Х	-	-	Х	-	-	-	-			
	B. unguis	-	-	Х	Х	-	-	-	-	-	-	Х	-	-	Х	Х	-	-	_			
	N. disturbata	-	-	-	Х	-	-	-	-	Х	-	Х	-	-	-	-	Х	-	_			
	E. brasiliensis	-	-	-	-	-	-	-	-	-	-	Х	-	-	-	-	-	-	-			
	E. tricomerus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Notonectinae	M. bentoi	-	-	-	-	Х	-	-	-	Х	-	Х	-	Х	-	-	-	-	-			
	M. brasiliensis	-	-	-	Х	-	-	Х	-	Х	-	-	-	Х	-	Х	-	-	-	1		
	M. membranacea	_	-	-	-	_	_	-	Х	Х	-	Х	-	Х	-	Х	_	-	_			

Table 1. Distribution of Notonectidae species based on their records in States. Normal font: previous records; bold font: new records; italic: same states, different localities.

			AL			BA	CE	PE	PI	SE			
Conservation Unit		APACC	APAP	EEM	RESEX	RBPT	PNCD	EEA	PNCA	PNSC	PNSI	RBSI	
Biome		A.F	A.F	A.F	A.F	A.F	С	С	С	C/C.S.	A.F/C	A.F	
Subfamily	Species												
Anisopinae	B.amnigenus	-	-	-	-	-	Х	Х	Х	X	-	-	
	B. femoralis	-	-	-	-	-	-	-	-	х	_	-	
	B. fuscipennis	_	-	-	-	-	-	Х	Х	-	-	-	
	B. koina	_	_	-	-	-	х	-	-	-	_	-	
	B. konta	х	-	Х	-	-	Х	Х	-	-	Х	-	
	B. mutabilis	_	_	-	-	-	-	-	-	х	х	-	
	B. pallipes	_	-	-	-	Х	-	-	Х	-	-	-	
	B. platycnemis	_	_	-	-	-	-	-	X	-	_	-	
	B. pseudomutabilis	_	-	-	-	-	х	-	-	-	_	-	
	B. salutis	х	Х	-	-	-	х	X	-	-	х	-	
	B. tarsalis	х	Х	-	-	Х	х	Х	Х	х	_	х	
	B. unguis	-	Х	-	-	-	-	Х	Х	х	_	х	
	N. disturbata	_	_	-	-	X	-	X	X	-	х	-	
Notonectinae	E. brasiliensis	-	-	-	-	-	х	-	-	-	_	-	
	E. tricomerus	_	_	X	-	-	-	-	-	-	_	-	
	M. bentoi	х	-	Х	-	-	Х	Х	Х	-	Х	-	
	M. brasiliensis	-	-	-	Х	-	-	Х	-	-	-	-	
	M. membranacea	-	-	-	-	-	Х	-	-	-	-	х	

Table 2. Distribution of Notonectidae species by their presence in Conservation Units sampled in this work. A.F.: Atlantic Forest; C. Caatinga; C.S. Cerrado Savannah.

Acknowledgements

We are grateful for the help provided by our colleagues during the fieldwork and for the support provided by the conservation units' staff members. We are also grateful for the help provided by our colleagues during the fieldwork and for the support provided by the conservation units' staffs. We would like to thank Dr. Higor Rodrigues and Dra. Silvia Mazzuconi for invaluable comments and contributions in reviewing the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The material examined for this study was collected thanks to resources provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; #429936/2016-8 and #421413/2017-4). Fieldwork was conducted with permits from the Sistema de Autorização e Informação em Biodiversidade (SISBIO; #60275 and #62159). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível

Superior - Brasil (CAPES) - Finance Code 001. FFFM benefited from grants provided by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (#E-26/201.362/2021 and #E-26/200.649/2023) and the CNPq (#301942/2019-6).

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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

Two new species of freshwater planarian from Hainan Island and Leizhou Peninsula, southern China (Platyhelminthes, Tricladida, Dugesiidae)

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Academic editor: Yander L. Diez Received: 19 December 2024 Accepted: 16 February 2025 Published: 1 April 2025

ZooBank: https://zoobank. org/56F6F2E2-46DA-4CAE-A598-D3CCA498A790

Citation: Wang L, Chang Y-F, Sun X-X, Sluys R, Liu D-Z, Dong Z-M, Chen G-W (2025) Two new species of freshwater planarian from Hainan Island and Leizhou Peninsula, southern China (Platyhelminthes, Tricladida, Dugesiidae). ZooKeys 1233: 289–313. https://doi.org/10.3897/ zookeys.1233.142976

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Abstract

Two new species of the genus Dugesia from Hainan Island and Leizhou Peninsula are described by applying an integrative approach, including morphological, karyological, histological, and molecular information. In the molecular phylogenetic tree, the two new species, Dugesia saccata Chen & Dong, sp. nov. and Dugesia aconcinna Chen & Dong, sp. nov., fall into an Eastern Palearctic/Oriental clade and an Oriental/Australasian clade, respectively, while sharing only a rather distant relationship. The separate specific status of the two new species is supported also by their genetic distances. Dugesia saccata is characterised by the presence of the following features: a sac-shaped expansion at the knee-shaped bend of the bursal canal; ventrally displaced ejaculatory duct with a subterminal opening; a duct between diaphragm and seminal vesicle; mixoploid karyotype with diploid complements of 2n = 2x = 16 and triploid complements of 2n = 3x = 24, with all chromosomes being metacentric. Dugesia aconcinna is characterised by the presence of the following features: asymmetrical openings of the oviducts into the bursal canal and the common atrium, with the left oviduct opening into the common atrium and the right oviduct opening into the most ventral, proximal portion of the bursal canal, at the point where the latter communicates with the common atrium; vasa deferentia separately opening into the posterior portion of the seminal vesicle; penis papilla of a very characteristic shape, with the part housing the connecting duct, diaphragm, and ejaculatory duct being a cylindrical structure with a blunt tip, while at its right-hand side sits a large penial fold that attaches to the base of the penis papilla; ejaculatory duct following a ventrally displaced course through the penis papilla, after which it opens at the tip of the papilla; presence of a duct between diaphragm and seminal vesicle.

Key words: Dugesia, new species, southern China, taxonomy, triclads

Introduction

To date, approximately 110 species of freshwater planarians of the genus *Dugesia* Girard, 1850 have been reported from the Afrotropical, Palearctic, Oriental and Australasian biogeographic regions (Dols-Serrate et al. 2024). Over the past five years, 14 species of *Dugesia* have been described from China, from which ten species occur in Southern China (Chen et al. 2022; Wang et al.

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Figure 1. Collection sites of Dugesia in Hainan Island and Leizhou Peninsula.

2024; Zeng et al. 2024; Zhu et al. 2024, and references therein). These recent taxonomic studies revealed a rich biodiversity in southern China, which forms a potential distribution hotspot for *Dugesia* (Solà et al. 2022; Wang et al. 2024).

Hainan Island is a tropical island and the second largest island in southern China from which many species of insects have been reported (Li et al. 2020, 2024). The island lies opposite to the Leizhou Peninsula, the third largest peninsula in China, separated from it by the Qinzhou Strait, which forms a natural geographic barrier between the island and the peninsula (Fig. 1). Its special physical geographic environment contributes greatly to the fact that Hainan Island forms one of China's hotspots of biodiversity (Bu et al. 2019). The species diversity on Hainan Island includes the freshwater planarians *Dugesia majuscula* Chen & Dong, 2021 and *D. semiglobosa* Chen & Dong, 2021. In the present study we describe a third new species of *Dugesia* for Hainan Island as well as the first and equally new species of *Dugesia* for the Leizhou Peninsula, by applying an integrative approach, including morphological, karyological, histological, and molecular information, albeit the chromosomes could be examined for only one of the new species.

Materials and methods

Specimen collection and culturing

Specimens were collected from under stones in streams or springs with the help of a paintbrush. After collection, the worms were transferred to plastic bottles filled with stream water that during transportation to the laboratory were placed in a cooler filled with an ice bag. In an automatic incubator (BOXUN BSP-800) the planarians were cultured in autoclaved tap water at 20 °C and fed with fresh beef liver once per week. The worms were starved for at least seven days before being used for karyotype and histological studies and DNA extraction. Images of their external morphology were obtained by using a digital camera attached to a stereo-dissecting-microscope (Leica M165C).

In order to distinguish between sympatric populations at the same collection site, all worms were at first carefully examined in the laboratory under the stereo-microscope and separated into groups based on morphological differences. Hereafter, randomly selected individuals from each group or population (at least 3–5 worms from each population, including sexual and asexual individuals), were cut into two fragments. The anterior fragments were used for DNA extraction, amplification, and sequencing; the posterior fragments were used for histological or karyological studies. Sexual individuals from each population were used to prepare histological sections, irrespective whether they had been collected sexually mature in the field or had sexualised in the laboratory.

Phylogenetic analysis and genetic distances

Procedures for DNA extraction, amplification and sequencing followed Wang et al. (2021a). The quality and quantity of DNA was determined by Nano-Drop oneC (Thermo Scientific). For both new species, four specimens were used to extract DNA, and four gene fragments were amplified by polymerase chain reaction (PCR), namely 18S ribosomal gene (*18S rDNA*, type II), 28S ribosomal gene (*28S rDNA*), ribosomal internal transcribed spacer-1 (*ITS-1*), and Cytochrome C oxidase subunit I (*COI*). Primers used for amplification and the PCR protocol are listed in Suppl. material 1. In total, we generated datasets consisting of four gene sequences (*18S rDNA*, *28S rDNA*, *ITS-1*, and *COI*) of the two new *Dugesia* species and available sequences of other *Dugesia* species from major portions of the geographic range of the genus, while *Schmidtea mediterranea* (Benazzi et al., 1975), *S. polychroa* (Schmidt, 1861), and *Recurva postrema* Sluys & Solà, 2013, were chosen as the outgroup taxa to perform phylogenetic analyses (Table 1).

Sequence analyses were done as described previously by Wang et al. (2024). In brief, 18S rDNA, 28S rDNA and ITS-1 sequences were aligned online with MAFFT (Online version 7.247) using the G-INS-i algorithm. Protein-coding sequences (i.e., COI) were translated into amino acid sequences in order to check for the presence of stop codons (with the NCBI's genetic code 9 - Flatworm Mitochondrial). For COI, sequences were aligned online with Translator X (Abascal et al. 2010, http://translatorx.co.uk) using FFT-NS-2 method, were checked by BioEdit 7.2.6.1 (Hall 1999) and, thereafter, back-translated to nucleotide sequences. Since automated removal of gap columns and variable regions has been reported to negatively affect the accuracy of the inferred phylogeny (Dessimoz and Gil 2010; Tan et al. 2015), the Gblocks option was disabled (Talavera and Castresana 2007). A total of three datasets were used in this study, viz., dataset I: ITS-1 (46 sequences; Table 1), dataset II: COI (52 sequences; Table 1), dataset III: concatenated sequences COI + ITS-1 + 28S rDNA + 18S rDNA (171 sequences; Table 1). The substitution saturation test for COI (using DAMBE 6, according to Xia et al. 2003) showed no sign of saturation.

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Table 1. GenBank accession numbers of sequences used in molecular analyses. New species indicated in boldface.

Species	COI	ITS-1	285	18S
D. aconcinna	PV055688	PV055833	PV055834	_
D. adunca	OL505739	OL527659	_	_
D. aethiopica	KY498845	KY498785	KY498806	KY498822
D. afromontana	KY498846	KY498786	KY498807	KY498823
D. ancoraria	OR326966	OR296750	OR225689	OR198141
D. arabica	OL410620	OK587374	OK491342	OK646637
D. arcadia	KC006971	KC007044	OK491318	KF308694
D. ariadnae	KC006972	KC007048	OK491317	OK646636
D. batuensis	OL410626	OK587362	OK491316	OK646630
D. benazzii	FJ646977 + FJ646933	FJ646890	MK712509	OK646628
D. bengalensis	_	FJ646897	_	_
D. bifida	KY498851	KY498791	KY498813	KY498843
D. biiuga	MH119630	-	_	MH113806
D. circumcisa	MZ147041	MZ146782	_	_
D. cretica	KC006976	KC007050	OK491340	KF308697
D. constrictiva	MZ871766	MZ869023	_	_
D. damoae	KF308768	KC007057	OK491310	OK646619
D. deharvengi	KF907820	KF907817	KF907824	_
D. effusa	KF308780	KC007058	0K491311	OK646618
D. elegans	KC006984	KC007063	OK491313	KF308695
D. etrusca	EJ646984 + EJ646939	EJ646898	OK491312	0K646617
D. gemmulata	01 632201	-	-	-
D. gibberosa	KY498857	KY498803	KY498819	KY498842
D. gonocephala	EJ646986 + EJ646941	EJ646901	D0665965	D0666002
D. granosa	01 410634	KY498795	KY498816	KY498833
D. henta	MK712639	MK713035	OK491309	0K646612
D. hoidi	OR650791	_	-	-
D. improvisa	KC006987	KC007065	OK491304	KF308696
D. japonica	FJ646990	FJ646904	D0665966	D83382
D. liauriensis	0L410632	OK587358	OK491353	OK646615
D. malickvi	KC006988	KC007068	OK491294	OK646585
D. majuscula	MW533425	MW533591	_	_
D. mariae	OR650829	-	_	_
D. musculosa	OR189184	OR205922	_	_
D. naiadis	KF308756	OK587343	OK491293	_
D. notogaea	FJ646993 + FJ646945	FJ646908	KJ599720	KJ599713
D. parasagitta	KF308739	KC007073	_	OK646577
D. pendula	OR195337	OR205921	_	_
D. pustulata	MH119631	OK587366	OK491355	MH113807
D. ryukyuensis	AB618488	FJ646910	OK491323	AF050433
D. saccata	PV055687	PV055830	PV055832	PV055831
D. sagitta	KC007006	KC007077	OK491320	OK646567
D. semiglobosa	MW525210	MW526992	_	_
D. sicula	KF308797	OK587339	OK491287	KF308693
D. sigmoides	KY498849	KY498789	KY498811	KY498827
D. sinensis	KP401592	_	_	_
D. subtentaculata	MK712628	MK713004	MK712501	AF013155
D. tumida	OL505740	OL527709	_	_
D. umbonata	MT176641	MT177211	MT177210	MT177214
D. verrucula	MZ147040	MZ146760	-	_
R. postrema	KF308763	_	MG457274	KF308691
S. mediterranea	JF837062	AF047854	DQ665992	U31085
S. polychroa	FJ646975 + FJ647021	-	DQ665993	AF013152

Bayesian information criterion (BIC) was implemented in PartitionFinder 2 (Lanfear et al. 2017) to estimate the best-fit partition schemes and models of dataset III. The best models for each gene and codon position were as follows: ITS-1: GTR + G;18S rDNA and 28S rDNA: GTR + I + G; COI: GTR + I + G for the first and third codon positions, and HKY + I + G for the second codon position. Bayesian inference analysis (BI) was run with MrBayes v. 3.2 (Ronquist et al. 2012) using two replicate runs with four chains for 5 million generations, sampling trees every 1000 generations. The convergence of runs was checked by monitoring that the standard deviation of split frequencies reached a value below 0.01, thus indicating that the runs had reached a stationary state. Following completion of each analysis, the first 25% of the generated trees were discarded as "burn-in". Maximum likelihood (ML) analysis with IQ-TREE (Minh et al. 2020) was used to perform 10,000 replicates of ultrafast bootstrap approximation (Hoang et al. 2018). BI and ML trees were visualised and edited using Figtree v. 1.4.3. Genetic distances, based on dataset I and dataset II, were calculated by MEGA 6.06 (Tamura et al. 2013) with the Kimura 2-parameter substitution model (Solà et al. 2013).

Histology and karyology

Histological sections were prepared as described previously by Dong et al. (2017). In brief, worms were fixed in Bouin's fluid for 24 h, and, subsequently, rinsed and stored in 70% ethanol. For histological study, specimens were dehydrated in an ascending series of ethanol solutions, after which they were cleared in clove oil and embedded in synthetic wax. Serial sections were made at intervals of 6 μ m and were stained with Hematoxylin-Eosin (cf. Winsor and Sluys 2018). Histological preparations of specimens have been deposited in the Zoological Museum of the College of Life Science of Henan Normal University, Xinxiang, China (ZMH-NU), and Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH).

The air-drying method was used to obtain karyological preparations (Wang et al. 2024), while the karyological analysis was done with a compound microscope (ZEISS, Axio Scope. A1) equipped with CoolCube digital camera (MetaSystems, Altlussheim, Germany). Karyograms were prepared using the IKAROS Karyotyping system (MetaSystems, Altlussheim, Germany, https:// metasystems-international.com/en/products/ikaros/), according to the protocols described by Wang et al. (2024). For details on preparation and analyses of karyology, see Extended methods in Suppl. material 2.

Results

Molecular phylogeny and genetic distances

Phylogenetic trees were constructed using the alignment of 4308 base pairs (bp), including 1564 bp for *18S rDNA*, 1383 bp for *28S rDNA*, 656 bp for *ITS-1*, and 705 bp for *COI* (Table 1). Four specimens were examined from each of the two new species, *Dugesia saccata* and *D. aconcinna*, and these showed no variation in either of the four gene sequences.

The BI and ML generated trees showed identical topologies, differing only in support values (Fig. 2, Suppl. material 3). The two new species fall into two major groups, viz., an Eastern Palearctic/Oriental group and an Oriental/Australasian

group, albeit these are statistically poorly supported and, thus effectively form one polytomous group of species. Nevertheless, within this group the clade of the two species *D. pendula* Chen & Dong, 2024 and *D. tumida* Chen & Sluys, 2022 shares a sister-group relationship with *D. saccata*, with rather good support (pp = 86, bs = 0.86). The four species *D. adunca*, *D. ancoraria* Zhu & Wang, 2024, *D. bengalensis* Kawakatsu, 1983, *D. notogaea* Sluys & Kawakatsu, 1998 and *D. aconcinna* clearly cluster together, forming a clade with high support (pp = 100, bs = 1.00).

The highest *COI* distance values between *D. saccata* and *D. aconcinna* and their congeners were 21.54% (with *D. bijuga* Harrath & Sluys, 2019) and 22.75% (with *D. naiadis* Sluys, 2013), respectively, while the lowest *COI* distance values were 8.78% and 10.87% (both with *D. deharvengi* Kawakatsu & Mitchell, 1989). Furthermore, there is a 18.48% *COI* difference between the two new species (Suppl. material 4). With respect to *ITS-1*, *D. saccata* and *D. aconcinna* showed the highest distance values with *D. pustulata* Harrath & Sluys, 2019 and *D. naiadis* Sluys, 2013, being 19.60% and 21.25%, respectively, and exhibited the lowest distance values with *D. pendula* (1.76%) and *D. adunca* (2.97%). For *ITS-1*, the molecular distance between the two new species was 10.85% (Suppl. material 5). Thus, the separate species status of *D. saccata* and *D. aconcinna* is well-supported by both molecular phylogenetics and genetic distances.

Systematic account

Order Tricladida Lang, 1884 Suborder Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguñà & Riutort, 1998 Family Dugesiidae Ball, 1974 Genus *Dugesia* Girard, 1850

Dugesia saccata Chen & Dong, sp. nov.

https://zoobank.org/079234B5-51CF-4876-AFCD-C83ADD28B46A Figs 1-6

Collection site, habitat, and reproduction. On 24 February 2018, specimens were collected from a freshwater stream in the Yingge Mountains, Hainan Island (Figs 1, 3A), which is located within a national Nature Reserve at an altitude of 430 m a.s.l.; air temperature was 24 °C and water temperature was 21 °C. In the population of *D. saccata*, all worms were asexual at collection in the field, and under laboratory conditions were fissiparous. During a period of ~ 4 months, each of the ten specimens sexualised; in the first month only two sexualised individuals were found, while at the end of the fourth month ten sexual animals were present. After 18 months of culturing, sexualised worms produced > 10 cocoons. The spherical cocoons (1.3 mm in diameter) were dark brownish and provided with a stalk. Thus far, none of the cocoons hatched, most likely infertile. During laboratory culturing, the sexualised worms sometimes lost their copulatory apparatus and, subsequently, returned to the asexual mode of reproduction.

Material examined. *Holotype* • ZMHNU-YZCB1, Yongzhong village (18°46'6"N, 109°38'42"E), alt. 430 m a.s.l., Wuzhishan City, Hainan Province, China, 24 February 2018, coll. G-W Chen, D-Z Dong and co-workers, sagittal sections on 28 slides.



