

Research Article

A new four-pored *Amphisbaena* Linnaeus, 1758 (Amphisbaenia, Amphisbaenidae) from the north of Espinhaço Mountain Range, Brazil

Síria Ribeiro^{1,20}, Alfredo P. Santos Jr^{1,20}, Isabelly G. Martins^{1,20}, Elaine C. S. Oliveira³, Roberta Graboski⁴⁰, Thiago Barbosa Da Silveira⁵, Matheus H. M. Benício⁶, Wilian Vaz-Silva⁷⁰

- 1 Universidade Federal do Oeste do Pará, Programa de Pós-Graduação em Biodiversidade, 68040-255, Santarém, Pará, Brazil
- 2 Laboratório de Ecologia e Comportamento Animal, Universidade Federal do Oeste do Pará, 68040-255, Santarém, Pará, Brazil
- 3 Instituto Chico Mendes de Conservação da Biodiversidade, Coordenação Regional Oeste do Pará, 68040-000, Santarém, Pará, Brazil
- 4 School of Zoology, Faculty of Life Sciences, the Steinhardt Museum of Natural History, Tel-Aviv University, Tel-Aviv, Israel
- 5 Tecsan Tecnologia e Saneamento Ltda Rua Dr. José Peroba, 275, Ed. Metrópolis Empresarial, Sala 201/202, Stiep, 41770-235, Salvador, Bahia, Brazil
- 6 Bahia Mineração S/A, Rodovia BA 156, s/n, Projeto Pedra de Ferro, 46400-000, Caetité, Bahia, Brazil
- 7 Pontifícia Universidade Católica de Goiás, Escola de Ciências Médicas e da Vida, Centro de Estudos e Pesquisas Biológicas, and Programa de Pós-Graduação em Ciências Ambientais e Saúde, 74605-010, Goiânia, Goiás, Brazil

Corresponding author: Síria Ribeiro (siherp@hotmail.com)



Academic editor: Uri García-Vázquez Received: 4 March 2024 Accepted: 18 July 2024 Published: 24 September 2024

ZooBank: https://zoobank. org/77F68F11-081E-4693-94BD-F211FFCFED62

Citation: Ribeiro S, Santos Jr AP, Martins IG, Oliveira ECS, Graboski R, Barbosa Da Silveira T, Benício MHM, Vaz-Silva W (2024) A new fourpored *Amphisbaena* Linnaeus, 1758 (Amphisbaenia, Amphisbaenidae) from the north of Espinhaço Mountain Range, Brazil. ZooKeys 1213: 1–27. https://doi.org/10.3897/ zookeys.1213.122265

Copyright: © Síria Ribeiro 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).

Abstract

A new species of *Amphisbaena* is described from the north of Espinhaço Mountain Range, municipality of Caetité, state of Bahia, Brazil. *Amphisbaena amethysta* **sp. nov.** can be distinguished from its congeners by the following combination of characters: (1) snout convex in profile, slightly compressed not keeled; (2) pectoral scales arranged in regular annuli; (3) four precloacal pores; (4) distinct cephalic shields; (5) 185–199 dorsal half-annuli; (6) 13–16 caudal annuli; (7) conspicuous autotomic site between 4th–6th caudal annuli; (8) 16–21 dorsal and ventral segments at midbody; (9) 3/3 supralabials; (10) 3/3 infralabials; and (11) smooth and rounded tail tip. The new species is the 71st species of genus with four precloacal pores, and the 22nd species from the Caatinga morphoclimatic domain. The identification of *Amphisbaena amethysta* **sp. nov.** indicates that the reptile fossorial fauna in the Espinhaço Mountain Range region is far from being completely known and that it may harbour a much greater diversity of endemic taxa.

Key words: Morphology, new species, phylogeny, taxonomy

Introduction

In the Caatinga 21 species of *Amphisbaena* have been recorded to date, with most being fully restricted to this morphoclimatic domain (Ribeiro and Eleutério 2023). Of these, four have wide distributions in Caatinga: *A. alba* Linnaeus, 1758, *A. lumbricalis* Vanzolini, 1996, *A. pretrei* Duméril & Bibron, 1839, and *A. vermicularis* Wagler, 1824, while nine have been found in a small number of localities: *A. anomala* (Barbour, 1914), *A. arenaria* Vanzolini, 1991, *A. bahiana* Vanzolini, 1964, *A. carvalhoi* Gans, 1965, *A. frontalis* Vanzolini, 1991, *A. fuliginosa*