Figure 2. Topology of the molecular phylogenetic tree obtained from Bayesian analysis of the concatenated dataset (dataset III). Numbers at nodes indicate support values (pp/bs). New species indicated by asterisks. Scale bar: substitutions per site.

Paratypes • RMNH.VER.22249.1, ibid., sagittal sections on 12 slides • RMNH. VER.22249.2, ibid., sagittal sections on 20 slides • ZMHNU-YZCB2, 3, 6, ibid., sagittal sections on 19, 18, and 26 slides, respectively • ZMHNU-YZCB5, ibid., horizontal sections on 16 slides • ZMHNU-YZCB8, ibid., transverse sections on 35 slides.

Diagnosis. *Dugesia saccata* is characterised by the presence of the following features: symmetrical openings of the oviducts into the most proximal section of the bursal canal, near the point where the latter communicates with the atrium; a sac-shaped expansion at the knee-shaped bend of bursal canal; vasa deferentia opening symmetrically into posterior portion of the seminal vesicle; ventrally displaced ejaculatory duct with subterminal opening; a duct between diaphragm and seminal vesicle; mixoploid karyotype, with diploid chromosome portraits of 2n = 2x = 14, and triploid complements of 2n = 3x = 21, with all chromosomes being metacentric.

Karyology. Seven intact specimens were randomly selected to prepare metaphase plates. In a total of 157 metaphase plates that were examined, 42 plates





exhibited diploid chromosome complements of 2n = 2x = 14, while in 104 plates chromosome complements were triploid with 2n = 3x = 21 chromosomes; chromosome complements on the remaining 11 plates could not be determined, due to either lack of well dispersed chromosomes or over-dispersed sets of chromosomes. All seven specimens exhibited mixoploid chromosome complements, with all chromosomes being metacentric. Karyotype parameters, including relative length, arm ratio, and centromeric index, are given in Table 2. Chromosomal plates and an idiogram are shown in Fig. 4.

Morphology. In sexualised living specimens, the body measured 14–22 mm in length and 1.3–1.6 mm in width. Triangular head provided with two blunt auricles and two eyes, which are placed in pigment-free spots. Each pigmented eyecup houses numerous photoreceptor cells. The dorsal surface is taupe, the ventral surface is paler in colour than the dorsal one (Fig. 3C).

Pharynx situated at the mid-region of the body, measuring ~ 1/5 of the body length. Mouth opening located at posterior end of the pharyngeal pocket. The outer pharyngeal musculature is composed of a subepithelial layer of longitudinal muscles, followed by a layer of circular muscles. The inner pharyngeal musculature consists of a thick subepithelial layer of circular muscles, followed by a thin layer of longitudinal muscles.

Chromosome	Relative length	Arm ratio	Centromeric index	Chromosome type
1	19.00 ± 0.41	1.09 ± 0.05	47.91 ± 1.08	metacentric
2	16.74 ± 0.46	1.24 ± 0.12	44.85 ± 2.44	metacentric
3	15.03 ± 0.25	1.15 ± 0.05	46.69 ± 0.98	metacentric
4	14.00 ± 0.31	1.14 ± 0.07	46.93 ± 1.52	metacentric
5	13.08 ± 0.43	1.09 ± 0.03	48.00 ± 0.65	metacentric
6	12.00 ± 0.11	1.14 ± 0.05	46.78 ± 1.06	metacentric
7	10.26 ± 0.61	1.15 ± 0.03	46.74 ± 0.68	metacentric

Table 2. Karyotype parameters (mean values and standard deviations) of Dugesia saccata.



Figure 4. *Dugesia saccata* A1, B1 metaphase plate and karyogram of triploid complement A2, B2 metaphase plate and karyogram of diploid complement C idiogram. Scale bars: 5 µm.

The hyperplasic ovaries are located at 1/3-1/5 of the distance between the brain and the root of the pharynx, occupying ~ 1/2 of the dorso-ventral space, with several scattered masses. The oviducts arise from the dorsal wall of the ovaries, then turn to the ventral side and run in a caudal direction to the level of the genital pore, after which they curve dorso-medially to open separately and symmetrically into the bursal canal, near the point where the latter communicates with the atrium (Fig. 5A). Cyanophil shell glands discharge their secretion into the vaginal region of the bursal canal, at the level of the oviducal openings.

The small, dorsally located testes are poorly developed and provided with only a few mature spermatozoa. As a consequence, we found spermatozoa to be present in the vasa deferentia only in specimens YZCB3, 5, 6, and 8, as well as in RMNH.VER.22249.2. Testicular follicles are arranged on either side of the midline of the body in four or five longitudinal zones, extending from the posterior level of the ovaries to almost the posterior end of the body.

At the level of the penis bulb, the sperm ducts curve towards the dorsal body surface, then penetrate the ventral wall of the penis bulb to open separately into the seminal vesicle. The precise approach of the ducts to the seminal vesicle differs somewhat between specimens. In the holotype one sperm duct exhibits a short dorso-ventral recurvature before opening into the proximal section of the duct that leads from the seminal vesicle to the diaphragm; the other duct opens at the same position but has a much more direct approach (Fig. 6). In



Figure 5. *Dugesia saccata*. Photomicrographs of transverse (**A**, **B**) and sagittal (**C**, **D**) sections. **A** Paratype ZMHNU-YZCB8, showing symmetrical openings of oviducts into the bursal canal **B** paratype ZMHNU-YZCB8, showing symmetrical openings of vasa deferentia into the seminal vesicle **C** holotype ZMHNU-YZCB1, showing copulatory bursa and bursal canal **D** holotype ZMHNU-YZCB1, showing penis papilla, vasa deferentia, seminal vesicle, diaphragm, and ejaculatory duct. Abbreviations: bc: bursal canal; cb: copulatory bursa; d: diaphragm; ed: ejaculatory duct; lod: left oviduct; ma: male atrium; od: oviduct; pp: penis papilla; rod: right oviduct; se, sac-shaped expansion of bursal canal; sv: seminal vesicle; vd: vas deferents. Scale bars: 100 μm.

paratype YZCB-6 there is an even more distinctly asymmetrical approach of the sperm ducts, with one duct having a dorsal approach, after a well-developed dorso-ventral recurvature, opening in the antero-lateral portion of the seminal vesicle, again close to the duct leading to the diaphragm. The other sperm duct does not show the recurvature and opens directly into the proximal section of the connecting duct between seminal vesicle and diaphragm. In contrast, in paratype YZCB-8 both ducts exhibit a well-developed recurvature before symmetrically opening into the latero-dorsal portions of the seminal vesicle, close to the point where the connecting duct opens into the seminal vesicle (Fig. 5B).

The sperm ducts are lined with nucleated cells and surrounded by a layer of circular muscles. The oval-shaped, rather large seminal vesicle is lined by a flat, nucleated epithelium and is surrounded by a layer of irregularly crosswise arranged muscle fibres. The postero-ventral section of the seminal vesicle gives rise to a duct that is lined by an infranucleated epithelium, which is underlain by a subepithelial layer of intermingled muscle fibres and via a small diaphragm opens into the ejaculatory duct (Figs 5D, 6). The small diaphragm is located at approximately the root of the penis papilla and receives the abundant secretion of erythrophil penis glands (Fig. 5D). The ejaculatory duct, which is lined with a cuboidal, infranucleated epithelium, is devoid of any discernible musculature and follows a ventrally displaced course through the penis papilla, opening





Figure 6. *Dugesia saccata.* Sagittal reconstruction of the copulatory apparatus of holotype YZCB1 **A** female copulatory apparatus **B** male copulatory apparatus. Abbreviations: bc: bursal canal; ca: common atrium; cb: copulatory bursa; cg: cement glands; cpg: cyanophil penial glands; d: diaphragm; ed: ejaculatory duct; epg: extrabulbar penial glands; go: gonopore; lod: left oviduct; ma: male atrium; mo: mouth; pb: penis bulb; pp: penis papilla; rod: right oviduct; se, sac-shaped expansion of bursal canal; spv: spermiducal vesicles; sg: shell glands; sv: seminal vesicle; vd: vas deferens. Scale bars: 100 µm.

subterminally at its tip (Figs 5D, 6). Cyanophil penis glands discharge abundant secretion into the central and distal portion of the ejaculatory duct.

Because of the ventrally displaced course of the ejaculatory duct, the penis papilla is asymmetrical, with its dorsal lip being considerably larger than the ventral one (Figs 5D, 6). The cylindrical penis papilla has an oblique, ventro-caudal orientation and is covered by a nucleated epithelium, which is underlain by a subepithelial layer of circular muscle, followed by a layer of longitudinal muscle fibres (Figs 5D, 6). The copulatory bursa is a large sac-shaped structure that may occupy the entire dorso-ventral space (paratype YZCB-6), while in other specimens it extends well over the central longitudinal axis of the body but does not fully occupy the dorso-ventral space (e.g., holotype YZCB-1; Fig. 6). The bursa is lined by a vacuolated epithelium with basal nuclei and is almost devoid of any surrounding musculature. The bursal canal arises from the postero-dorsal wall of the bursa, after which it runs in a caudal direction to the left side of the male copulatory apparatus (Figs 5C, 6A). The rather broad bursal canal occupies ~ 1/4 of the dorso-ventral space. At the level of the gonopore it decreases somewhat in diameter, whereafter it gives rise to a saccate posterior extension (Figs 5C, 6). The antero-ventral, knee-shaped bend in the bursal canal arises from the ventral wall of the saccate portion and communicates with the common atrium (Figs 5C, 6).

The bursal canal is lined with a ciliated epithelium with basal nuclei. Particularly the dorsal wall of the canal may be thrown into several folds. It is noteworthy that the ventral wall of the bursal canal is lined with cylindrical cells, whereas the dorsal wall is composed of cuboidal or even flat cells; the saccate expansion is also lined with a low epithelium. The bursal canal is surrounded by a subepithelial layer of longitudinal muscles, followed by a layer of circular muscle that is particularly well developed on the ventral wall of the canal; an extra outer layer of longitudinal musculature, forming the ectal reinforcement, extends from the atrium to 2/3 on the bursal canal.

The common atrium communicates with a gonoduct, which is lined by a columnar epithelium and receives the openings of erythrophil cement glands (Fig. 6).

Etymology. The specific epithet is derived from the Latin noun *saccus*, bag, and alludes to the sac-shaped expansion at the knee-shaped bend of the bursal canal.

Discussion. There is one character that immediately sets *D. saccata* apart from all of its known congeners, the sac-shaped expansion on the posterior section of the bursal canal, near the knee-shaped bend of the canal. This is slightly reminiscent of a situation in Dugesia aethiopica Stocchino, Corso, Manconi & Pala, 2002, in which the posterior section of the bursal canal, immediately before receiving the separate openings of the oviducts, is expanded in lateral direction and gives rise to several large folds (Sluys 2007). However, this is merely a superficial resemblance to the situation in D. saccata, and these two species also differ in many other features. For example, D. aethiopica shows a horizontal approach of the bursal canal to the atrium, which represents a rare feature among species of Dugesia and is also absent in D. saccata. In Dugesia arabica Harrath & Sluys, 2013 the bursal canal is considerably expanded as well as highly folded in the region of the oviducal openings (Harrath et al. 2013). However, this situation differs from that in D. saccata in that the expansion sits near the oviducal openings, whereas in D. saccata the sac-shaped expansion occurs dorsally, or entally to the openings of the oviducts into the bursal canal. Another difference between these two species concerns the presence of a duct between seminal vesicle and diaphragm in D. saccata and absence of such a duct in D. arabica.

Two characteristic features of *D. saccata* may be found also in other species of *Dugesia*, a ventrally displaced ejaculatory duct with subterminal opening and the presence of a duct between the seminal vesicle and the diaphragm. Besides *D. saccata*, these two character states are also expressed, among others, in the three Chinese species *D. majuscula*, *D. umbonata* Song & Wang, 2020, and *D. verrucula* Chen & Dong, 2021 (Song et al. 2020; Wang et al. 2021a, b). However, in

contrast to *D.* saccata, in both *D.* majuscula and *D.* umbonata the ejaculatory duct has a subterminal dorsal opening at the tip of the penis papilla, while *D.* verrucula exhibits a permanent dorsal bump near the root of the penis papilla, which is absent in *D.* saccata. Another difference concerns the presence of a large muscularised hump on the dorsal surface of the bursal canal of *D.* umbonata, which is absent in *D.* saccata. Although these three species (*D.* umbonata, *D.* verrucula, and *D.* saccata) belong to the same clade, they are molecularly well-differentiated. On the other hand, while *D.* saccata, *D.* pendula, *D.* tumida, and *D.* majuscula belong to the same small clade, they are anatomically well differentiated.

In *Dugesia* species, the basic chromosome number concerns three types, 7, 8, and 9. Previous studies have shown that in China number 8 is the most frequent type, while 7 is much rarer (Wang et al. 2024). In that respect, it is noteworthy that the basic chromosome number in *D. saccata* is n = 7, which is shared only with *D. pendula*, *D. hepta* Pala, Casu, & Vacca, 1981, *D. batuensis* Ball, 1970, and *D. ryukyuensis* Kawakatsu, 1976 (Kawakatsu et al. 1976; Pala et al. 1981; Khang et al. 2017; Wang et al. 2024). However, *D. pendula* exhibits an aneuploid plus mixoploid karyotype, with diploid (2n = 2x = 14 + 0-1 B-chromosome) and triploid (2n = 3x = 21 + 0-1 B-chromosome) sets, while *D. batuensis* exhibits six metacentric chromosomes and one subtelocentric chromosome, and *D. ryukyuensis* shows six metacentric chromosomes and one submetacentric chromosome, which is the case also in *D. hepta*. In contrast, *D. saccata* exhibits a mixoploid karyotype with diploid (2n = 3x = 21) sets, with all chromosomes being metacentric, thus contrasting with the chromosome complements of the other species.

In fact, *D. saccata* produced infertile cocoons and only showed asexual reproduction by means of fission, which corresponds with its poorly developed or hyperplasic ovaries and the triploid chromosome complement. It has been established that in such abnormal ovaries the oocytes are anomalous, thus preventing regular oogenesis (Harrath et al. 2014).

Dugesia aconcinna Chen & Dong, sp. nov.

https://zoobank.org/1DBF6C63-D76D-46F8-B958-E807694E8639 Figs 1, 2, 7-9

Collection site, habitat, and reproduction. On 4 January 2019, the specimens were collected from a stream in the Qingfeng village, Leizhou Peninsula (Figs 1, 7A, B), which is a volcanic spring at an altitude of 56 m a.s.l.; air temperature was 22 °C and water temperature was 19 °C. With respect to the *D. aconcinna* population, six mature worms and five asexual worms were collected in the field. After ~ 5 months under laboratory conditions, all of the immature worms sexualised, although none of the worms (sexual in the field and the sexualised ones in the laboratory) produced any cocoons.

Material examined. *Holotype* • ZMHNU-TPYC5, Qingfeng village (21°14'33"N, 110°9'49"E), alt. 56 m a.s.l., Suixi County, Guangdong Province, China, 4 January 2019, coll. Z-M Dong, L Wang and J-Z Chen, sagittal sections on 14 slides. *Paratypes* • RMNH.VER.22250.1, ibid., sagittal sections on 11 slides • RMNH. VER.22250.2, ibid., sagittal sections on 10 slides • ZMHNU-TPYC1-3, 6, 7,11, ibid., sagittal sections on 14, 29, 15, 5, 21, 14 slides • ZMHNU-TPYC9, ibid., horizontal sections on 23 slides • ZMHNU-TPYC8, ibid., transverse sections on 19 slides.





Diagnosis. *Dugesia aconcinna* is characterised by the presence of the following features: live, mature animals rather small; asymmetrical openings of the oviducts into the common atrium; vasa deferentia separately opening into the posterior portion of the seminal vesicle; penis papilla of a very characteristic shape, with the part housing the connecting duct, diaphragm, and ejaculatory duct being a cylindrical structure with a blunt tip, while at its right-hand side sits a large penial fold that attaches to the base of the penis papilla; ejaculatory duct following a ventrally displaced course through the penis papilla, after which it opens at the tip of the papilla; presence of a duct between diaphragm and seminal vesicle.

Description. Body of both asexual and sexual live specimens is quite small, with the sexual worms being only 6-9 mm in length and 1.0-1.2 mm in width. The low-triangular head is provided with two blunt auricles and two eyes, which are placed in pigment-free spots. Each pigmented eyecup houses numerous photoreceptor cells. The dorsal surface is yellow-brown, with many brown pigment granules and pale blotches all over the dorsal surface; the ventral surface is paler than the dorsal body surface (Fig. 7C).

Pharynx situated in the mid-region of the body, measuring ~ 1/6 of the body length. Mouth opening located at the posterior end of the pharyngeal pocket. Outer pharyngeal musculature is composed of a thin, subepithelial layer of lon-

gitudinal muscles, followed by a thin layer of circular muscles; no extra inner layer of longitudinal muscles was observed. The inner pharyngeal musculature consists of a thick, subepithelial layer of circular muscle, followed by a thin layer of longitudinal muscle.

In those specimens in which we were able to examine the ovaries, most of the gonads were not hyperplasic (specimens TPYC3, 5, 7, 8, and RMNH.VER.22250.1), excepting specimens TPYC6, and 11, and RMNH.VER.22250.2. In general, the oval ovaries are situated at 1/3-1/4 of the distance between the brain and the root of the pharynx, occupying ~ 1/4 of the dorso-ventral space. The oviducts arise from the dorsal wall of the ovaries, then turn to the ventral side and run in a caudal direction to the level of the genital pore. Subsequently, the left oviduct bends dorsally to open into the common atrium, while the right oviduct exhibits a much more pronounced curvature towards the dorsal body surface, after which it recurves in antero-ventral direction to open into the most ventral, proximal portion of the bursal canal, at the point where the latter communicates with the common atrium (Figs 8A, 9A). Thus, the right oviducal branch opens dorsally to the left one. The oviducts are lined with a columnar, infranucleated epithelium.

The sac-shaped copulatory bursa lies immediately behind the pharyngeal pocket and may occupy the entire dorso-ventral space or, at least, a considerable portion of it. The bursa is lined with a columnar, vacuolated epithelium with basal nuclei and is devoid of any surrounding musculature (Figs 8B–D, F, 9). Near its communication with the postero-dorsal section of the bursa, the bursal canal is rather narrow and may occupy ~ 1/8 of the dorso-ventral space (Figs 8F, 9A). From thereon, the canal expands somewhat in diameter while it runs in a caudal direction to the left side of the male copulatory apparatus. At the level of the gonopore, the posterior section of the canal exhibits a rather abrupt, ventrally directed bend, after which it opens into the common atrium (Fig. 9A). The bursal canal is lined with cylindrical, infranucleated, ciliated cells and is surrounded by a subepithelial layer of longitudinal musculature, forming the ectal reinforcement, extends from the copulatory bursa to the atrium. Erythrophil shell glands open into the vaginal region of the bursal canal, near the oviducal openings.

The numerous, well-developed testes are situated dorsally and provided with mature spermatozoa. On either side of the midline of the body, testicular follicles are arranged in eight or nine longitudinal zones and extend from the posterior level of the ovaries to almost the posterior end of the body.

At the level of the pharyngeal pocket, the vasa deferentia expand to form spermiducal vesicles, which are packed with mature spermatozoa (Fig. 9). Upon reaching the large penis bulb, the vasa deferentia turn dorso-medially and quickly decrease very much in diameter while penetrating the wall of the bulb. Subsequently, the sperm ducts open separately and symmetrically into the mid-posterior section of the seminal vesicle, near the point where it communicates with the connecting duct that leads to the diaphragm. The sperm ducts are lined with a nucleated epithelium and surrounded by a layer of circular muscle.

The voluminous, oval seminal vesicle is lined by a flat, nucleated epithelium and is surrounded by a layer of intermingled muscle fibres. The seminal vesicle occupies ~ 2/5 of the dorso-ventral space and is located in the ventral portion of the penis bulb, close to the ventral body surface (Figs 8B–D, 9). Although the penis bulb is rather shallow, it is, nevertheless, a large structure, occupying







Figure 8. Dugesia aconcinna. Photomicrographs of sagittal (**B**–**D**), transverse (**A**, **E**) and horizontal (**F**) sections. **A** Paratype ZMHNU-TPYC8, showing penis papilla, common atrium, left oviduct and right oviduct **B** holotype ZMHNU-TPYC5, showing copulatory bursa, seminal vesicle, vasa deferentia, penis papilla, penis fold, male atrium, common atrium, and genital pore **C** holotype ZMHNU-TPYC5, showing copulatory bursa, seminal vesicle, diaphragm, penis papilla, penial fold, male atrium, and common atrium **D** holotype ZMHNU-TPYC5, showing copulatory bursa, seminal vesicle, diaphragm, ejaculatory duct, penial fold, male atrium, and common atrium **E** paratype ZMHNU-TPYC8, showing penis papilla, ejaculatory duct, penial fold, male atrium, and bursal canal **F** paratype ZMHNU-TPYC9, showing copulatory bursa, bursal canal, and penis fold. Abbreviations: bc: bursal canal; ca: common atrium; cb: copulatory bursa; d: diaphragm; ed: ejaculatory duct; go: gonopore; lod: left oviduct; ma: male atrium; pf: penial fold; pp: penis papilla; rod: right oviduct; sv: seminal vesicle; vd: vas deferens. Scale bars: 100 µm.

almost the entire dorso-ventral space (Figs 8B–D, 9). A relatively long and broad duct connects the seminal vesicle with a small diaphragm, the latter leading to the ejaculatory duct (Figs 8C, D, 9). This interconnecting duct is lined by an infranucleated epithelium and is surrounded by a layer of intermingled muscle fibres. The small diaphragm is located at the level of the root of the penis papilla and receives the abundant secretion of erythrophil penis glands (Figs 8C, D, 9). Both







Figure 9. *Dugesia aconcinna*. Sagittal reconstruction of the copulatory apparatus of holotype TPYC5. **A** Female copulatory apparatus **B** penial fold **C** male copulatory apparatus. Abbreviations: bc: bursal canal; ca: common atrium; cb: copulatory bursa; cg: cement glands; cpg: cyanophil penial glands; d: diaphragm; ed: ejaculatory duct; epg: extrabulbar penial glands; go: gonopore; lod: left oviduct; ma: male atrium; mo: mouth; pf: penial fold; pp: penis papilla; rod: right oviduct; spv: spermiducal vesicles; sg: shell glands; sv: seminal vesicle; vd: vas deferens. Scale bars: 100 µm.

the connecting duct and the ejaculatory duct run a ventrally displaced course through the penis papilla, with the relatively broad ejaculatory duct opening at the tip of the papilla (Figs 8D, F, 9). The ejaculatory duct is lined with a cuboidal, infranucleated epithelium and is devoid of any surrounding musculature.

The penis papilla has a very characteristic shape. The part that houses the connecting duct, diaphragm, and ejaculatory duct is a cylindrical structure with a blunt tip. This seems to be a rather symmetrical portion of the papilla but it should be noted that it concerns a lateral, left-hand part of the penis papilla. The right-hand part of the papilla develops a large penial fold (Figs 8E, 9B) that attaches to the base of the penis papilla. Penis papilla and penial fold are covered with a nucleated epithelium, which is underlain by a subepithelial layer of circular muscle, followed by a layer of longitudinal muscle fibres (Figs 8B–F, 9).

The genital atrium is divided into a common atrium and male atrium. The common atrium communicates with a gonoduct, which leads to the ventral gonopore; the gonoduct is lined by a columnar epithelium and receives the openings of cement glands (Fig. 9).

Etymology. The specific epithet is derived from the Latin adjective *aconcinna*, asymmetrical, and alludes to the asymmetrical penial fold as well as the asymmetrical oviducal openings into the bursal canal.

Discussion. A good number of *Dugesia* species possesses so-called penial annexes in the form of penial folds, which sometimes were indicated by the term adenodactyl. However, the term adenodactyl should not be applied to these penial annexes (Stocchino et al. 2017). Penial folds are located at the base of the penial papilla and are usually covered by the musculature of the penis bulb; folds may be of the parenchymatic-muscular type or of the parenchymatic-musculo-glandular type (Stocchino et al. 2017). Furthermore, penial folds may be located at both the ventral and dorsal side of the penis papilla, albeit the ventral fold may be smaller than the dorsal one, or a single fold may be restricted to the dorsal, dorso-lateral, or lateral portion of the papilla. Such a single fold is present in ~ 20 species of Dugesia. For the present comparative discussion, it suffices to concentrate on those species that exhibit a dorso-lateral or lateral penial fold more or less comparable to that of D. aconcinna, viz., D. arcadia de Vries, 1988, D. benazzii Lepori, 1951, D. golanica Bromley & Benazzi, 1991, D. hoidi Dols-Serrate, Stocchino & Riutort, 2024, D. iranica Livanov, 1951, D. libanica Bromley & Benazzi, 1991, D. mariae Stocchino, Dols-Serrate & Riutort, 2023, D. minotauros de Vries, 1984. However, all of these species differ from D. aconcinna in the absence of a connecting duct between the seminal vesicle and the diaphragm, perhaps excepting D. izuensis Katô, 1943 (cf. Sluys et al. 1998: table II; Dols-Serrate et al. 2024). For D. izuensis a diaphragm was not described but it is presumed that the abundant openings of eosinophilic penial glands approximately halfway into the ejaculatory duct (Kato 1950; Kawakatsu 1983) coincides with the location of the diaphragm, which is presumably very small. However, in other aspects D. izuensis is rather different from D. aconcinna. For example, in D. izuensis the penis papilla is a massive and plump structure, whereas the papilla in D. aconcinna is cylindrical. Other differences concern the penial fold. In D. izuensis the fold has the shape of a conical papilla with the central part filled with cyanophilic secretion, in contrast to the flap-like fold of *D. aconcinna* that lacks any secretions.

With respect to the shape and position of its penial fold, *D. aconcinna* resembles *D. benazzii*, *D. hoidi*, and *D. mariae*, all of which possess a flap-like penial

fold that extends dorso-laterally of the penis papilla, which holds true also for D. minotauros (de Vries 1984; Dols-Serrate et al. 2024). However, in all of these species the fold is situated on the left side of the penis papilla, in contrast to D. aconcinna in which the fold extends over the right side of the papilla. But there are also other differences. In D. benazzii and D. hoidi, the two vasa deferentia follow highly asymmetrical trajectories before opening, equally asymmetrically, into the seminal vesicle. In contrast, in both D. mariae and D. aconcinna the sperm ducts follow symmetrical trajectories. In D. iranica the penial fold sits also on the left side of the penis papilla, albeit it is not a flap-like fold, but a conical structure of variable size, which sometimes may be as large as the penis papilla; it is of the musculo-glandular type. All of this is different from the situation in D. aconcinna. Similarly to D. aconcinna, D. libanica possesses also a relatively long, cylindrical penis papilla with a blunt tip, with at its right-hand side a well-developed penial fold. In contrast to D. aconcinna, the fold of D. libanica is not flap-like but consists of a pear-shaped papilla, located dorsally to the right of the penis papilla, that may reach a length of ~ 3/4 of the penial papilla (Bromley and Benazzii 1991). Notably, none of above-mentioned species belongs to the same clade as D. aconcinna. Although, these species (D. adunca, D. ancoraria, D. bengalensis, and D. notogaea) are closely related molecularly, they can be easily distinguished from *D. aconcinna* by anatomical features.

General discussion

Molecular phylogenetic trees based on the concatenated dataset showed a basically identical topology with previous studies (Stocchino et al. 2017; Wang et al. 2021a, 2022; Chen et al. 2022). The clade comprising the Malagasy and Afrotropical/Mediterranean taxa is sister to the other major lineage in the phylogenetic tree of the genus *Dugesia*, comprising representatives from all other regions of the world. The Western Palearctic group shares a sister-group relationship with the Eastern Palearctic/Oriental/Australasian group. However, for *Dugesia*, the available molecular data and markers are limited, and the known species of China are scarce. In turn, this causes several nodes to have low support values in BI and ML trees (Fig. 1, Suppl. material 3).

Previous studies showed that the lowest *COI* and *ITS-1* distance values between species are usually higher than 6% and 1%, respectively (Lázaro et al. 2009; Solà et al. 2013). *Dugesia saccata* showed the lowest *COI* and *ITS-1* distances with *D. deharvengi* and *D. pendula*, being 8.78% and 1.76%, respectively. *Dugesia aconcinna* showed the lowest *COI* and *ITS-1* distances with *D. deharvengi and D. adunca*, being 10.87% and 2.97%, respectively. Therefore, *D. aconcinna* and *D. saccata* are well-separated from their congeners, which further supports their separate specific status as suggested by the morphological and phylogenetic analyses.

It is noteworthy that although *D. saccata*, *D. semiglobosa*, and *D. majuscula* are from Hainan Island, *D. saccata* shares only a distant relationship to *D. semiglobosa*. Furthermore, *D. saccata* differs anatomically greatly from *D. semiglobosa*, in that *D. saccata* has a duct between the seminal vesicle and the diaphragm, whereas *D. semiglobosa* has two diaphragms without a duct (Wang et al. 2021b). In contrast to *D. semiglobosa*, *D. saccata* exhibits a rather close relationship with *D. majuscula*, but the ejaculatory duct in *D. majuscula* has a dorsal opening at the tip of the papilla, contrasting with the subterminal and ventral opening in *D. saccata* (Wang et al. 2021b). Our results suggest that different *Dugesia* lineages were already present in the Hainan area prior to its isolation as an island.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

All handling procedures were strictly compliant with the current Animal Protection Law of China. This study did not involve endangered or protected species. No approvals were required for collections of specimens from the locations in this study. Ethical approvals are not required at Henan Normal University, Xinxiang University, or Naturalis Biodiversity Center for research conducted on invertebrates such as flatworms used in this study.

Funding

This work was supported by the National Natural Science Foundation of China (grant numbers: 32270501, 32470463, 32070427), the Major Public Welfare Project of Henan Province (grant number: 201300311700), Postdoctoral Research Project of Henan Province (grant number: HN2022136), the Foundation for the Key Research Program of Higher Education of Henan Province (25A180017), and by the Puyang Field Scientific Observation and Research Station for Yellow River Wetland Ecosystem, Henan Province.

Author contributions

Guang-Wen Chen and Zi-Mei Dong conceived and designed the study. Guang-Wen Chen, Zi-Mei Dong and Lei Wang sampled the specimens. Lei Wang and Xin-Xin Sun made the histological sections. Guang-Wen Chen, Zi-Mei Dong, Lei Wang, De-Zeng Liu, and Ronald Sluys analysed the histological sections. Lei Wang prepared the reconstruction drawings. Lei Wang, Yi-Fang Chang and Xin-Xin Sun performed the molecular analyses. Lei Wang prepared and examined metaphase plates. Lei Wang, Yi-Fang Chang and Xin-Xin Sun cultured and fed the worms. Lei Wang wrote the first draft of the manuscript. Guang-Wen Chen, Zi-Mei Dong and Ronald Sluys reviewed, revised and finalized the manuscript. All authors read and approved of the final manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information. Holotypes and paratypes of the two new species were deposited in the Zoological Museum of the College of Life Science of Henan Normal University, Xinxiang, China (ZMHNU), and Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH).

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Supplementary material 1

Primer sequences used for PCR amplification

Authors: Lei Wang, Yi-Fang Chang, Xin-Xin Sun, Ronald Sluys, De-Zeng Liu, Zi-Mei Dong, Guang-Wen Chen

Data type: docx

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Link: https://doi.org/10.3897/zookeys.1233.142976.suppl1

Supplementary material 2

Extended methods

Authors: Lei Wang, Yi-Fang Chang, Xin-Xin Sun, Ronald Sluys, De-Zeng Liu, Zi-Mei Dong, Guang-Wen Chen

Data type: docx

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Link: https://doi.org/10.3897/zookeys.1233.142976.suppl2

Supplementary material 3

Molecular phylogenetic tree obtained from ML analysis of the concatenated dataset

Authors: Lei Wang, Yi-Fang Chang, Xin-Xin Sun, Ronald Sluys, De-Zeng Liu, Zi-Mei Dong, Guang-Wen Chen

Data type: tif

- Explanation note: Numbers at nodes indicate support values (pp). New species indicated in red. Scale bar: substitutions per site.
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Link: https://doi.org/10.3897/zookeys.1233.142976.suppl3

Supplementary material 4

Genetic distances for COI

Authors: Lei Wang, Yi-Fang Chang, Xin-Xin Sun, Ronald Sluys, De-Zeng Liu, Zi-Mei Dong, Guang-Wen Chen

Data type: xls

- Explanation note: Highest and lowest distance values between the two new species and congeners indicated in blue and red, respectively. Green: distance value between the two new species.
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Link: https://doi.org/10.3897/zookeys.1233.142976.suppl4

Supplementary material 5

Genetic distances for ITS-1

Authors: Lei Wang, Yi-Fang Chang, Xin-Xin Sun, Ronald Sluys, De-Zeng Liu, Zi-Mei Dong, Guang-Wen Chen

Data type: xls

- Explanation note: Highest and lowest distance values between the two new species and congeners indicated in blue and red, respectively. Green: distance value between the two new species.
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Link: https://doi.org/10.3897/zookeys.1233.142976.suppl5



Research Article

Revision of the genus *Labidolanguria* Fowler, 1908 (Coleoptera, Erotylidae, Languriinae), with descriptions of a new species and two new combinations

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Abstract

The oriental genus *Labidolanguria* Fowler, 1908, previously remained unknown in China, is revised in this study. A new species, *Labidolanguria liangi* Huang, **sp. nov.**, is described and illustrated from the Xizang Autonomous Region. Two new combinations are proposed: *Labidolanguria apicata* (Zia, 1959), **comb. nov.**, and *L. sauteri* (Fowler, 1913), **comb. nov.** This genus now comprises four recognized species in Asian, and a key is provided for their identification. The relationship of the genus *Labidolanguria* to closely related genera is discussed.

Key words: China, Cucujoidea, identification key, lizard beetle, new record, taxonomy

Introduction

The Genus Labidolanguria has been monotypic since Fowler (1908) established it in the subfamily Languriinae. In the present study, a new species was discovered and is described here as Labidolanguria liangi **sp. nov.** Additionally, two species formerly described in another genus are transferred into the genus Labidolanguria: Tetraphala sauteri (Fowler, 1913) and Tetraphala apicata (Zia, 1959). The number of the species of Labidolanguria is increased from one to four, which are mostly distributed in the Himalayan region and southwestern China. An identification key for all known Labidolanguria species is also provided.

Materials and methods

The material studied is deposited in the following collections:

IZAS	Institute of Zoology, Chinese Academy of Sciences, Beijing, China
MNHN	Muséum national d'Histoire naturelle, Paris, France
NHMUK	Natural History Museum, London, United Kingdom
SDEI	Senckenberg Deutsches Entomologisches Institut, Müncheberg,
	Germany



Academic editor: Alexander Kirejtshuk Received: 14 July 2024 Accepted: 3 March 2025 Published: 1 April 2025

ZooBank: https://zoobank. org/7FDE27BF-6B00-4166-A0D7-F19619D28B40

Citation: Huang Z-Z, Yang X-K, Ge S-Q (2025) Revision of the genus *Labidolanguria* Fowler, 1908 (Coleoptera, Erotylidae, Languriinae), with descriptions of a new species and two new combinations. ZooKeys 1233: 315–323. https://doi. org/10.3897/zookeys.1233.132046

Copyright: © Zheng-Zhong Huang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Specimens used in this study were relaxed in distilled water for 12–24 h prior to dissection of the genitalia and mouthparts. Detached parts were soaked in 10% KOH solution for 12–24 h at room temperature, rinsed with distilled water, and dissected in 75% ethanol under a Nikon SMZ1000 stereomicroscope. All photographs of adults were taken by a Canon 5D Mark III digital camera equipped with a Canon MP-E 65 mm macro lens. The images were stacked with Helicon Focus v. 6.7.1 and edited with Adobe Photoshop CS6 to correct contrast, brightness, and imperfections. We utilized a Zeiss Axio Zoom.V16 Fluorescence Stereo Zoom Microscope equipped with an AxioCam MRc 5 camera to acquire images of the male and female genitalia. Subsequently, we conducted photomontage in Zen 2012 (blue edition) imaging software.

The holotype and paratypes of *L. liangi* sp. nov. and *L. apicata* (Zia, 1959) comb. nov. are deposited in IZAS. The syntype of *L. sauteri* (Fowler, 1913) comb. nov. is deposited in SDEI. The holotype of *Labidolanguria mucronata* Fowler, 1908 is deposited in NHMUK.

Taxonomy

Genus Labidolanguria Fowler, 1908

Labidolanguria Fowler, 1908: 9. Type species: Labidolanguria mucronata Fowler, 1908 by monotypy.

Diagnosis. Antennal club composed of 4–6 antennomeres. Dorsal body surface with green or deep -green metallic luster. Compound eyes large and finely faceted. Pronotum with basal pronotal carina. Elytral epipleura absent, apex of elytra acute, with outer angle of elytra somewhat acute and produced, sutural angle acute but not produced. Prosternal process rectangular, with two long grooves along each side.

Distribution. China (Xizang, Yunnan, Sichuan, Taiwan), India (Nilgiri Hills).

Remarks. When Fowler (1908) established this genus, he only compared it with the genus *Pentelanguria* Crotch, 1876. *Labidolanguria* and *Pentelanguria* share a similar elytral apex, which is acutely pointed. Apart from the smaller and more slender antennae in *Labidolanguria*, the two genera can also be distinguished by the prosternal process, which is smooth and flat in *Pentelanguria*, whereas in *Labidolanguria* it is grooved laterally.

The genera *Labidolanguria* and *Tetraphala* exhibit strong similarities in the structure of the antennae, pronotum, and prosternal process. However, they can be differentiated by the elytral apex, which is consistently truncate and bears small denticles in *Tetraphala*, while in *Labidolanguria* it is acute and lacks denticles.

Key to species of genus Labidolanguria

- 1 Club composed of the last four antennomeres (Fig. 3A, B)2

- Body deep copper green, with little metallic luster; outer angle of elytra not acute, sometimes with several denticles (Figs 1D, 2A–D)...... L. liangi sp. nov.

- Club composed of the last six antennomeres; abdomen with black or green metallic spots (Fig. 1C)L. apicata (Zia, 1959), comb. nov.

Labidolanguria mucronata Fowler, 1908

Fig. 1A

Labidolanguria mucronata Fowler, 1908: 9. Type locality: India, Nilgiri Hills. Type depository: NHMUK.

Type material examined. *Holotype*: Type [round label] // Type [red rectangular label] // Nilgiri Hills//Andrewes/Bequest. / B. M. 1922-221. // 623 [in red] // *Labi-dolanguria mucronata* Fowler/ TYPE [handwriting]//QR code NHMUK 010800985

Additional material examined. WALLARDI(Travancore) // MUSEUM PARIS/ Ex. Coll. M. MAINDRON/ Coll. G. BABAULT 1930 // Labidolanguria mucronata Fowl. / A.Villiers det.; Himalaya/ oriental/ R. P. Bertrand // MUSEUM PARIS/ Coll. L. BEDEL 1922; Travancore/ Inde [handwriting] // MUSEUM PARIS/ 1930/ COLL SICARD

Distribution. India.

Diagnosis. The antennal club of this species is only composed of the last four antennomeres, and the outer angle of elytra is more acute in comparison with that of other species in this genus.

Labidolanguria sauteri (Fowler, 1913), comb. nov.

Fig. 1B

Tetralanguroides sauteri Fowler, 1913: 133. Type locality: China, Taiwan (Taihorin, Kosempo). Type depository: SDEI.

Tetralanguria sauteri: Villiers1945: 290.

Tetraphala sauteri: Leschen and Wegrzynowicz 1998: 241.

Type material examined. *Syntype*: Kosempo/ Formosa/ H. Sauter, 1909 // 7. IX// Fowler det // *Tetralanguroides sauteri* Fowler. / TYPE // Syntypus [red label] // DEI Coleoptera / #200777.

Additional material examined. 6 exx. Formosa/ Taihorin / 1911; Kosempo/ Formosa/ 7. IX.1909.

Distribution. China (Taiwan).

Diagnosis. This species can be easily recognized by its antennal club composed of five antennomeres. The outer and sutural angles of the elytra are not very acute.

Labidolanguria apicata (Zia, 1959), comb. nov. Fig. 1C

Tetralanguria apicata Zia, 1959: 236. Type locality: China: Sichuan. Type depository: IZCAS.