Linnaeus, 1758, *A. hastata* Vanzolini, 1991, *A. heathi* Schmidt, 1936, and *A. ig-natiana* Vanzolini, 1991. In addition, five species are known only from the type locality: *A. arda* Rodrigues, 2003, *A. caetitensis* Almeida, Freitas, Silva, Valverde, Rodrigues, Pires & Mott, 2018, *A. longinqua* Teixeira Junior, Dal Vechio, Recoder, Cassimiro, Sena & Rodrigues, 2019, *A. mongoyo* Teixeira Junior, Dal Vechio, Recoder, Cassimiro, Sena & Rodrigues, 2019, and *A. uroxena* Mott, Rodrigues, Freitas & Silva, 2008 (Vanzolini 1992; Teixeira Jr et al. 2014; Costa et al. 2015; Almeida et al. 2018; Ribeiro and Eleutério 2023).

In the last two decades, six species of *Amphisbaena* have been identified in the high-altitude areas of the Espinhaço Mountain Range in Bahia and Minas Gerais: *A. bahiana, A. longinqua, A. metallurga* Costa, Resende, Teixeira Jr, Dal Vechio & Clemente, 2015, *A. mongoyo, A. uroxena*, and *A. caetitensis* (Costa et al. 2015; Almeida et al. 2018; Teixeira Junior et al. 2019). Four of which are known to be closely related phylogenetically (Teixeira Junior et al. 2019), while the position of the *A. metallurga* awaits analysis; and that of *A. caetitensis* awaits resolution of the phylogenetic relationship (Almeida et al. 2018). Such clustering points to a probable centre of endemism for this type of geological formation.

During a faunal rescue in the south of state of Bahia, Brazil, conducted as part of environmental activities carried by BAMIN (Bahia Mineração mining company), specimens of a species of *Amphisbaena* with four precloacal pores were collected, which could not be identified as belonging to any known amphisbaenid species. Accordingly, it was concluded that they represented a new taxon, which is described below.

Materials and methods

We analysed 48 specimens of non-identified Amphisbaena from the municipality of Caetité, state of Bahia, Brazil. The type series was deposited in the herpetological collection from Pontifícia Universidade Católica de Goiás (CEPB), municipality of Goiânia, state of Goiás, Brazil. For morphological comparisons we used data from 370 analysed specimens of Amphisbaena (see list SI1). Additional morphological data was taken from the literature. The taxonomy follows the nomenclature of Guedes et al. (2023) for species (but not including subspecies). Nomenclature for the cephalic scales and for meristic data follow Gans and Alexander (1962). Morphometric data follow the methodology of Perez et al. (2012). Head scale morphometric data were taken with digital callipers (precision 0.01 mm) on the right side of specimens. Head length was measured from the tip of the rostral shield to the anterior margin of the first dorsal half-annulus; and ventral length of head was measured from tip of rostral shield to the anterior margin of first body annulus. Snout-vent length was measured with the aid of a nylon line and subsequently measured with a millimetre rule. Counts for dorsal and ventral half-annuli were made on the right side of each specimen. Incomplete half-annuli were not included in the total count. Observations on specimen and annuli counts were made with the aid of a stereomicroscope. Infralabials, supralabials, and parietal shields variations are treated as "right/left".

Our molecular data matrix comprises 81 terminals for six genes: three mitochondrial genes (16S- 16S Large Subunit Ribosomal RNA gene; 12S - Small Subunit Ribosomal RNA gene; and nd2 - NADH Dehydrogenase 2 gene) and three nuclear genes (*c-mos* – Oocyte maturation factor Mos; *bdnf* – Brain-derived neurotrophic factor; and *rag1* – Recombinant activating gene 1). We sequenced four new DNA fragments (two for 12S and two for 16S) for two specimens of *Amphisbaena amethysta* sp. nov. (see Suppl. material 1: table S1). We also included sequences available in GenBank (https://www.ncbi.nlm.nih.gov) for 79 species of Amphisbaenia. We rooted our phylogenetic tree using Lacertidae (*Lacerta media* Lantz & Cyrén, 1920).