Tetraphala apicata: Leschen and Wegrzynowicz 1998: 241.



Figure 1. Type materials of all species of *Labidolanguria* with labels pinned under each specimen **A***L*. *mucronata* Fowler, 1908, holotype **B***L*. *sauteri* (Fowler, 1913), comb. nov., syntype **C***L*. *apicata* (Zia, 1959), comb. nov., holotype **D***L*. *liangi* sp. nov., holotype. Scale bars: 5.0 mm.

Type material examined. *Holotype*: Sichuan Emei mountain, 1100 m, 1955. VI.22 [In Chinese] / leg. Wu Le [In Chinese] // [in Russian] // HOLOTYPE [red label] // IOZ(E)221361 [blue label] // *Tetralanguria apicata* m. / det. Zia Yonyon] 1957 [In Chinese]; Paratype: Sichuan Emei mountain, 580–1100 m, 1955.VI.22 [In Chinese] // leg. Huang Tian-rong [In Chinese] // [in Russian] // ALLOTYPE [green label] // IOZ(E)221362 [blue label].

Additional material examined. 1 \bigcirc 1 \bigcirc CHINA: Yunnan Malipo County Xiajinchang Village Zhongzhai / 2015.VI.7 leg. Huang Z.Z. / IZCAS// N 23°07.213' / E 104°49.378' / alt. 1855 m / IZCAS.

Distribution. China (Sichuan and Yunnan).

Diagnosis. This species is unique within the genus for its abdominal color pattern; all three ventrites from II to IV have a black or green metallic spot in the middle of the basal margin. The last ventrite has a black or green metallic luster. The abdominal coxal lines are long and parallel.

Labidolanguria liangi Huang, sp. nov.

https://zoobank.org/8DDDFDC6-E22B-4332-B3EB-99A6B159DE14 Figs 1D, 2A-D, 3A-L

Type material. CHINA • *Holotype* male; Xizang Prov., Motuo, CAS; 1980.V.19; Leg. Jin Gen-tao, Wu Jian-yi; 24207168; HOLOTYPE, *Labidolanguria liangi*, det. Huang Z.Z. [red label]. *Paratypes.* **CHINA** • 1 $^{\circ}$; Xizang Prov., Motuo Dexing; 970 m; CAS; 1980.VI.1; Leg. Jin Gen-tao, Wu Jian-yi; 24204767 • 1 $^{\circ}$; Xizang Prov., Motuo Dexing; 970 m; CAS; 1980.VI.1; Leg. Jin Gen-tao, Wu Jian-yi; 24204769 • 1 $^{\circ}$; Xizang Prov., Motuo Kabu; 1200 m; CAS; 1980.V.10; Leg. Jin Gen-tao, Wu Jian-yi; 24203357 • 1 $^{\circ}$; Xizang Prov., Motuo Yadong; 1250 m; CAS; 1980.V.25; Leg. Jin Gen-tao, Wu Jian-yi; 24204760 • 1 $^{\circ}$; Xizang Prov., Motuo Aniqiao; 1200 m; CAS; 1979.VII.21; Leg. Jin Gen-tao, Wu Jian-yi; 24201228 • 2 $^{\circ}$; Xizang Prov., Motuo Beibeng; 800–900 m; CAS; 1983.V.15; Leg. Han Yin-heng • 1 $^{\circ}$; Xizang Prov., Motuo; 2013.VIII.02–03;



Figure 2. *Labidolanguria liangi* sp. nov. A, B holotype, male A habitus, dorsal view B same, ventral view C, D same, paratype, female C same, dorsal view D same, ventral view. Scale bars: 5.0 mm.



Figure 3. Labidolanguria liangi sp. nov. A holotype, male antenna B paratype, female antenna C holotype, male mesotibia D paratype, female mesotibia E–I holotype, male genitalia E male genital, lateral view; J median lobe, dorsolateral view G apex of median lobe, dorsal view I tegmen, dorsal view H tegmen, lateral view J–L female genitalia, paratype J ovipositor, dorsal view K same, dorsolateral view L spermatheca, lateral view.

Leg. Bai Xing-long, Shan Jun-sheng; Heibei university museum • 13; Xizang Prov., Motuo, Beibeng country; 2013.VII.30; Leg. Bai Xing-long, Shan Jun-sheng; Heibei university museum • 13; Xizang Prov., Motuo County, Renqinbeng mount; 1314 m; 2015.VIII.26D; IOZCAS; 29.3175°N, 95.3333°E; Leg. Liang Hong-bin, Wang Ming-qiang • 149, 33; Xizang Prov., Motuo County, Deguo Bridge; 837 m; 2015.VIII.18;

IOZCAS; 29.4019°N, 95.3772°E; Leg. Huang Zheng-zhong • 2♀♀, 1♂; Xizang Prov., Motuo County, Yarang Village; 792 m; 2015.VIII.23; IOZCAS; 29.2964°N, 95.2772°E; Leg. Huang Zheng-zhong • 3♂♂, 2♀♀; Xizang Prov., Motuo County Dexing Country; 796 m; 2015.VIII.25; IOZCAS; 29.3203°N, 95.2911°E; Leg. Huang Zheng-zhong • 3♂♂, 3♀♀; Xizang Prov., Motuo County Motuo Town Lagong Tea plantation; 1253 m; 2021.VI.16D2; IOZCAS; 29.3191°N, 95.3158°E; sweep-net method; Leg. Liang Hong-bin, Xu Yuan et al. • 13, 299; Xizang Prov., Motuo County Motuo Town Lagong Tea plantation; 1294 m; 2021.VI.8D1; IOZCAS; 29.3187°N, 95.3157°E; Leg. Liang Hong-bin, Xu Yuan • 2 3 ざ; Xizang Prov., Motuo County, Beibeng Country, Gelin Village; 1758 m; 2021.VI.15D1; IOZCAS; 29.2134°N, 95.1717°E; Leg. Liang Hongbin, Xu Yuan • 13; Xizang Prov., Motuo County, Beibeng Country, Jiangxin Road 2 km; 830 m; 2021.VI.12N1; IOZCAS; 29.2325°N, 95.1461°E; light trap method; Leg. Liang Hong-bin et al. • 12; Xizang Prov., Linzhi City, Motuo County, Beibeng Country, Jiaga Valley; 677 m; 2021.VI.13; IOZCAS; 29.2540°N, 95.1965°E; Leg. Liu Hong • 1; Xizang Prov., Motuo County, Beibeng Country, Jiangxin Village; 764 m; 2021. VI.13; IOZCAS; 29.2240°N, 95.1311°E; Leg. Liu Hong.

Distribution. Known only from Motuo County (China, Xizang Autonomous Region).

Diagnosis. This species resembles *L. mucronata* in body structures, but it is distinguished by its darker, duller coloration, and the apex of the elytra which is not as acute. Moreover, *L. liangi* exhibits sexual dimorphism at the base of the mesotibia: the male mesotibial segment terminates medially with some denticulations and a prominent terminal tooth, while the female lacks these features.

Description. Body length 12.1–17.1 mm, width 2.4–3.3 mm. Body with coppery, metallic luster. Ventral side dark or black with metallic luster. Abdomen deep or pale brown; middle of ventrites I–IV nearly black and with deep green metallic luster; last ventrite always black and with metallic luster. Coxae and trochanters red-brown; femora and tibiae with green metallic luster. Tarsomeres I–III with indigo metallic luster. Antennomeres I–VII with green metallic luster; antennomeres VIII–XI with indigo metallic luster (Fig. 3A, B).

Body slender and subparallel. Head with dense punctures, coarsest near eyes but finer between eyes. Antennae 11 antennomeres; club pubescence composed of four antennomeres. Compound eyes medium-sized, finely faceted. Clypeofrontal suture obvious; clypeus wider than long, with dense punctures. Anterior edge of clypeus straight or sometimes concave in middle.

Pronotum slightly convex, nearly rectangular, longer than broad, finely punctate; sides subparallel, but middle part of lateral pronotal carina invisible from above. Anterior angle round, thick, and not produced; posterior angle not acute but produced, reaching elytral humeri. Basal fovea deep, with one pair of short, deep lateral fovea. Basal margin complete and clear.

Prosternum coarsely punctate and plicated, without setae. Prosternal process long and trapezoidal, weakly convex in middle, with fine punctures. Each lateral side with deep groove; apex of prosternal process broad.

Scutellar shield liguliform, with round apex. Elytra parallel before apex, regularly striate-punctate, and with intervals with fine punctation. Apices of elytra tapering and acute; sutural angle distinctly acute and produced; outer angle of elytra not acute as sutural angle but more produced.

Mesoventrite coarsely and densely punctate. Median suture of metaventrite not reaching apex of metaventral process. Abdomen finely punctate,



Figure 4. A, B the environment at the time of collecting, in Motuo County C Labidolanguria liangi sp. nov. feeding on spores on surface of fern leaf, and (inset) spores on beetle mouthparts. Photographs by Dr. Zheng-Zhong Huang.

with one pair of long, parallel coxal lines, reaching half of ventrite 1. Last ventrite with dense, yellow setae at apex. Male genitalia similar to those congeners in the genus. Median lobe long and slender, slightly curved. Apex of median lobe somewhat acute (Fig. 3E–G); internal sac invisible. Paramere slender, with apex not produced and with short yellow setae (Fig. 3H, I). Ovipositor short, with apex sclerotized and acute, without stylus and setae (Fig. 3J, K). Spermathecal capsule heart-shaped (Fig. 3L).

Dimorphism. The sexual dimorphism of this species is mainly reflected in the varying number of spines along the inner side of the mesotibia in males, with sometimes large, prominent terminal spines (Fig. 3C, D). This feature is not found in other species of the genus and is very rare even at the subfamily Languriinae in general. Some languriines exhibit sexual dimorphism in the form of protuberances on the profemur of males, such as in the genera *Doubledaya* and *Callilanguria* (Huang et al. 2018).

Etymology. The specific epithet honors Dr. Hong-bin Liang, a specialist of Carabidae from the Institute of Zoology, Chinese Academy of Sciences, for his outstanding leadership and contribution during multiple expeditions in Yunnan and Xizang provinces.

Host plant. In the field, we observed that this new species is relatively common in shrublands mixed with plants outside Motuo County and along the Renqingbeng Mountain Road (Fig. 4A, B). It lives on *Diplazium* sp. (family Athyriaceae) and was observed feeding on spores of this fern (Fig. 4C).

Variation. The variation of this species is mainly reflected in its body color and the spines on the mesotibia of males. Early collected specimens generally have a brownish body with a weak metallic luster. However, the specimens we collected in Motuo in recent years have a relatively dark -green body with a slight metallic luster. The differences in the spines on the inner side of the mesotibia of males among different individuals are also large, some are obvious, while others are not very prominent.

Discussion

Compared with the fungivorous erotylids, herbivorous languriines are rare in the family, yet their hosts remain largely unknown. Toki et al. (2020) recorded and summarized the hosts of some known species of adult and larval languriines.

Some species of *Tetraphala* feed on ferns, and consistent with our unpublished data, the species of ferns they feed on belong to different families or genera.

Fowler (1908) established this genus and considered it similar to *Pentelan-guria*, and both morphological characteristics and feeding habits indeed suggest that *Labidolanguria* and *Tetraphala* are rather closely related. The only difference between them is in shape of the elytral apex, which is why these two new combinations of *Labidolanguria* were initially assigned to *Tetralanguria*. (*Tetralanguria* was treated as a synonym of *Tetraphala* by Leschen and Wegrzynowicz 1998). In terms of feeding habits, both genera include relatively rare insects that feed on ferns. According to the latest review of phytophagous Coleoptera (Fuentes-Jacques et al. 2021), groups that feed on ferns account for 0.5% of currently recorded species with known feeding habits. *Labidolanguria* is here treated as a genus separate from *Tetraphala*, although it might be better to regard it as a subgenus. Further research on the phylogenetic relationships within Langurinae is essential to clarify this problem.

Acknowledgements

We sincerely thank all the curators, Mr Maxwell Barclay (NHMUK), Dr. Konstantin Nadein (SDEI), Dr. Theirry Deuve (MNHN), and Dr. Kui-yan Zhang (IZAS) for facilitating the first author's examination of the specimens. We also thank Dr. Hongbin Liang (IZAS) for leading many memorable expeditions. We would like to express our sincere gratitude to Dr. Szymon Konwerski (Adam Mickiewicz University, Poznań) and other two anonymous reviewers for their valuable comments and suggestions, which have greatly improved the quality of this manuscript. We are also deeply thankful to the editors, Dr. Alexander Kirejtshuk (Zoological Institute of the Russian Academy of Sciences, St. Petersburg) and Robert Forsyth (copy editor of ZooKeys), for their guidance and support throughout the editorial process.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was supported by the Institute of Zoology, Chinese Academy of Sciences (Grant No. 2023IOZ0310) and by the Second Tibetan Plateau Scientific Expedition and Research Program (STEP) under Grant No. 2019QZKK05010600.

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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

Acanthocephalans from freshwater fishes in northeast Thailand with the description of a new species of the subgenus *Acanthosentis* Verma & Dutta, 1929 (Acanthocephala, Quadrigyridae)

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Academic editor: David Gibson Received: 10 September 2024 Accepted: 13 November 2024 Published: 3 April 2025

ZooBank: https://zoobank.org/ CB90A0A9-4D96-4801-8359-AF1EB722A993

Citation: Lisitsyna O, Oros M, Ribas A, Poonlaphdecha S, Barčák D (2025) Acanthocephalans from freshwater fishes in northeast Thailand with the description of a new species of the subgenus *Acanthosentis* Verma & Dutta, 1929 (Acanthocephala, Quadrigyridae). ZooKeys 1233: 325–348. https://doi.org/10.3897/ zookeys.1233.136533

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Abstract

During an ichthyoparasitological survey in northeast Thailand in 2015, four species of acanthocephalans were found in four species of freshwater fishes. Adult stages of Pallisentis rexus Wongkham & Whitfield, 1999 and Raosentis sp. (Eoacanthocephala, Quadrigyridae) were collected from Channa striata (Anabantiformes, Channidae) and Mystus mysticetus (Siluriformes, Bagridae), respectively, and cystacanths of Arhythmorhynchus sp. (Palaeacanthocephala, Polymorphidae) were found on the visceral organs of M. albolineatus (Siluriformes, Bagridae). Adult acanthocephalans of the subgenus Acanthosentis Verma & Dutta, 1929 isolated from Barbonymus schwanenfeldii (Cypriniformes, Cyprinidae) were morphologically distinct from all described species of the subgenus in the arrangement of rings of tegumental spines in two fields with a more or less pronounced distance between them, and by the presence of a dome-shaped process with a ring of small spines at the base at the posterior end in females. Molecular data were generated for three species and phylogenetic analysis based on the 18S rDNA clearly supported the generic identification of Acanthogyrus (Acanthosentis) barbonymi sp. nov. and P. rexus. While the phylogenetic position of the former species within the genus could not be clarified, the latter species formed a well-supported sister lineage in a clade with isolates of four congeneric species. Acanthogyrus (Acanthosentis) barbonymi sp. nov. is formally described, the first genetic data for P. rexus are generated, a species of the genus Raosentis Datta, 1947 is reported for the first time outside the Indian subcontinent, and a key to the species of the latter genus is presented.

Key words: Acanthogyrus (Acanthosentis), Arhythmorhynchus, DNA, fish helminths, integrative taxonomy, *Pallisentis*, *Raosentis*

Introduction

Besides their classic recognition as causative agents of human and animal diseases, parasites are also integral components of ecosystems where they play remarkable roles in food webs, affect behavior, fitness, and survival of their hosts, and thus significantly contribute to forming a community structure

(Hudson et al. 2006). Considering helminths, acanthocephalans are among the most neglected group despite their global distribution and often high abundances, complex life cycles which cross-link different trophic levels, and other interesting attributes that make them promising models in studies of evolution of parasitism, host-parasite interactions, and ecotoxicology (Sures et al. 1999; Near 2002; Perrot-Minnot et al. 2023). Some species may also be pathogenic to their hosts, including humans (Mathison et al. 2021).

Acanthocephalans have been reported in several studies on fish parasites in Thailand, most of them dealing with commercial fish species (Wongkham and Whitfield 1999, 2004; Mard-Arhin et al. 2001; Lerssutthichawal and Supamattaya 2005; Yooyen et al. 2006; Chaiyapo et al. 2007; Purivirojkul and Areechon 2008; Sriwongpuk 2017; Juntaban et al. 2021), rarely with non-commercial ones (Tunya and Wongsawad 2019). In total, 826 species of freshwater and brackish fish belonging to 88 families have been recorded in Thailand (Froese and Pauly 2024) and acanthocephalans have been reported from only 18 freshwater fish species, belonging to ten families. In many of these faunistic papers, the identification was not supported with descriptions or drawings, nor with molecular data. Several studies have focused on the distribution or the ecology of acanthocephalans (Pearse 1933; Farooqi and Sirikanchana 1987; Wongkham and Whitfield 1999, 2004; Amin and Taraschewski 2003; Wahab et al. 2021).

To date, four species of acanthocephalans have been found in freshwater fishes in Thailand: *Pallisentis nagpurensis* (Bhalerao, 1931) from the Asian swamp eel *Monopterus albus* (Zuiew) and Bronze featherback *Notopterus notopterus* (Pallas); *P. ophiocephali* (Thapar, 1930) from Striped snakehead *Channa striata* (Bloch); *P. rexus* Wongkham & Whitfield, 1999 from *Ch. striata* and *M. albus*; and *Acanthogyrus* (*Acanthosentis*) siamensis (Farooqi & Sirikanchana, 1987) from the Silver barb *Barbonymus gonionotus* (Bleeker) (= *Puntius gonionotus*). Additionally, six acanthocephalans have been identified to genera: *Acanthogyrus* (*Acanthosentis*) sp., *Arhythmorhynchus* sp., *Pallisentis* sp, *Polyacanthorhynchus* sp., *Polymorphus* sp., and *Sphaerechinorhynchus* sp. (Mard-Arhin et al. 2001; Yooyen et al. 2006; Purivirojkul and Areechon 2008; Phalee 2018; Juntaban et al. 2021).

This paper presents data on an ichthyoparasitological survey in northeast Thailand in 2015, which were generated by an integrative approach combining alpha taxonomy and molecular phylogeny. The original findings involve the description of one new species of the subgenus *Acanthosentis*, the first record of the genus *Raosentis* outside India, and the first genetic data of the widespread species *Pallisentis rexus*. A key for identification for *Raosentis* species and phylogenetic analysis of the family Quadrigyridae are also presented.

Materials and methods

Specimen collection and morphological examination

The specimens studied in the present work were collected in Udon Thani and Nong Khai provinces of Thailand in April and May 2015 by examination of freshly captured fishes. Acanthocephalans were washed in tap water, kept in the refrigerator overnight to erect the proboscis, and fixed in non-denatured 70% ethanol. For light microscopy, temporary slides mounted in Berlese's medium were prepared. Line drawings were made using a drawing tube of Leica DM 5000B light microscope (Leica Microsystems, Wetzlar, Germany). All measurements in the text are in micrometers (μ m) unless otherwise stated. Trunk length does not include proboscis, neck, or evaginated bursa. The width of the body is given as the maximum width. The ordinal number of the hooks in the longitudinal row is indicated in brackets when describing the dimensions of the blades and the roots of the hooks.

For scanning electron microscopy, specimens were dehydrated in an ethanol series and dried in hexamethyldisilazane (HMDS). Subsequently, the specimens were sputter coated with gold and captured with a JEOL JSM 6510LA (JEOL Ltd., Tokyo, Japan).

The scientific and common names of the fish hosts follow FishBase (Froese and Pauly 2024). Selected specimens have been deposited in the Helminthological collections of the Institute of Parasitology, Biology center of the Czech Academy of Sciences, Budweiss, Czechia (**IPCAS**) and the Natural History Museum, London, UK (**NHMUK**) (see Suppl. material 1).

Molecular phylogenetic analysis

Total genomic DNA was extracted with Qiagen DNeasy Blood & Tissue kit from the middle part or posterior half of the body of the four Acanthosentis and three Pallisentis specimens (i.e., hologenophores); a complete cystacanth of Arhythmorhynchus was used as paragenophore (see Pleijel et al. 2008). The PCR amplification was targeted on three ribosomal nuclear markers and mitochondrial cytochrome c oxidase I (COI) using the primers of Garey et al. (1996) for 18S rRNA gene, ZX1 and 1500R primers (Olson et al. 2003; Bray et al. 2009) for 28S rRNA gene, LCO 1490 and HCO 02198 (Folmer et al. 1994) for partial COI gene. The primers used for amplification of the complete ITS region, forward (5'-GGAAGTAAAAGTCGTAACAAG-3') and reverse (5'-GATATGCTTAARTTCAGCGGG-3'), are reversed/complementary versions of ZX1 and WormB (Littlewood and Olson 2001; Bray et al. 2009). The PCR products were verified on agarose gel by electrophoresis and enzymatically purified (Werle et al. 1994). The templates were sequenced by the Sanger method, at least two raw reads were de-novo aligned to create contiguous sequences, which were manually inspected for ambiguous positions. Newly generated sequences were deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

The phylogenetic relationships within the family Quadrigyridae (Gyracanthocephala) were calculated based on 18S rRNA gene, the marker with reasonable number of available sequences. The dataset was aligned using MAFFT v. 7.490 employing the algorithm E-INS-i (Katoh et al. 2002; Katoh and Standley 2013). The 18S alignment, after manual removal of misaligned regions due to gaps, was 1,375 bp long. The optimal nucleotide substitution model TN+F+G4 was calculated in ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017) using AICc criterion. Phylogeny was estimated in IQtree 2.0.5. using ultrafast bootstrapping of 1,000 replicates (Hoang et al. 2018; Minh et al. 2020).

Results

Species descriptions

Eoacanthocephala Quadrigyridae

Acanthogyrus (Acanthosentis) barbonymi sp. nov.

https://zoobank.org/C3E9FFBA-329F-4D0B-B70E-FDDB85AC2909 Figs 1-3

Type host. Tinfoil barb *Barbonymus schwanenfeldii* (Bleeker) (Cypriniformes, Cyprinidae).

Type locality. Nong Khai Inland Fisheries Research and Development Center, Had Sai Thong village, Nong Khai province, Thailand (17°55.318'N, 102°36.230'E).

Site of infection. Intestine.

Infection rates. Prevalence 87.0%, intensity 7–67.

Type material. Deposited in the Helminthological collections of IPCAS and NHMUK (Coll. nos. IPCAS A-145 and NHMUK 2025.1.8.1-15).

Molecular data. The sequences of nuclear 18S rRNA (1,767 bp), 28S rRNA (1,188 bp) and the mitochondrial COI (657 bp) genes of *Acanthogyrus (Acanthosentis) barbonymi* sp. nov. were deposited in the GenBank database (Acc. nos. PQ636375–PQ636378, PQ636383–PQ636385, PQ631040, PQ631041).

Etymology. Species name is derived from the scientific name of the host.

Morphology. Quadrigyridae with features of genus *Acanthogyrus* and subgenus *Acanthosentis*. Acanthocephalans white in color, medium size, usually sickle-shaped, with maximum width in anterior third of body (Figs 1–3). Female larger than male. Anterior body part with two fields of tegumental spines in rings with rosette-shaped root processes (Figs 1B, 2A, D, E). Number of giant tegumental nuclei not constant. Proboscis small, round, armed with 18 hooks in three rows, six hooks in each row. Sensory pore in base of proboscis (Figs 1A, B, 3B). Hooks of the anterior row large, with simple massive roots directed posteriorly, located irregularly, three slightly anteriorly, three slightly posteriorly (Fig. 1D). Hooks of middle row twice as small as hooks of anterior row, with complex forked roots directed anteriorly. Hooks of basal row smallest, with simple roots directed posteriorly (Fig. 1A, D). Neck conical. Proboscis receptacle with single-layer muscular wall, with cephalic ganglion at bottom. Lemnisci almost equal in length, extending to middle of body. Genital pore terminal in both sexes (Figs 1, 2B, C, 3D).

Male (based on nine mature specimens with sperm, measurements of the holotype specimens followed with measurements of type series). Trunk 3.54 (2.78–4.93) mm long, 618 (585–921) wide (Fig. 1B). Tegumental spines of anterior field with nine (9–12) rings, 16 (16–20) spines in each ring. Posterior rings of anterior field incomplete dorsally, basal ring with 2–10 spines. Length of spine blades 13 (12–16), diameter of rosette root 15 (12–16). Distance between spines fields (154–189). Somatic spines of posterior field with nine (8–10) rings, 12 (12–15) spines in each ring. Posterior rings of posterior spines field incomplete dorsally, basal ring with five or six spines. Length of spine blades 7 (7–14), diameter of rosette root 14 (12–24).



Figure 1. Line drawing of *Acanthogyrus* (*Acanthosentis*) *barbonymi* sp. nov. ex *Barbonymus schwanenfeldii* (Bleeker) from Thailand **A** proboscis of male, note sensory pore **B** total view of male **C** posterior end of mature female **D** hooks of proboscis **E** posterior end of immature female. Abbreviations: SP – sensory pore. Scale bars: 100 µm (**A**, **D**); 500 µm (**B**, **C**, **E**).



Figure 2. Light microscope photographs of *Acanthogyrus* (*Acanthosentis*) barbonymi sp. nov. ex Barbonymus schwanenfeldii (Bleeker) from Thailand **A** anterior part of female **B** posterior part of male with eggs in the cavity of the bursa **C** posterior ends of both sexes during copulation **D**, **E** tegumental spines **F** eggs. Scale bars: 300 μm (**A**–**C**); 50 μm (**D**–**F**).

Body wall with six (5-7) giant tegumental nuclei, four (2-4) dorsal, and two (1-3) ventral. Proboscis 73 $(73-99) \times 108$ (108-138). Length of hook blades of anterior row 62-63 (51-69), middle 26 (21-26), basal 18 (18-24). Length of hook roots of anterior row 26-28 (25-45), middle 19 (16-20), basal 10

(10–15). Neck 74 (72–110) long, wide in anterior part 86 (86–105), in posterior part 126 (126–163). Proboscis receptacle 424 (370–467) × 91 (91–155). Lemnisci do not reach anterior testis, 874 (483–1,499) × 71 (44–92) and 834 (473–1,561) × 76 (44–98). Organs of reproductive system in posterior half of body. Testes oval, in tandem, anterior larger than posterior. Anterior testis 620 (429–1,078) × 399 (313–605), posterior testis 477 (378–702) × 356 (221–590). Cement gland oval, adjacent to posterior edge of posterior testis, 339 (267–575) × 302 (261–628). Almost round cement reservoir posteriorly to cement gland, 171 (121–280) × 179 (153–312). Saefftigen's pouch club-shaped, 249 (432–621) × 82 (82–173). Vas deferens elongated, 393 (393–474) × 124 (119–124). Type specimen with leaf-shaped penis 94 × 42. Evaginated bursa 361 (335–361) × 229 (229–343).

Female (based on 13 specimens, 7 with eggs, 6 without eggs). Trunk 5.60-11.72 mm long, 0.86-1.45 mm wide. At base of caudal process 4-8 very small spines in one ring (Fig. 3E). Tegumental spines of anterior field with 10 or 11 rings, 16-22 spines in each ring. Posterior rings of anterior field incomplete dorsally, basal ring with 8-11 spines. Length of spine blades 12-15, diameter of rosette root 15-20. Distance between spines fields 100-262. Somatic spines of posterior field with 10-12 rings, 13-17 spines in each ring. Number of spines in ring decreases towards basal ring to 3-12. Length of spine blades 10-13, diameter of rosette root 13-20. Body wall with 6-10 giant tegumental nuclei, 2-6 dorsal, 2-5 ventral. Proboscis 112-136 × 133-170 (Figs 1A, 3C). Length of hook blades of anterior row 59-78, middle 28-34, basal 26-27. Length of hook roots of anterior row 33-45, middle 18-31, basal 15-17. Neck 42–129 long, width of anterior part 105–124, width of posterior part 166–320. Proboscis receptacle 361-560 × 124-147. Lemnisci 1.29-1.54 mm long, 83-154 wide. Female reproductive tract in posterior part of body, 962-1,400 long. Vagina with two sphincters (Fig. 1C, E). Eggs fusiform, elongate, no polar prolongation of fertilization membrane, 21–24 × 8–10 (Fig. 2F). Posterior end of female forms somewhat pronounced dome-shaped process, 180-343 × 198-356 (Fig. 3D) with complete or incomplete ring of 4-8 small spines at its base. At the moment of copulation, the male's bursa embraces the domeshaped caudal process of the female (Fig. 2C), and the eggs are injected into the cavity of the male bursa. After copulation, the bursa invaginates and some of the eggs may temporarily remain in cavity of bursa (Fig. 2B).

Remarks. To date, 57 species have been described in the subgenus *Acanthosentis* of the genus *Acanthogyrus*, mainly parasites of freshwater fish in South and Southeast Asia (Amin 2005, 2013; Naidu 2012; Saxena et al. 2013; Amin et al. 2017; Mohd-Agos et al. 2021; Rana and Kaur 2023). *Acanthogyrus (Acanthosentis) barbonymi* sp. nov. differs from most species of the subgenus in the arrangement of rings of tegumental spines in two fields with a more or less pronounced distance between them, as well as the presence of a dome-shaped process with a ring of tiny spines at the base at the posterior end of the females. The arrangement of spines in two fields is characteristic for two species of the subgenus *Acanthosentis*, *A. (A.) multispinus* (Wang, 1966), described from the silver carp *Hypophthalmichthys molitrix* (Valenciennes) from China (Wang 1966) and *A. (A.) bispinosa* Rana & Kaur, 2023 from the mrigal carp *Cirrhinus mrigala* Hamilton and the orangefin labeo *Labeo calbasu* Hamilton in Malaysia. However, *A. (A.) barbonymi* sp. nov. differs from *A. (A.) multispinus* in two characteristics:



Figure 3. Scanning electron micrographs of Acanthogyrus (Acanthosentis) barbonymi sp. nov. ex Barbonymus schwanenfeldii (Bleeker) from Thailand **A** anterior part of female **B** lateral view on proboscis of female, note the sensory pore (arrowhead) **C** subapical view on proboscis of female **D** caudal process of female posterior end **E** tiny spine at base of caudal process. Scale bars: 100 μ m (**A**); 20 μ m (**B**, **C**); 50 μ m (**D**); 5 μ m (**E**).

i) posterior rings of tegumental spines of *A*. (*A*.) *barbonymi* sp. nov. are incomplete in both fields, and the rings of the posterior field do not extend to the middle of the body; while the rings of spines in both fields of *A*. (*A*.) *multispinus* are complete and the rings of the posterior field of spines reach the posterior end of the body; ii) the proboscis hooks of the middle row *A*. (*A*.) *barbonymi* sp. nov.

are half the size of the hooks of the apical row; while in *A*. (*A*.) *multispinus*, the size of the proboscis hooks gradually decreases from the apical to the basal row. *A*. (*A*.) *barbonymi* sp. nov. differs from *A*. (*A*.) *bispinosa* in the number of rings of spines, with 9–12 rings of spines in the anterior field, 8–11 rings of spines in the posterior field versus 7–10 rings of anterior spines and 23–38 rings of posterior spines in *A*. (*A*.) *bispinosa* (Rana and Kaur 2023).

One species of the subgenus Acanthosentis, A. (A.) siamensis (Farooqi & Sirikanchana, 1987) Amin, 2005, has been found in the silver barb Barbonymus gonionotus (Bleeker) (= Puntius gonionotus) in Thailand (Farooqi and Sirikanchana 1987). Acanthogyrus (A.) barbonymi sp. nov. and A. (A.) siamensis have similarities in the size of the proboscis hooks and the shape of the female caudal process; however, they differ in the number of rings of body spines: 20–26 in A. (A.) barbonymi sp. nov. vs 3–4 in A. (A.) siamensis.

Recently, Mohd-Agos et al. (2021) described three new species of the subgenus Acanthosentis from Barbonymus schwanenfeldii from Lake Kenyir in Malaysia, namely A. (A.) kenyirensis Mohd-Agos, Mohd-Husin, Zakariah, Yusoff, Wahab, Jones, Hassan, 2021, A. (A.) tembatensis Mohd-Agos, Mohd-Husin, Zakariah, Yusoff, Wahab, Jones, Hassan, 2021 and A. (A.) terengganuensis Mohd-Agos, Mohd-Husin, Zakariah, Yusoff, Wahab, Jones, Hassan, 2021. Although described from the same fish host, A. (A.) barbonymi sp. nov. differs from these three species in the size of the proboscis hooks, and the hooks of the middle and basal rows of A. (A.) barbonymi sp. nov. have similar lengths and are approximately half as long as the hooks of the anterior row, whereas in A. (A.) kenyirensis, A. (A.) tembatensis, and A. (A.) terengganuensis the hooks of the anterior and middle rows are of comparable length and more than twice as long as the hooks of the basal row. Additionally, A. (A.) barbonymi sp. nov. clearly differs from them in other morphological characters: i) A. (A.) barbonymi sp. nov. has 4-11 giant nuclei in the tegument whereas A. (A.) kenyirensis and A. (A.) terengganuensis have no giant nuclei in the tegument; ii) the tegumental spines of A. (A.) barbonymi sp. nov. form two fields, with 9-12 rings of spines in the anterior field and 8-11 rings of spines in the posterior field whereas tegumental spines in A. (A.) kenyirensis and A. (A.) tembatensis are in one field with eight or nine rings; iii) A. (A.) kenyirensis, A. (A.) tembatensis, and A. (A.) terengganuensis have a unique collar ring on the neck and a muscular-like structure on both sides of proboscis attached to the ring whereas this structure absent in A. (A.) barbonymi sp. nov.; iv) females of A. (A.) barbonymi sp. nov. have a caudal process with tiny spines in one ring at its base whereas details of the caudal process in females of the three species from Malaysia were not mentioned. These morphological differences suggest that A. (A.) barbonymi sp. nov. is not conspecific with A. (A.) kenyirensis, A. (A.) tembatensis, nor A. (A.) terengganuensis.

Mohd-Agos et al. (2021) generated a sequence of ITS region from each of the three Malayan species (MK184204, MK184205, MK069588; 589–813 bp); a single COI sequence of *A*. (*A*.) *kenyirensis* (MN833316; 633 bp) was submitted to the GenBank by these authors, but this was not included in their work. COI sequences of our hologenophore specimens were almost identical (99.0% pairwise similarity) with the unpublished sequence of *A*. (*A*.) *kenyirensis*. The ITS marker could not be used for reliable phylogenetic analysis because we were unable to generate sequences of sufficient length. However, comparison of short (131 bp)

long) ITS sequences of our three hologenophores showed the highest pairwise similarity of *A*. (*A*.) *barbonymi* sp. nov. with *A*. (*A*.) *terengganuensis* (95.8%), followed by *A*. (*A*.) *kenyirensis* (89.2%) and *A*. (*A*.) *tembatensis* (83.2%). Since the analyses of ITS and COI markers provided inconsistent results, this could indicate misidentification of the specimens from Malaysia used for genotyping.

Pallisentis rexus Wongkham & Whitfield, 1999

Figs 4, 5

Host. Striped snakehead *Channa striata* (Bloch) (Anabantiformes, Channidae). Locality. Nong Samrong Lake, Nong Samrong Town, Udon Thani province,

Thailand (17°27.065'N, 102°45.791'E) and a fish farm in Kong Nang village, Tha Bo Town, Nong Khai province, Thailand (17°54.190'N, 102°35.211'E).

Site of infection. Intestine.

Infection rates. Prevalence 84.9%, intensity 1–35.

Molecular data. The nuclear18S rRNA (1,735 bp), 28S rRNA (1,085 bp), ITS (774 bp) and the mitochondrial COI (623 bp) genes sequences of *Pallisentis rex-us* were deposited in the GenBank database (Acc. nos. PQ636379–PQ636381, PQ636386–PQ636388, PQ636390, PQ636391, PQ631042–PQ631044).

Morphology (based on 5 males, 7 females). Medium-sized, white acanthocephalans. Anterior part of trunk with two fields of spines in rings (Figs 4A, 5A). Distance between anterior and posterior fields 105–151. Proboscis length less than its width (Fig. 4A). Proboscis with four rows of hooks, 12 hooks in each row. Hooks size decreases from apical to basal row. Proboscis receptacle with single-layer muscular wall, with cephalic ganglion in middle part. Neck conical. Lemnisci longer than proboscis receptacle. Gonopore terminal in both sexes.

Male. Trunk 4.03–7.3 mm long, 389–446 wide. Tegumental spines of anterior field with 14 rings, 14–18 spines in each. Length of spines 27-31 (Fig. 4C). Somatic spines of posterior field in 20-32 rings, anterior rings complete, ten spines in each, posterior rings incomplete, 2–4 in each. Length of spines 27-38 (Fig. 4D). Proboscis $164-214 \times 253-289$ (Figs 4A, 5B). Length of hook blades of anterior row 69-81, second 59-69, third 38-42, basal 25-33. Length of hook roots of anterior row 47-58, second 39-44, third 30-49, basal 19-25. Proboscis receptacle $419-594 \times 98-160$. Neck 164-186 long. Lemnisci $921-1,359 \times 70-98$. Organs of reproductive systems in posterior half of trunk (Fig. 4B). Testes I $575-695 \times 172-200$, II $636-654 \times 125-198$. Single cement gland $1,079 \times 115-183$, cement reservoir $121-432 \times 153-200$. Saefftigen's pouch club-shaped, $307-478 \times 115-125$.

Female. Trunk 10.00–12.55 mm long, 264–405 wide. Tegumental spines of anterior field with 12–15 rings, anterior rings complete, 14–20 spines in each, posterior rings incomplete dorsally, 6–14 in each. Length of spines 25–34. Somatic spines of posterior field with 40–47 rings, ten spines in anterior rings, 3–11 in posterior rings. Length of spines 39–48. Proboscis 110–216 × 206–319. Length of hook blades of anterior row 74–85, second 63–74, third 45–52, basal 31–39. Length of hook roots of anterior row 48–67, second 43–65, third 35–46, basal 27–29. Proboscis receptacle 520–760 × 105–166. Neck 184–275 long. Lemnisci 1,094–1,115 × 55–72. Reproductive tract 398–531. Egg fusiform, elongate, no polar prolongation of fertilization membrane, 92–102 × 42–53 (Fig. 4E).



Figure 4. Light microscope photographs of *Pallisentis rexus* Wongkham & Whitfield, 1999 ex *Channa striata* (Bloch) from Thailand **A** anterior part of female **B** posterior part of male **C** tegumental spines of anterior field **D** tegumental spines of posterior field **E** eggs. Scale bars: 300 µm (**A**, **B**); 50 µm (**C**–**E**).

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Figure 5. Scanning electron micrographs of *Pallisentis rexus* Wongkham & Whitfield, 1999 ex *Channa striata* (Bloch) from Thailand **A** anterior part of male **B**, **C** two views on proboscis of male. Scale bars: 100 µm (**A**); 50 µm (**B**, **C**).

Remarks. *Pallisentis rexus* (Eoacanthocephala, Quadrigyridae) was described from the striped snakehead *Channa striata* (Bloch) in the Chiang Mai Basin in Thailand (Wongkham; Whitfield, 1999). Later, immature specimens of this species were found in the Asian swamp eel, *Monopterus albus* (Zieuw) from Bangkok, Thailand (Amin; Taraschewski 2003). Adult *P. rexus* were also found in *Channa* sp. from a river in northern Taiwan (Lisitsyna et al. 2023).

Raosentis sp.

Figs 6, 7A

Host. Mystus mysticetus Roberts (Siluriformes, Bagridae).
Locality. Flood area of the Dan canal near Daeng Ban Non Du village, Udon Thani Province, Thailand (17°32.891'N, 103°03.831'E).
Site of infection. Intestine.



Figure 6. Line drawing of *Raosentis* sp. ex *Mystus mysticetus* Roberts from Thailand **A** anterior part of male **B** total view of male **C** hooks of proboscis. Scale bars: 100 μ m (**A**); 500 μ m (**B**); 50 μ m (**C**).



Figure 7. Light microscope photographs **A** anterior part of male of *Raosentis* sp. ex *Mystus mysticetus* Roberts from Thailand **B** total view on cysthacanth of *Arhythmorhynchus* sp. ex body cavity of *Mystus albolineatus* Roberts from Thailand. Scale bars: 100 µm.

Infection rates. Prevalence 12.5% (1/8), intensity 1.

Morphology (based on one male with sperm). Quadrigyridae with characters of genus *Raosentis*: i) proboscis with four rows of hooks with an unequal number of hooks in anterior and posterior rows; ii) a large area without hooks between second and third rows of proboscis hooks.

Male. Small acanthocephalan, white, fusiform, 4.11 mm long, 471 maximum width in anterior quarter of body (Fig. 6B). Anterior part of body with in 28 rings of small tegumental spines, 50-54 spines in each ring. Field of spines 1.16 mm long beyond level of posterior edges of lemniscus, do not reach level of anterior margin of anterior testis. Distance between anterior ring of spines and next rings 77. Distance between 2^{nd} and 17^{th} rings of spines 18-22, distance between rings of spines increases posteriorly to 55-61. Proboscis 186×206 , with 27 hooks in 4 rows, 6 or 7 spines in each row (Fig. 6A, C). Hooks of anterior row located irregularly, three slightly anteriorly, three slightly posteriorly. Hooks of two anterior rows large, with simple roots directed posteriorly (Fig. 4A, C). Their blades and roots $2-3 \times$ larger than blades and roots of two posterior rows.

Hooks of third and basal rows separated from hooks of the two anterior rows by 35, their roots also simple and directed posteriorly. Hooks blades of anterior row 91, second 78–92, third 20–29, basal 17–21. Hook roots of anterior row 46, second 50–52, third 21–23, basal 17–20. The neck is pronounced, 74 long, width in anterior part 87, in posterior part 135. Proboscis receptacle 524 × 129, with single-layer muscular wall, with cephalic ganglion 79 × 47 at bottom. Lemnisci 796 × 67, extend beyond proboscis receptacle, not reaching level of anterior edge of anterior testis. Organs of reproductive system 2.45 mm long, occupying 60% of body length, in its posterior part. Testes oval, tandem, anterior larger than posterior. Anterior testis 423 × 216, posterior 401 × 208. Cement gland elongated, 443 × 167, adjacent to posterior edge of posterior testis. Pearshaped cement reservoir under cement gland, 190 × 72, branching posteriorly into two vas deferens. Saefftigen's pouch absent. Bursa in invaginated state 685 long. Gonopore terminal.

Remarks. To date, all seven species of the genus Raosentis Datta, 1947 have been described from freshwater fishes in India (Naidu 2012). Raosentis sp. from Thailand differs from all other species of the genus by a slender elongated body more than 4 mm in length in the male (the body length of the male of other species of the genus does not exceed 3.3 mm) (Naidu 2012) as well as by the significant distance of the anterior rings of tegumental spines from the following rings of spines. Raosentis sp. morphologically resembles R. lucknowensis Saxena, Gupta, Johri, Ramakant, 2014, described from Mystus vittatus (Bloch) in River Gomti (Lucknow, India) by the number of rings of tegumental spines (28 in Raosentis sp., 28-30 in R. lucknowensis, in other species of the genus the number does not exceed 20) and by the length of the blades of the proboscis hooks of the two anterior rows (78–92 in our specimen, 70–90 in R. lucknowensis) (Saxena et al. 2014). However, these two species differ in the number of proboscis hooks (6, 7, 7, 7 in Raosentis sp. vs 8, 8, 8, 8 in R. lucknowensis), the size of the blades of the third and fourth rows (20–29 and 17–21 in Raosentis sp. vs 30-40 and 30-40 in R. lucknowensis, respectively). We suppose that Raosentis sp. might be a new species of the genus Raosentis, however, we waive its formal description since only a single specimen was studied. At the same time, we consider it appropriate to present a key for identifying the species of the genus.

Key to species of the genus Raosentis

1 Anterior ring of tegumental spines at a clear distance from the next rings of spines. Male body length is > 4 mm Raosentis sp. Spines located in the anterior body in a continuous field. Body length of 2 Spines in anterior body in 16–30 rings4 _ 3 Spines in anterior body in 9 rings. Organs of male reproductive system occupy 2/3 of body length, anterior testis at a distance from bottom of the proboscis sheath and posterior edge of the lemniscusR. thapari Rai, 1967 Spines in anterior body in 12 rings. Organs of male reproductive system occupy 3/4 of body length, anterior edge of anterior testis at level of posterior end of proboscis receptacle and posterior edge of the lemnisci...... R. godavarensis Vankara & Vijayalakshmi, 2009

- 5 The field of spines extends to level of anterior testis6

- Tegument has 3 giant nuclei dorsally and 3 ventrally. Length of blades of anterior hooks 80–90 µm
- Proboscis with 6 or 7 in a row. Length of hook blades 85–115 μm, 70–95 μm, 25–35 μm, 25–30 μm respectively *R. podderi* Datta, 1947

Palaeacanthocephala Polymorphidae

Arhythmorhynchus sp.

Fig. 7B

Host. Mystus albolineatus Roberts (Siluriformes, Bagridae).

Locality. Flood area of the Dan canal near Daeng Ban Non Du village, Udon Thani Province, Thailand (17°32.891'N, 103°03.831'E).

Site of infection. Body cavity.

Infection rates. Prevalence 1/1, intensity 6.

Molecular data. Nuclear 18S rRNA (1,703 bp), 28S rRNA (1,132 bp), ITS (847 bp), and mitochondrial COI (603 bp) genes sequences of *Arhythmorhynchus* sp. were deposited in the GenBank database (Acc. nos. PQ636382, PQ636389, PQ636392, PQ631045).

Note. Six cysthacanths of the genus *Arhythmorhynchus* were found in the body cavity of one bagrid catfish *Mystus albolineatus* Roberts. Five of them were in capsules with invaginated proboscis, one cysthacanth, female, had an evaginated proboscis, facilitating its morphological examination.

Morphology. Trunk 1.27 mm long with maximum width at level of middle of proboscis receptacle 391. The front part of body with one field of spines. Its extent same ventrally and dorsally. Spines blades 21 long. Cylindrical proboscis with expansion in middle part. Proboscis 513 × 157 with 16 longitudinal rows of hooks, 22–23 hooks in each row. Sizes of hooks do not differ dorsally and ventrally. Hooks in anterior eight or nine rows large, with simple massive roots directed posteriorly. Hooks in next 13 rows spine-shaped, with short roots processes directed posteriorly. Length of hook blades: 33–40 (hook 2), 35-41 (3), 37-40 (4–7), 38-41 (8), 32-34 (9), 20-22 (10), 17-21 (11), 15-19 (12), 16-20 (13), 16-19 (14), 16-18 (15), 16-17 (16), 16 (17–23). Length of hook roots: 23-29 (2), 31 (3), 32-33 (4), 32 (5), 34-39 (6), 39-41 (7), 40-43 (8), 27-39 (9). Length of root processes of next hooks 8-12. Hooks

in last one or two rows without roots. Neck retracted. Proboscis receptacle with double-layer muscular walls, 630 × 120 with oval cephalic ganglion in middle part. Lemnisci thin, ribbon-shaped, convoluted, 712 × 38, longer than proboscis receptacle. Vagina with two sphincters. Posterior body end slightly retracted. Genital pore terminal.

Remarks. The definitive hosts of these acanthocephalans are gulls and waders (Charadriiformes), and fish are paratenic hosts. To date, 24 valid species have been described in the genus Arhythmorhynchus (Amin 2013). Only three species have more than 20 hooks in a longitudinal row on the proboscis: A. xeni Atrashkevich, 1978 described from Terek sandpiper Xenus cinereus (Güldenstädt, 1775) in Siberia, A. longicollis (Villot, 1875) Lühe, 1912 described from gulls in Europe, and A. limosae Edmonds, 1971 described from godwit Limosa lapponica in Townsville, Queensland, Australia (Golvan 1956; Edmonds 1971; Atrashkevich 1978). The cystacanths of Arhythmorhynchus sp. from Thailand differs from all three species in the number of longitudinal rows of hooks (16 in Arhythmorhynchus sp. vs 19-20 in A. xeni and A. limosae, 22-24 in A. longicollis), as well as in the ratio of large spine-shaped hooks (8-9 and 12-13 in Arhythmorhynchus sp., 14-15 and 11-12 in A. xeni and A. limosae, respectively). The number of large hooks is similar in *Arhythmorhynchus* sp. and A. longicollis, 8-9 and 9-10 respectively; however, the length of the blades of the largest hooks in Arhythmorhynchus sp. is smaller than in A. longicollis (40-41 vs 48-50). Thus, morphological differences prevent us from classifying Arhythmorhynchus sp. as any of the known species of the genus.

Molecular phylogeny

In total, 24 new sequences of four genetic markers for three species of Acanthocephala from freshwater fishes in Thailand were generated and deposited in GenBank (Acc. nos. PQ636375–PQ636382, PQ636383–PQ636389, PQ636390–PQ636392, PQ631040–PQ631045). Intraspecific genetic variability was observed only for the COI marker and was limited to a single substitution for both *A*. (*A*.) *barbonymi* sp. nov. and *P. rexus*. The BLASTn comparison of the nuclear markers showed the highest similarity of *A*. (*A*.) *barbonymi* sp. nov. with isolates of *A*. (*A*.) cf. *tilapiae* and *A*. (*A*.) *bilaspurensis* (96.5–97.5% for 18S rRNA gene and 90.0–90.3% for 28S rRNA gene), while COI sequences of the new species were 99.0% identical with *A*. (*A*.) *kenyirensis* (MN833316). The best matches of *P. rexus* sequences were 98.6% similarity in 18S rRNA gene with *P. nandai* (MW164853, MW164854), 94.8% similarity in 28S rRNA gene with *Pallisentis* sp. (MW421633), and 72.8% similarity with *P. celatus* (Van Cleave, 1928) (NC_022921).

Phylogenetic analysis of the family Quadrigyridae clearly defined two highly supported clades. The first clade grouped isolates of *Acanthogyrus*, including *A.* (*A.*) *barbonymi* sp. nov.; however, weak support of internal nodes did not allow to define interrelationships within this genus. The second clade grouped the isolates of *Pallisentis*, and *P. rexus* formed well-supported sister lineage to the clade of *P. nandai* (MW164853), *P. paranandai* (MW723432), *P. roparensis* (MW421631), *P. nagpurensis* (MN400426), *P. himachalensis* (OM480738), and *P. longus* (OM480740) (Fig. 8).



Figure 8. Phylogenetic relationships within the family Quadrigyridae inferred from the dataset of the 18 rRNA gene marker. The values near nodes were calculated by ultrafast bootstrapping in IQtree, only supports > 90 are shown. Scale bar indicates number of substitutions per site.

Discussion

The list of fish acanthocephalans in Thailand has been expanded to ten species with the addition of the two taxa characterized in this work, the new species A. (A.) barbonymi sp. nov., and a putative new species of Raosentis, the genus currently containing seven described species (Naidu 2012). Pallisentis rexus was originally described from C. striata in Chiang Mai basin in Thailand and later recorded in Taiwan. Arhythmorhynchus sp. previously had been reported from the goonch Bagarius bagarius (Hamilton) from the Mekong River in Chiang Rai province, Thailand (Purivirojkul and Areechon 2008), but the authors did not provide a description nor drawings, so we cannot compare our species with the acanthocephalans described in that study. In total, three species of Pallisentis, two species of the subgenus Acanthosentis, and five other genera are currently known from fishes in Thailand. This reflects a paucity of parasitological surveys of fish in this geographical area. A greater number of taxa has only been recorded in Vietnam, where 15 species have been found in freshwater teleosts (Arthur and Te 2006; Amin et al. 2014): Acanthocephalus parallelcementglandatus Amin, Heckmann & Ha, 2014, Acanthogyrus (Acanthosentis) indicus (Tripathi, 1959), Cathavacanthus bagarii Moravec & Sey, 1989, Cleaveius longirostris Moravec & Sey, 1989, Dendronucleata spp., Micracanthorhynchina hemiculterus (Demshin, 1965), Neoechinorhynchus (Hebesoma) spiramuscularis Amin, Heckmann & Ha, 2014, Neotegorhynchus (as Brentisentis) cyprini (Yin & Wu, 1984), Pallisentis spp., Paradentitruncus longireceptaculis Moravec

& Sey, 1989, *Pseudoacanthocephalus coniformis* Amin, Heckmann, Ha, 2014, and *Pseudorhadinorhynchus vietnamensis* Moravec & Sey, 1989. Two species of fish, *M. albolineatus* and *B. schwanenfeldii* have been added to the list of fish that are hosts of acanthocephalans. Considering that only 20 species of fish have been confirmed as hosts, species diversity of acanthocephalans in freshwater fishes in Thailand remains largely unexplored.

The new species *A*. (*A*.) *barbonymi* sp. nov. is described herein from *B. schwanenfeldii*, the same host from which three other members of the genus were described recently in Malaysia (Mohd-Agos et al. 2021). These authors provided morphological characterization of the three; however, available genetic data are inconsistent and hardly comparable with ours (see Remarks). As our requests to borrow type specimens have not been met to date, the specimens collected in Thailand were described as a new species based on significant morphological differences from the three species from Malaysia.

Amin et al. (2000) published a taxonomic revision of the genus *Pallisentis*, in which three subgenera *Pallisentis*, *Brevitritospinus*, and *Demidueterospinus* Amin, Heckmann, Ha, Luc & Doanh, 2000 were erected based mostly on the relative proportions of their proboscis hooks. However, their validity has not been supported by recent molecular phylogenetic analyses (Chaudhary et al. 2019; Gautam et al. 2020; Amin et al. 2021; Rana and Kaur 2021). Similarly, our phylogenetic analysis indicates that the subgenera *Brevitritospinus* and *Pallisentis* are not natural, monophyletic groups; two available isolates of the subgenus *Brevitritospinus* clustered with three isolates of *Pallisentis*.

Golvan (1959) listed Acanthogyrus Thapar, 1927 and Acanthosentis Verma & Datta, 1929 as subgenera of Acanthogyrus. Amin (1985) accepted their subgeneric statuses, while Golvan (1994) considered Acanthogyrus and Acanthosentis to be separate genera. Herein, we follow the most recent classification of Amin (2013), where these two taxa are listed as subgenera, which may be distinguished based on the number of proboscis hooks: six hooks in each of three rows in Acanthosentis and eight hooks in each of three rows in Acanthogyrus. In our phylogenetic analysis, the type species of the subgenus Acanthogyrus, A. (A.) acanthogyrus and eight species of the subgenus Acanthosentis, including A. (A.) barbonymi sp. nov., were clustered in a well-supported clade. However, low supports of internal nodes did not allow us to estimate phylogenetic interrelationships within the clade and prevent us to confirm or refute the validity of these subgenera.

It is obvious that further research is necessary to resolve phylogenetic relationships within the order Gyracanthocephala. The currently available genetic data are not sufficient to provide comprehensive phylogenetic hypothesis due to the low coverage of taxa and possible incongruence of genetic markers. Another obstacle is the general absence of vouchers in museum collections available for the evaluation of morphological structures. The generation of the sequences of multiple genetic markers that are linked to morphological vouchers deposited in international collections reachable by all taxonomists needs to be implemented (see Perrot-Minnot et al. 2023).

Finally, this work provides detailed morphological characterization and genetic data for several taxa, enriching our knowledge about the acanthocephalan fauna of fishes in Thailand. Moreover, both hosts, *C. striata* and *B. schwanenfeldii*, are commercially important fishes used as food and ornamental fish, respectively. The heavy infestation of these fishes with acanthocephalans observed in this study suggests that these parasites may have a negative impact on fish farming in Thailand.

Acknowledgements

We would like to thank Tiwarat Thalerngkietleela from Inland Fisheries Research and Development Division, Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand, for facilitating our field work. We are also grateful to the reviewers for their helpful suggestions.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was funded by the EU Next Generation EU through the Recovery and Resilience Plan for Slovakia under the project No. 09103-03-V01- 00016 and the Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences (project No. VEGA 2/0130/24).

Author contributions

AR, OL, and MO conceived the study; AR and SP organized and funded field collections; OL performed the morphological studies; DB performed parasites isolation from fishes, scanning electron microscopy, and phylogenetic analyses; OL and DB wrote the first draft; all authors read, edited, and approved the manuscript.

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Data availability

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

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Supplementary material 1

Voucher specimens and their deposition

Authors: Daniel Barčák, Olga Lisitsyna

Data type: xlsx

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Link: https://doi.org/10.3897/zookeys.1233.136533.suppl1



Research Article

A new species of *Docosia* Winnertz, 1864 and new records of fungus gnats (Diptera, Bolitophilidae, Keroplatidae and Mycetophilidae) from North Africa

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Abstract

A new species of Mycetophilidae in the genus *Docosia* Winnertz, 1864 is described, *Docosia tazekkae* Chandler, El Mouden & Belqat, **sp. nov.**, with the addition of eighteen new records of fungus gnats to the fauna of North Africa. Most of the new taxa of fungus gnats are found in Morocco, especially in the National Park of Tazekka, where a total of 69 species are recorded for the first time, thus enriching the fauna of these gnats in the Middle Atlas region; the greatest species richness is observed in caves. All these findings attest to the high biodiversity of the fungus gnats in Morocco, which, among the North African countries, has the best-known fauna.

Key words: Biodiversity, caves, description, distribution, Middle Atlas, Morocco, National Park of Tazekka, taxonomy

Introduction

Fungus gnats are a highly diverse group of Diptera. Traditionally they include six families in the superfamily Sciaroidea: Bolitophilidae, Diadocidiidae, Ditomyiidae, and Keroplatidae, which were classified earlier as subfamilies of Mycetophilidae (Edwards 1925), before being later recognised as independent families (Väisänen 1984b; Matile 1990; Hippa and Vilkamaa 2005). The fungus gnats group encompasses also the Mycetophilidae and Rangomaramidae families, however, the latter's composition remains unclear. The fungus gnats include approximately 1500 species in the Palaearctic region alone (Søli et al. 2000) and more than 5000 species distributed worldwide (Pape et al. 2011). As for their morphology, adults are generally recognised by their hump-backed appearance, stout and elongated coxae and well-developed tibial spurs. According to Chandler (1999, 2022), all fungus gnat families are known to inhabit older, well-established forests and wooded biotopes rich in mature and decaying trees. Most species develop in fungi or decaying wood. Their larvae



Academic editor: Vladimir Blagoderov Received: 25 June 2024 Accepted: 6 December 2024 Published: 7 April 2025

ZooBank: https://zoobank.org/ D81D6D85-754F-4533-8FF5-C68977898D55

Citation: El Mouden MA, Chandler PJ, Saidoun I, Driauach O, Belqat B (2025) A new species of *Docosia* Winnertz, 1864 and new records of fungus gnats (Diptera, Bolitophilidae, Keroplatidae and Mycetophilidae) from North Africa. ZooKeys 1233: 349–379. https://doi.org/10.3897/ zookeys.1233.130502

Copyright: © Mohamed Amin El Mouden et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). feed on decaying plant material, fungal mycelia in rotten wood, mushrooms, and in some special cases, on some bryophytes or are predaceous on other insects (Vockeroth 1981; Søli et al. 2000; Jakovlev 2011). The more humid the forests, the more they are favoured, and adults are usually most abundant in sheltered habitats (such as caves or shady biotopes) and near streams (Chandler 1999, 2022). Despite being primarily forest dwellers, fungus gnats can be recorded in various ecosystems, usually associated with fungal habitats (Kerr 2008); fewer species are therefore found in warm, dry and more open biotopes (Chandler 1999). Adults of fungus gnats often occur in large numbers and play important roles, especially in the forest environment food web (Kerr 2008).

The fungus gnat fauna of North Africa was hitherto represented by 114 species from 41 genera and three families: Bolitophilidae (one species from one genus), Keroplatidae (19 species from 10 genera) and Mycetophilidae with 94 species from 30 genera (Becker 1907; Enderlein 1913; Lundström 1916; Madwar 1935; Burghele-Balacesco 1967, 1972; Laštovka and Matile 1974; Matile 1977; Gagné 1981; Väisänen 1984a, 1984b; Bechev 1989; Caspers 1991; Chandler 1994; Chandler and Ribeiro 1995; Chandler and Gatt 2000; Chandler and Blasco-Zumeta 2001; Chandler et al. 2006; Banamar et al. 2020, Driauach et al. 2022; El Mouden et al. 2024). However, the distribution of these species over the five countries of North Africa is unbalanced and unequal; the fungus gnat fauna of some countries of the region, for example, Libya, is unknown, and that of Egypt is poorly defined with only two Keroplatidae species from one genus. As for Algeria and Tunisia, the knowledge of this dipteran group is modest as there have been no specific studies of fungus gnats in those countries, with all records having been obtained casually by general recording of Diptera. In Algeria, there are records of 29 species of fungus gnats from 17 genera comprising one species of Bolitophilidae from one genus, five species of Keroplatidae from three genera and 23 species of Mycetophilidae belonging to 13 genera. Meanwhile, the Tunisian fauna gathers 34 fungus gnat species from 23 genera belonging to two families: Keroplatidae, with nine species from seven genera and Mycetophilidae, with 25 species distributed in 16 genera (see Table 2).

Among the North African countries, Morocco has the best-known fungus gnat fauna. While only ten species of Mycetophilidae and two species of Keroplatidae were recorded from Morocco before 2020, the recent studies led by Banamar et al. (2020), Driauach et al. (2022), and El Mouden et al. (2024) improved the knowledge of this group by recording the following species as new to Morocco: 66 species of Mycetophilidae, ten species of Keroplatidae, and one species of the family Bolitophilidae. Overall, the Moroccan fauna was thus increased to 76 species of Mycetophilidae distributed in 28 genera, 12 species of Keroplatidae belonging to seven genera, and one species of Bolitophilidae. The largest share of these records belongs to the Rif area, while only a few records were from other regions of the Moroccan territory. Consequently, this paper presents the first concentrated study of this dipteran group from the Middle Atlas region, one of the most unstudied areas of Morocco. Thus, a total of 69 fungus gnat species (1 species of Bolitophilidae, 3 species of Keroplatidae, and 65 species of Mycetophilidae), of the 71 species mentioned in this study, are recorded for the first time in the Middle Atlas Mountains. Among the fauna identified, the Mycetophilidae genus Allodia Winnertz, 1864, is new to Morocco, while the genus Synplasta Skuse, 1890 is new to both Morocco and North Africa. Furthermore, out of the 71 studied fungus gnat species, 19 species (1 species of Keroplatidae and 18 species of Mycetophilidae) are recorded for the first time in Morocco, of which 18 are new to North Africa. Therefore, as expected in our previous study (El Mouden et al. 2024), Morocco appears to be home to more endemic fungus gnat species as this paper presents the description of the new species *Docosia tazekkae* sp. nov., which raises the number of endemic species of Mycetophilidae of Morocco to six species in total. *Docosia tazekkae* sp. nov. resembles many other species of Mediterranean *Docosia* Winnertz, 1864, although it is distinguished by some morphological characters detailed in this paper.

Material and methods

Area of study

The current study is situated in Morocco, one of the "five" countries of North Africa, a geographic entity that has no single accepted definition. It has been regarded by some as stretching from the Atlantic shores of Morocco in the west to the Suez Canal and the Red Sea in the east. Others have limited it to the countries of Morocco, Algeria, and Tunisia, a region known by the French during colonial times as Afrique du Nord and by the Arabs as the Maghrib ("West"). Our study took part in four Moroccan protected areas but mainly in the National Park of Tazekka (PNTZK), one of the ten protected Parks of Morocco, located in the northernmost eastern part of the Middle Atlas, near the city of Taza. It was created by Visiriel Decree of July 11, 1950, to protect all the natural resources of Jbel Tazekka summit (culminating at 1,980 metres), and more particularly, the cedar forest (Cedrus atlantica), which stands solely on this peak. Since its creation, the Tazekka National Park has expanded from originally 680 hectares (1950) to 13,737 ha (2004) to ensure the protection of more faunal and floral biodiversity by protecting more nearby natural areas and hydrological resources (Elgazzane 2019). The second national park that is part of the Middle Atlas region is the National Park of Ifrane (PNI). It is located at the central part of the Middle Atlas Mountains, almost entirely within the Sebou watershed. It was created in 2004 and initially included 53,800 ha. In 2008, the PNI was extended to 125,000 ha including 65,290 ha of forests which is equal to almost all the forests of the province of Ifrane. It contains, thus, 10% of the world surface of the Atlas cedar which classifies it in the heart of the Atlas Cedar Biosphere Reserve (Ismaili Alaoui et al. 2022). In the Rif region, our study included The National Park of Talassemtane (PNTLS), which is characterised by a significant biome for faunal and floral diversity in Morocco and North Africa. It is overlooking the Mediterranean Sea and takes part of the Intercontinental Biosphere Reserve of the Mediterranean. The National Park of Talassemtane is biogeographically located in the Rif region western part of northern Morocco, in a landlocked area of the Western Rif chain of the Tangier-Tetouan-Al Hoceima region. It covers an area of 58,022 ha distributed between two provinces of Chefchaouen (78%) and Tétouan (22%) and extends over nine rural communes including six belonging to the province of Chefchaouen (Tassif, Talembote, Bab Taza, Beni Selmane and Bni Derkoul, Tassift) and three to that of Tétouan (El Hamra, Oulad Ali Manson and Al Ouad) (Aoulad-Sidi-Mhend et al. 2020). Not far away from the National Park of Talassemtane, the territory of the Natural Park of Bouhachem (PPNB), which takes its name from the Bouhachem mausoleum existing in the area, is located in the northwest of Morocco in the biogeographic region of the Western Rif, precisely on the southern side of the Mediterranean basin. The PPNB is entirely included in the Tangier-Tétouan region, where it extends over six rural communes belonging to the three provinces of Tétouan, Larache and Chefchaouen: Béni Leit and Al Ouad (Province of Tétouan), Derdara, Laghdir and Tanakoub (Province of Chefchaouen) and Tazroute (Province of Larache). It covers an area of 75,497 ha of which 50,113 ha belongs to the province of Chefchaouen, and 40–50% of the total area belongs to the forest domain (Bachar et al. 2021). It constitutes a rural region characterised by a distinct identity and a wealth of natural and cultural heritage. However, this balance remains fragile and increasingly threatened. Consequently, it has been incorporated into heritage preservation initiatives by public authorities.

Methodology

Collecting was performed in 33 sampling sites (Table 1, Fig. 1) of which 28 were distributed over mountainous areas of the National Park of Tazekka, such as forests, rivers, lakes, waterfalls, entrances of caves, and five additional sites



Figure 1. Map of study areas within Morocco **A** the NP of Talassemtane (in red) and the Natural Park of Bouhachem (in blue) **B** the NP of Tazekka **C** the NP of Ifrane: localities within corresponding national parks are indicated by red stars and nearby cities with triangles .

 Table 1. Sampling sites (in alphabetical order) hosting the species collected in the four

 Moroccan protected areas, with altitudes and geographical coordinates.

Station		Geographical coordinates Latitude, Longitude
	Elevation (m)	
Rif Mountains	·	
National Park of Talassemtane		
1. Forêt Malâab Tizimezzan	1452	35.1156, -5.1388
2. Oued Bni Mhamed	1314	35.1603, -5.1269
3. Oued Farda	420	35.2392, -5.1743
Natural Park Project of Bouhachem	้	
4. Forêt après Amsemlil	1127	35.2579, -5.4347
Middle Atlas Mountains	'	
National Park of Tazekka		
5. Aïn Admam	1453	34.0530, -4.1512
6. Barrage Bab Lota	615	34.0003, -4.3111
7. Cascade Ras Lma	985	34.1480, -4.0090
8. Forêt Admam	1500	34.0479, -4.1497
9. Forêt Bab Boudir	1450	34.0716, -4.1216
10. Forêt Bouhayati	1430	34.0928, -4.0987
11. Forêt gouffre Friouato	1503	34.1045, -4.0714
12. Gouffre Izora	1430	34.0944, -4.0985
13. Grotte Bouhayati	1424	34.0871, -4.1071
14. Grotte Bouslama	1449	34.0879, -4.1118
15. Lacune Bab Boudir	1474	34.0786, -4.1288
16. Maison forestière	806	34.0663, -4.2709
17. Oued Aïn Chabab	1389	34.0735, -4.1333
18. Oued Azhar	801	34.0456, -4.2686
19. Oued Boussbaâ	597	34.0053, -4.2924
20. Oued El Ghannaj	958	34.0571, -4.2118
21. Oued Lagziri	650	34.1026, -4.2870
22. Oued Lek'hal	1053	34.0806, -4.1567
23. Oued Sidi Boulaâla	1097	34.0831, -4.1580
24. Oued Tametrhouste	1413	34.0598, -4.0632
25. Oued Taourirt	1343	34.0729, -4.1308
26. Pont Oued Lek'hal	1053	34.0825, -4.1572
27. Pont Ras Lma	1972	34.1476, -4.0093
28. Ruisseau Aïn Chabab	1261	34.0762, -4.1475
29. Ruisselet Ras Lma	1022	34.1457, -4.0110
30. Vallée des cerfs	1353	34.0469, -4.1863
31. Vallée des oiseaux	1318	34.1302, -4.0310
32. Village Bab Boudir	1579	34.0685, -4.1243
National Park of Ifrane		
33. Forêt Bab Lkhayl	1655.6	33.5320, -5.1081

distributed between the three other protected Parks (PNTLS, PNI, and PPNB). The sampling results contained a total of 4,216 specimens of Mycetophilidae (3,817 males and 399 females), eight specimens of Keroplatidae, all of them males, and one male of Bolitophilidae; all the specimens were collected us-

ing the sweeping technique. The material was collected by members of the Group Belqat, PhD students: I. Saidoun, K. Aattouch, M. Beni-Eich, M. El Ouahabi, and the first author during the period 10 February 2020–2 May 2023 and was preserved in 96% ethanol. The material was sorted by the first author and was identified to generic level with the help of Dr. P. Beuk. Species identifications were done by Dr. P.J. Chandler. The examined material will be deposited in the collection of Diptera of the Department of Biology, Faculty of Sciences, University Abdelmalek Essaadi, Tétouan (**UAE-FST**). The holotype of the newly described species will be deposited at the Natural History Museum, London, UK (**NHMUK**). An annotated list of fungus gnat species reported from Morocco in the present study is provided, in alphabetical order, with the mention of the North African distribution of each species.

Taxonomy

Family Bolitophilidae Genus *Bolitophila* Meigen, 1818

Bolitophila saundersii (Curtis, 1836)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2402.

North African distribution. Algeria (Burghele-Balacesco 1967); Morocco (Driauach et al. 2022).

Family Keroplatidae Subfamily Macrocerinae Rondani, 1856 Genus *Macrocera* Meigen, 1804

Macrocera fasciata Meigen, 1804

Material examined. MOROCCO – Middle Atlas Region • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2403.

North African distribution. Algeria (Becker 1907); Morocco (Driauach et al. 2022).

Subfamily Keroplatinae Rondani, 1856 Tribe Orfeliini Malloch, 1917 Genus *Neoplatyura* Malloch, 1928

Neoplatyura biumbrata (Edwards, 1913)

Material examined. MOROCCO – Middle Atlas Region • 3♂♂; Grotte Bouslama; 19/05/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2404 • 1♂; Pont Ras Lma; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2405.

North African distribution. Morocco (Driauach et al. 2022).

Genus Orfelia Costa, 1857

Orfelia fasciata (Meigen, 1804)

Material examined. MOROCCO - Middle Atlas Region • 13; Cascade Ras Lma; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2406 • 13; Oued Lek'hal; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2407 • 1³; Oued El Ghannaj; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2408.

North African distribution. New to Morocco and North Africa.

Family Mycetophilidae Subfamily Gnoristinae Edwards, 1925 Genus Boletina Staeger, 1840

Boletina lundstroemi Landrock, 1912

Material examined. MOROCCO – Middle Atlas Region • 1∂; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2409.

North African distribution. New to Morocco and North Africa.

Genus Coelosia Winnertz, 1864

Coelosia fusca Bezzi, 1892

Material examined. MOROCCO – Middle Atlas Region • 1♂, 2♀♀; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2410.

North African distribution. Morocco (Banamar et al. 2020).

Genus Docosia Winnertz, 1864

Docosia gilvipes (Walker, 1856)

Material examined. MOROCCO - Middle Atlas Region • 6순간; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2411 • 12; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2412 • 13; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2413 • 6♂♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2414.

North African distribution. Morocco (Banamar et al. 2020).

Docosia melita Chandler & Gatt, 2000

Material examined. MOROCCO - Middle Atlas Region • 3 순간; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2415 • 1 중; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2416 • 2 중 중; Oued Sidi Boulaâla; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2417 • 5 중 중; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2418 • 1 중; Ruisseau Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2419 • 1 중; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2419 • 1 중; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2420 • 1 중; Oued Lgziri; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2420 • 1 ठ; Oued Lgziri; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2420 • 1 ठ; Oued Lgziri; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2420 • 1 ठ; Oued Lgziri; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2420 • 1 ठ; Oued Lgziri; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2420 • 1 ठ; Oued Lgziri; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2420 • 1 ठ; Oued Lgziri; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2421.

North African distribution. Morocco (El Mouden et al. 2024).

Docosia tazekkae Chandler, El Mouden & Belqat, sp. nov. https://zoobank.org/A58CAAC4-8605-4E8B-BA05-860D8DF141E5

Type material. *Holotype*. MOROCCO – Middle Atlas Region • ♂ (mounted in DMHF, terminalia on a slide); Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; collected using sweep net; NHMUK. *Paratypes*. MOROCCO – Middle Atlas Region • 1♂; Forêt Bab Lkhayl; 2/V/2023; M. Beni-Eich, K. Aattouch and M.A. El Mouden leg; collected using sweep net; UAE-FST MA23/2401. – Rif Region • 1♂; Forêt après Amsemlil; 10/II/2020; Group Belqat leg; collected using sweep net; UAE-FST R22/2450 • 1♂; Forêt Malâab Tizimezzan; 12/V/2022; M. Beni-Eich, K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; collected using sweep net; UAE-FST R22/2451 • 2♂♂ (mounted in DMHF, terminalia of one Oued Bni Mhamed specimen on a slide); Oued Bni Mhamed; 11/V/2022; M. Beni-Eich, K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; collected using sweep net; UAE-FST R22/2449 • 1♂; Oued Farda; 11/III/2023; I. Saidoun leg; collected using sweep net; UAE-FST R22/2452.

Diagnosis. This species resembles several other Mediterranean species of this genus, including *D. melita* Chandler & Gatt, 2000 previously recorded from Morocco (El Mouden et al. 2024) in its dark body colouration, mainly yellow legs and bare laterotergites. It belongs to a group of species with a simple gonostylus bearing a row of spines on its inner margin. Within this group it is most similar to *D. kerkini* Kurina & Ševčík, 2011, described from Greece (Kurina and Ševčík 2011) which also has a medioventral humped process of the apical margin of the gonocoxites bearing a cluster of short spinose setae on each side preapically. In *D. kerkini* the row of the spines on the gonocoxites is shorter and narrower with a deeper medial excavation and more tight patches of stout setae; tergite 9 is also differently shaped, broadened apically with a concave apical margin.

Description. Male. Wing length 3.0-3.2 mm. *Colouration.* Head including antennae and body including terminalia all black. Legs mostly yellow. Coxae dark on basal part (extreme base of fore and mid coxa, ~ 1/3-1/2 of hind coxa); trochanters dark, hind femur narrowly dark at base and tip. Wing clear, with costa and radial veins dark, other veins pale. Haltere yellow. *Head.* Antenna longer than head and thorax together, with slender flagellomeres > 2 × as long as broad. Ocelli situated close to eye margin. *Thorax.* Laterotergite bare. *Legs.* Mid tibia with four short anterior and three or four longer dorsal setae, almost as long as tibial width. Hind tibia with 10-12 short anterior and six longer dorsal

setae, about as long as tibial width, with a few short setae in line beyond them. **Wing.** Sc ends in R ~ 1/2 or a little more to base of Rs. R₁ 2–2.5 × length of r-m. **Terminalia** (Fig. 2). Tergite 9 short and broad, rounded apically with long setae on apical margin. Cercus with tiers of close-set combs of retinacula (12 combs in holotype, 13 in paratype figured). Gonostylus simple, narrowed apically, with long fine scattered setae on outer surface and a row of eight strong spinose setae on inner margin, the most distal longest, the proximal two or three shorter than the rest. Gonocoxites with a medioventral humped process of the apical



Figure 2. *D. tazekkae* Chandler, El Mouden & Belqat, sp. nov. Male terminalia posterior view (tergite dorsally, apical margin of gonocoxites ventrally) of **A** holotype and same of **B** paratype from Oued Bni Mhamed. Holotype terminalia in **C** dorsal and **D** lateral views.

margin, bearing an irregular cluster of short spinose setae on each side preapically, the bare part beyond these spines with a shallow apical emargination; the apical margin on each side bearing a row of short strong setae and a narrow internal flange bearing long fine setae.

Female. Unknown.

Etymology. After the name of the Tazekka National Park, which shelters the new species in the Bab Boudir Forest locality (Fig. 3).

Habitat. The holotype of this species was collected in the remains of a forest that neighbours the holiday centre of Bab Boudir, in the heart of the Tazekka National Park (Fig. 3). The sampling took place at ca 0800 hrs on a sunny morning (25 °C), with low humidity (17%) and moderate wind speed (14 Km/h) in a dense part of the forest where the tree branches seem to meet as they are very close to each other, over a very small river (Bab Boudir rivulet), with calming low speed waters. The dense vegetative cover creates a shadowy, sheltered and fairly cold spot where sun rays can barely penetrate.

Genus Megophthalmidia Dziedzicki, 1889

Megophthalmidia amsemlili Chandler, Belqat & Banamar, 2024

Literature records. Morocco – **Middle Atlas Region •** 1♂; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2401 (El Mouden et al. 2024).

North African distribution. Morocco (El Mouden et al. 2024).



Figure 3. Moroccan habitat of *Docosia tazekkae* Chandler, El Mouden & Belqat, sp. nov. Environments of the Forêt Bab Boudir within the PNTZK **A** Bab Boudir forest **B** Bab Boudir rivulet.

Genus Synapha Meigen, 1818

Synapha fasciata Meigen, 1818

Material examined. MOROCCO – Middle Atlas Region • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2423.

North African distribution. Morocco (Banamar et al. 2020).

Genus Tetragoneura Winnertz, 1846

Tetragoneura sp.

Material examined. MOROCCO – Middle Atlas Region • 1♀; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2424.

Subfamily Leiinae Edwards, 1925 Genus *Leia* Meigen, 1818

Leia arcana Chandler, Belqat & Driauach, 2024

Material examined. MOROCCO - Middle Atlas Region • 13; Forêt gouffre Friouato; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2426 • 7승군; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2427 • 31 ථ ථ; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2428 • 1 ; Ruisselet Ras Lma; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2429 • 4♂♂; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2430 • 1 ; Village Bab Boudir; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2431 • 3 신강; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2425 · 3 소강; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2432 • 10∂∂; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2433 • 9건강; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2434 • 13; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2435 • 3 강강; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2436.

North African distribution. Morocco (El Mouden et al. 2024).

Leia beckeri Landrock, 1940

Material examined. MOROCCO – Middle Atlas Region • 1♀; Oued Boussbaâ; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2437.

North African distribution. Algeria (Becker 1907 as *L. bifasciata*; Hackman et al. 1988; Chandler 2009); Morocco (Banamar et al. 2020).

Leia bimaculata (Meigen, 1804)

Material examined. MOROCCO - Middle Atlas Region • 13; Forêt Bouhayati; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2438 · 2건강; Forêt gouffre Friouato; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2439 • 1∂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2440 • 1 ; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2441 • 13; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2442 • 3건강; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2443 • 13; Pont Oued Lek'hal; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2444 • 1∂; Ruisseau Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2445 • 1∂; Oued Tametrhouste; 21/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2446 • 13; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2447.

North African distribution. Algeria (Burghele-Balacesco 1972); Morocco (Chandler et al. 2006; Banamar et al. 2020).

Genus Novakia Strobl, 1893

Novakia scatopsiformis Strobl, 1893

Material examined. MOROCCO – **Middle Atlas Region** • 6 \Im ; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2448 • 3 \Im ; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2449 • 2 \Im ; Vallé des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2450 • 13 \Im ; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2450 • 13 \Im ; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2451 • 2 \Im ; Barrage Bab Lota; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2451 • 2 \Im ; Barrage Bab Lota; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2451 • 2 \Im

North African distribution. Tunisia (Enderlein 1913; Hackman et al. 1988; Chandler 1994); Morocco (Banamar et al. 2020).

Novakia simillima Strobl, 1910

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2453.

North African distribution. Morocco (Banamar et al. 2020).
Subfamily Mycetophilinae Newman, 1834 Tribe Exechiini Edwards, 1925 Genus *Allodia* Winnertz, 1864

Allodia ornaticollis (Meigen, 1818)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2454.

North African distribution. New to Morocco and North Africa.

Genus Brevicornu Marshall, 1896

Brevicornu fissicauda (Lundström, 1911)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2455.

North African distribution. New to Morocco and North Africa.

Brevicornu griseicolle Staeger, 1840

Material examined. MOROCCO - Middle Atlas Region • 740∂∂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2457 • 161 강강; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2458 • 13; Pont Ras Lma; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2459 • 6♂♂; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2460 • 1∂; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2456 • 26 3; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2461 • 2♂♂; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2462 • 2 소강; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2463 • 13; Pont Oued Lek'hal; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2464 • 2 승강; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2465 • 5건강; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2466.

North African distribution. Morocco (Banamar et al. 2020).

Brevicornu intermedium (Santos Abréu, 1920)

Material examined. MOROCCO – Middle Atlas Region • 214♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2467 • 134♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2468 • 1♂; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2469 • 29♂♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2470 • 1♂; Oued Sidi Boulaâla; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2471 • 11♂♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2472 • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2473.

North African distribution. Morocco (Banamar et al. 2020).

Brevicornu sericoma (Meigen, 1830)

Material examined. MOROCCO – **Middle Atlas Region** • 3373; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2474 • 1363; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2475 • 43; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2476 • 13; Lacune Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2476 • 13; Lacune Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2477 • 433; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2478 • 13; Pont Oued Lek'hal; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2479 • 13; Ruisseau Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2480 • 1933; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2481 • 533; Oued Azhar; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2481 • 533; Oued Azhar; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2483 • 13; Oued Azhar; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2483 • 13; Oued Azhar; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2483 • 13; Oued El Ghannaj; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2483.

North African distribution. Tunisia (Chandler et al. 2006); Morocco (Banamar et al. 2020).

Brevicornu verralli (Edwards, 1925)

Material examined. MOROCCO – Middle Atlas Region • 17 යි ; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2485 • 13 යි ; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2486 • 3 යි ; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2484 • 1 ; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2487.

North African distribution. Tunisia (Chandler 1994) and Morocco (Banamar et al. 2020).

Genus Cordyla Meigen, 1803

Cordyla crassicornis Meigen, 1818

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2489 • 2♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2490 • 1♂; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2491 • 1♂; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2488 • 1♂; Oued Tametrhouste; 21/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2492 • 2♂♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2488 • 1♂; Oued Tametrhouste; 21/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2492 • 2♂♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2493 • 1♂; Oued Azhar; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2494 • 1♂; Maison forestière; 23/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2495.

North African distribution. Morocco (Banamar et al. 2020).

Cordyla murina Winnertz, 1864

Material examined. MOROCCO - Middle Atlas Region • 1♂; Forêt gouffre Friouato; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2496 • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2497 • 2♂♂; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2498 • 1♂; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2499 • 1♂; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2499 • 1♂; Vallée des cerfs; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/2499 • 1♂; Vallée des cerfs; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24100.

North African distribution. Morocco (Banamar et al. 2020).

Genus Exechia Winnertz, 1864

Exechia dorsalis (Staeger, 1840)

Material examined. MOROCCO – Middle Atlas Region • 2♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24101 • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24102 • 1♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24103 • 3♂♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24104.

North African distribution. Morocco (Banamar et al. 2020).

Exechia fulva Santos Abreu, 1920

= Rymosia exornata Séguy, 1941.

Material examined. MOROCCO – Middle Atlas Region • 191♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24106 • 555♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24107 • 1♂; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24105 • 19♂♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24108 • 1♂; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24109 • 3♂♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24110.

North African distribution. Tunisia (Chandler and Ribeiro 1995); Morocco (Séguy 1941; Chandler and Ribeiro 1995; Banamar et al. 2020).

Exechia fusca (Meigen, 1804)

Material examined. MOROCCO – Middle Atlas Region • 257♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24111 • 119♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24112 • 3♂♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24113 • 2♂♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24114.

North African distribution. Algeria (Chandler and Ribeiro 1995); Tunisia (Chandler 1994; Chandler and Ribeiro 1995); Morocco (Banamar et al. 2020).

Exechia spinuligera Lundström, 1912

Material examined. MOROCCO – Middle Atlas Region • 3♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24115 • 2♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24116.

North African distribution. New to Morocco and North Africa.

Exechia neorepanda Lindemann, 2021

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24117.

North African distribution. New to Morocco and North Africa.

Genus Pseudexechia Tuomikoski, 1966

Pseudexechia trivittata (Stæger, 1840)

Material examined. MOROCCO – Middle Atlas Region • 3♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24118 • 2♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24119.

North African distribution. New to Morocco and North Africa.

Pseudexechia tuomikoskii (Kjærandsen, 2009)

Material examined. MOROCCO – Middle Atlas Region • 10♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24120 • 7♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24121.

North African distribution. Morocco (Banamar et al. 2020).

Genus Rymosia Winnertz, 1864

Rymosia beaucournui Matile, 1963

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24122.

North African distribution. Tunisia (Chandler 1994); Morocco (Chandler and Ribeiro 1995; Chandler et al. 2006; Banamar et al. 2020).

Rymosia ebejeri Chandler & Belqat, 2024

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24123.

North African distribution. Morocco (El Mouden et al. 2024).

Rymosia pseudocretensis Burghele-Balacesco, 1967

Material examined. MOROCCO – Middle Atlas Region • 2♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24124.

North African distribution. Algeria (Burghele-Balacesco 1967); Morocco (Chandler 1994; Chandler et al. 2006; Banamar et al. 2020).

Genus Stigmatomeria Tuomikoski, 1966

Stigmatomeria crassicornis (Stannius, 1831)

Material examined. MOROCCO – Middle Atlas Region • 9♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24125 • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24126 • 1♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24127.

North African distribution. Tunisia (unpublished record, leg. H. Malicky, pers. comm. from N. Caspers, 14 April 2004); Morocco (Banamar et al. 2020).

Genus Synplasta Skuse, 1890

Synplasta gracilis (Winnertz, 1864)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24128 • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24129.

North African distribution. New to Morocco and North Africa.

Tribe Mycetophilini Newman, 1834 Genus Mycetophila Meigen, 1803

Mycetophila britannica Laštovka & Kidd, 1975

North African distribution. Morocco (Chandler and Ribeiro 1995; Banamar et al. 2020).

Mycetophila edwardsi Lundström, 1913

Material examined. MOROCCO – Middle Atlas Region • 36♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24137 • 11♂♂, 5♀♀; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24138 • 1♂; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24136 • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24139. North African distribution. Morocco (Banamar et al. 2020).

Mycetophila marginata Winnertz, 1864

Material examined. MOROCCO – **Middle Atlas Region** • 2133, 519; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24140 • 3233; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24141 • 133; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24142 • 1333; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24142 • 13333; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24143.

North African distribution. Morocco (Banamar et al. 2020).

Mycetophila mitis (Johannsen, 1912)

Material examined. MOROCCO – **Middle Atlas Region** • 12♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24144 • 3♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24145 • 2♂♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24146.

North African distribution. New to Morocco and North Africa.

Mycetophila perpallida Chandler, 1993

Material examined. MOROCCO - Middle Atlas Region • 1⁽²⁾; Forêt Bouhayati; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24147 • 16⁽³⁾; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24148 • 13⁽³⁾; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24149 • 5⁽³⁾; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24150.

North African distribution. Cited from North Africa without further details (Chandler 2009); Morocco (Banamar et al 2020).

Mycetophila pictula Meigen, 1830

Material examined. MOROCCO – **Middle Atlas Region** • 143, 209; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24152 • 13, 49; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24153 • 19; Forêt Bab Boud-ir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST

MA22/24151 • 2 승승, 3 우 우; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24154.

North African distribution. Morocco (Chandler and Ribeiro 1995; Banamar et al. 2020).

Mycetophila pumila Winnertz, 1864

Material examined. MOROCCO – Middle Atlas Region • 13, 15qq; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24156 • 533; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24157 • 13; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24158; • 13; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24158; • 13; Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24155.

North African distribution. New to Morocco and North Africa.

Mycetophila sordida van der Wulp, 1874

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24159.

North African distribution. Morocco (Chandler 1994; Banamar et al. 2020).

Mycetophila spectabilis Winnertz, 1864

Material examined. MOROCCO - Middle Atlas Region • 23♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24160 • 5♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24161 • 1♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24162 • 1♂; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24163 • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24164.

North African distribution. Morocco (Banamar et al. 2020).

Mycetophila strigatoides (Landrock, 1927)

Material examined. MOROCCO – Middle Atlas Region • 2♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24165 • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24166.

North African distribution. Tunisia (Chandler 1994); Morocco (Banamar et al. 2020).

Mycetophila unicolor Stannius, 1831

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24167.

North African distribution. Morocco (Banamar et al. 2020).

Mycetophila vittipes Zetterstedt, 1852

Material examined. MOROCCO – **Middle Atlas Region** • 5, Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24169 • 5, G, 5, Q; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24170; • 1, Forêt Bab Boudir; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24168.

North African distribution. Morocco (Banamar et al. 2020).

Genus Phronia Winnertz, 1864

Phronia biarcuata (Becker, 1908)

Material examined. MOROCCO – Middle Atlas Region • 4ථ්ථ; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24171 • 15ථ්ථ; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24172.

North African distribution. Tunisia (Chandler 1994); Morocco (Chandler 1994; Chandler and Ribeiro 1995; Chandler et al. 2006; Banamar et al. 2020).

Phronia nitidiventris (van der Wulp, 1858)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24173.

North African distribution. Morocco (Banamar et al. 2020).

Phronia tenuis Winnertz, 1864

Material examined. MOROCCO – Middle Atlas Region • 9♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24174 • 19♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24175 • 2♂♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24176 • 2♂♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24177.

North African distribution. Algeria, Tunisia (Chandler 1994); Morocco (Banamar et al. 2020).

Phronia willistoni Dziedzicki, 1889

Material examined. MOROCCO – Middle Atlas Region • 1♂; Forêt Bab Boudir; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24178.

North African distribution. Morocco (Banamar et al. 2020).

Genus Sceptonia Winnertz, 1864

Sceptonia flavipuncta Edwards, 1925

Material examined. MOROCCO – Middle Atlas Region • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24179.

North African distribution. New to Morocco and North Africa.

Sceptonia intestata Plassmann & Schacht, 1990

Material examined. MOROCCO – Middle Atlas Region • 1♂; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24180 • 3♂♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24181.

North African distribution. Morocco (Banamar et al. 2020).

Sceptonia membranacea Edwards, 1925

Material examined. MOROCCO – Middle Atlas Region • 1♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24182 • 2♂♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24183.

North African distribution. Morocco (Banamar et al. 2020).

Genus Trichonta Winnertz, 1864

Trichonta apicalis Strobl 1897

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24184.

North African distribution. New to Morocco and North Africa.

Trichonta clavigera Lundström, 1913

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24185.

North African distribution. Tunisia (unpublished record, leg. H. Malicky, pers. comm. from N. Caspers, 14 April 2004); new to Morocco.

Trichonta foeda Loew, 1869

Material examined. MOROCCO – Middle Atlas Region • 2♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24186 • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24187.

North African distribution. Morocco (Banamar et al. 2020).

Trichonta vitta (Meigen, 1830)

Material examined. MOROCCO - Middle Atlas Region • 5 ぷぷ; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24188 • 3 ぷぷ; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24189 • 1ぷ; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24190 • 3 ぷぷ; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24191 • 1ぷ; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24192 • 1ぷ; Forêt Admam;

North African distribution. Algeria (Gagné 1981; Hackman et al. 1988; Chandler 1994); Morocco (Banamar et al. 2020).

Genus Zygomyia Winnertz, 1864

Zygomyia humeralis (Wiedemann, 1817)

Material examined. MOROCCO – Middle Atlas Region • 8♂♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24193 • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24194 • 1♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24195 • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24196.

North African distribution. Tunisia (unpublished record, leg. H. Malicky, pers. comm. from N. Caspers, 14 April 2004); Morocco (Banamar et al. 2020).

Zygomyia valeriae Chandler, 1991

Material examined. MOROCCO – Middle Atlas Region • 3♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24197.

North African distribution. New to Morocco and North Africa.

Zygomyia valida Winnertz, 1864

Material examined. MOROCCO – Middle Atlas Region • 11 33; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24198 • 433; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24199 • 13; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24200.

North African distribution. Morocco (Banamar et al. 2020).

Subfamily Mycomyinae Edwards,1925 Genus *Mycomya* Rondani, 185

Mycomya prominens (Lundström, 1913)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24201 • 3♂♂; Oued Aïn Chabab; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24202.

North African distribution. Morocco (El Mouden et al. 2024).

Mycomya pygmalion Väisänen, 1984

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouhayati; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24203.

North African distribution. Morocco (Banamar et al. 2020).

Mycomya tenuis (Walker, 1856)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24204.

North African distribution. New to Morocco and North Africa.

Mycomya tumida (Winnertz, 1864)

Material examined. MOROCCO – Middle Atlas Region • 1♂; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24205.

North African distribution. Morocco (Banamar et al. 2020).

Subfamily Sciophilinae Rondani, 1840 Genus *Azana* Walker, 1856

Azana anomala Staeger, 1840

Material examined. MOROCCO - Middle Atlas Region • 6♂♂; Forêt Bouhayati; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24206 • 1♂; Forêt gouffre Friouato; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24207 • 2♂♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24208 • 1♂; Village Bab Boudir; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24209 • 1♂; Oued Taourirt; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24210 • 1♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24210 • 1♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24210 • 1♂; Aïn Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24211 • 1♂; Forêt Admam; 22/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24212 • 1

North African distribution. Algeria (Becker 1907 as *A. altera*; Hackman et al. 1988); Morocco (Banamar et al. 2020).

Genus Sciophila Meigen, 1818

Sciophila geniculata Zetterstedt, 1838

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24213.

North African distribution. New to Morocco and North Africa.

Sciophila insolita Santos Abreu, 1920

Material examined. MOROCCO – Middle Atlas Region • 1♂; Gouffre Izora; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24214.

North African distribution. New to Morocco and North Africa.

Sciophila parviareolata Santos Abreu, 1920

Material examined. MOROCCO – Middle Atlas Region • 1♂; Grotte Bouslama; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24215 • 1♂; Vallée des oiseaux; 19/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24216 • 1♂; Grotte Bouhaya-

ti; 20/V/2022; K. Aattouch, M. El Ouahabi and M.A. El Mouden leg; UAE-FST MA22/24217.

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Discussion

Fungus gnats of North Africa in numbers

The newly recorded species from Morocco have raised the total number of North African fungus gnat species from 114 to 133. While the number of Bolitophilidae species remains unchanged, one new species has been added to the Keroplatidae family, bringing the total to 20 species. The Mycetophilidae family has seen the most significant increase, growing from 94 to 112 species, and now includes an additional genus, making a total of 31 genera (Table 2).

Morocco, with the greatest number of total species, has more than 80% of the North African fungus gnats fauna (Table 2). In contrast, neighbouring countries such as Algeria and Tunisia account for only 20% and 25% of the total regional species, respectively, based on non-systematic study data. Interestingly, there is no published or examined collection data available for the fungus gnats of Libya, while Egypt has only two recorded Keroplatidae species.

	Bolitophilidae		Keroplatidae		Mycetophilidae		Total	
	Species	Genera	Species	Genera	Species	Genera	Species	Genera
North Africa	1	1	20	10	112	31	133	42
Morocco	1	1	13	7	94	30	108	38
Algeria	1	1	5	3	23	13	29	17
Tunisia	0	0	9	7	25	16	34	23
Libya	0	0	0	0	0	0	0	0
Egypt	0	0	2	1	0	0	2	1

 Table 2. Summary of Bolitophilidae, Keroplatidae, and Mycetophilidae species and genera recorded from the North African countries.

However, the similar climates of Morocco, Algeria, and Tunisia, with their comparable geological compositions, such as the extension of the High Atlas Mountains into Algeria and Tunisia, suggest that more species of fungus gnats may be found in these regions. By any manner, given the limited research and knowledge about fungus gnats in other North African countries, this part of the discussion serves as a preliminary comparison of species presence and richness across the region.

Fungus gnat distribution relative to surrounding areas

It is striking that all the previously described species that are newly recorded from Morocco have a widespread Palearctic distribution. Although, the influence of Western European distribution and Mediterranean distribution seems to have an equal impact since all of the species newly cited in our study are in common with Western Europe and with the Mediterranean region. As usual,

faunistic exchanges with the Iberian Peninsula are always higher, as much as in this current study, emphasising that more than 63% of the 18 species newly recorded for Morocco in this study are in common with those of the Iberian Peninsula (Chandler and Báez 2002). Approximately 26% of the species are shared with the North Atlantic islands' region, a noteworthy figure considering the significant role of endemism in shaping the fauna of these islands As for North Africa, only one species newly recorded from Morocco seems to be in common with the rest of the area, which can be explained by the lack of comparable studies in other North African countries. Finally, Docosia tazekkae Chandler, El Mouden & Belgat, sp. nov. described in this paper, in addition to the other five Mycetophilidae species recently described in El Mouden et al. (2024), raise the percentage of the species of fungus gnat recorded only from Morocco so far (Mycetophilidae family particularly) from 0% to 5.5% (El Mouden et al. 2024). This percentage is considered to be highly important, and it highlights the potential of finding more species of fungus gnats new to science from Morocco.

Fungus gnats of the caves

Several species of fungus gnats are known to inhabit cavities and especially cave entrances for the perfect climatic conditions they offer which correspond to the optimum life conditions for this dipteran group of insects. The results of our study pointed in the same direction since 55 species of fungus gnats of the 71 species recorded were found in the three cave entrances we managed to sample: Grotte Bousslama, Gouffre Izora, and Grotte Bouhayati, which are all located in the PNTZK. The first cave, Grotte Bousslama, is situated in the heart of a dense forest, a natural, well-preserved area. While Gouffre Izora is positioned near a managed rest area, one of the few well-maintained rest areas of the PNTZK, it still belongs to a forested habitat with a high level of protection. The entrances to the two caves are sheltered by a significant vegetative cover, which makes them the perfect ecosystem for the fungus gnats; this is supported by the high number of findings in each locality: 45 species from 16 genera were present at Grotte Bousslama, while 41 species from 18 genera were found to inhabit Gouffre Izora. The third cave, Grotte Bouhavati, is generally a natural hole in the ground under the limit of a forest area devoid of vegetation at its entrance, where a total of 21 species from nine genera were collected from this cave. Most of these species are found also in other types of habitats within the park, while a total of 26 species were found nowhere else but in caves. In other words, more than one-third (37%) of the PNTZK fungus gnat species were recorded only in caves.

However, most of these species are not primarily cave dwellers. According to literature records from other geographical areas and to the former survey on Mycetophilidae of Morocco by Banamar et al. (2020), they have been found in other different biotopes. Members of this family are well known to enter caves for aestivation or hibernation. We believe that the reason for this marked effect on our results was the prevailing climate. During the period of sampling in late May, the temperature was quite high, commonly between 27 °C and 37 °C. Consequently, the majority of fungus gnats seek refuges like these cavities when external temperatures are high to shelter from the heat.

Conclusions

This study contributes significantly to our knowledge of the biodiversity of the fungus gnats known from Morocco and North Africa. Two genera and 19 species were recorded for the first time from Morocco which increases the number of species in the fungus gnat fauna from 89 to 108. As for North Africa, 18 of these species and one genus are considered new to the region so the fungus gnat species number jumps from 114 to 133. Also, the discovery of the new *Docosia* species from Morocco together with the five mycetophilid species previously described by El Mouden et al. (2024), highlights biogeographic and conservation interest areas of the studied areas. If this indicates anything, it is that the North African region still hides more secrets about the fungus gnats than it shows, either as new records or even as species new to science. This should stimulate the appetite for more hard work and more profound studies in every country of the region to lead to curtain raising on the discoveries that are waiting for us.

Acknowledgements

We warmly thank Dr. Paul Beuk (Natuurhistorisch Museum Maastricht, de Bosquetplein 6-7, NI-6211kj Maastricht, The Netherlands) for his tremendous help in the early stages of this study. We extend our deepest gratitude to both reviewers, Jevgeni Jakovlev and Olavi Kurina, for their thorough and constructive feedback, which has been crucial in refining this manuscript. Our appreciation also goes to the chief editor, Vladimir Blagoderov, for his invaluable support and guidance. Additionally, I thank the entire editorial team at ZooKeys for their professionalism and dedication.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

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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