DNA was extracted from liver tissue using the PureLink extraction kit (Invitrogen, Massachusetts, USA), following the manufacturer's protocol. Sequences were amplified by Polymerase Chain Reaction (PCR) using the primers 12S and 16S as described by Graboski et al. (2023) following the amplification protocols described in Kearney and Stuart (2004) and Mott and Vieites (2009). Amplified fragments were purified with shrimp alkaline phosphatase and exonuclease I (GE Healthcare, Piscataway, NJ, USA). Both strands were sequenced on an Applied Biosystems 3500 Series Genetic Analyzer (Thermo Fisher Scientific, USA) at Laboratório de Genética e Biodiversidade da Universidade Federal de Goiás, Brazil. Both strands were quality-checked and, when necessary, edited manually. Consensus for both strands was generated using Geneious Prime 2022.1.1 (https://www.geneious.com).

Sequences were aligned using MAFFT 1.3.6 (Katoh and Standley 2013) through a plugin implemented in Geneious Prime. The 16S and 12S sequences were aligned under the E-INS-I algorithm, while nd2 and nuclear genes were aligned under the G-INS-i algorithm. We used default parameters for gap opening and extension. The protein-coding gene alignments were visually checked using Geneious Prime to verify that all sequences follow the correct reading frame. All genes were concatenated using Geneious Prime.

We used PartitionFinder 2 (Lanfear et al. 2016) to identify the combined best-fitting of partitioning schemes and models of molecular evolution. Our input matrix was divided in 14 partitions (coding genes were partitioned by codon positions and each rRNA was analysed as a separate partition) and was analysed using the greedy option. We performed a run allowing the program to select (using the Akaike Information Criterion with correction: AIC) for molecular evolution models implemented on RAxML (models GTR and GTR+G). We performed a maximum likelihood (ML) analysis using RAxML 8.2.3 (Stamatakis 2014). The ML tree was estimated using the RAxML algorithm that conducts a rapid bootstrap analysis and searches for best scoring ML tree in the same run (option -f a). We ran 1000 bootstrap replicates, and the best scoring ML tree was estimated 200 times using as a starting tree each fifth bootstrap tree. We also calculate uncorrected genetic distance (p-distance) using PAUP 4.0 (Swofford 2003). We considered only bootstrap values above 70% as a strong support.

Results

Amphisbaena amethysta sp. nov.

https://zoobank.org/E34029C4-6E1A-4E87-89C7-99E70AC1B95A Figs 1-4

Type material. *Holotype*: • male; CEPB 2311; municipality of Caetité, state of Bahia, BRAZIL; [14°21'31"S, 42°32'19"W; 1012 m above sea level (a.s.l.)]; collected on 1 November 2022 by Faunal Rescue Team Tecsam (F. Santos, P. Belufi, and G. Nascimento). *Paratypes*: • All from Caetité, Bahia, Brazil; collected by Faunal Rescue Team Tecsam (R. Assunção, A. Hirota, T. Silveira, F. Santos, P. Belufi, and G. Nascimento) • Female; CEPB 2301; (14°21'53"S, 42°32'20"W; 1013 m a.s.l.); 8 June 2022 • Male; CEPB 2302; (14°01'16"S, 42°31'06"W; 1082 m a.s.l.); 13 October 2021 • Female; CEPB 2303; (14°21'50"S, 42°32'20"W; 1013 m a.s.l.); 26 May 2022 • Female; CEPB 2308; (14°19'49"S, 42°32'44"W; 1011 m a.s.l.); 8 September 2022 • Female; CEPB 2327; (14°19'52"S, 42°32'42"W; 1011 m a.s.l.); 1 October 2022 • Female; CEPB 2331; (14°19'45"S, 42°32'45"W; 1011 m a.s.l.); 30 August 2022 • Male; CEPB 2346; (14°22'08"S, 42°32'17"W; 923 m a.s.l.); 6 October 2022 • Male; CEPB 2379; (14°21'27"S, 42°32'20"W; 1033 m a.s.l.); 26 January 2023 • Female; CEPB 2381; (14°21'27"S, 42°32'20"W; 1033 m a.s.l.); 26 January 2023.

Referred specimens. All from Caetité, Bahia, BRAZIL; collected by Faunal Rescue Team Tecsam (R. Assunção, A. Hirota, T. Silveira, F. Santos, P. Belufi, and G. Nascimento) · Female; CEPB 2298; (14°21'53"S, 42°32'20"W; 1013 m a.s.l.); 8 June 2022 • Female; CEPB 2299; (14°21'53"S, 42°32'20"W; 1013 m a.s.l.); 8 June 2022 · Male; CEPB 2300; (14°21'51"S, 42°32'21"W; 1011 m a.s.l.); 25 May 2022 • Female; CEPB 2304; (14°21'53"S, 42°32'20"W; 1013 m a.s.l.); 8 June 2022 • Female; CEPB 2305; (14°21'53"S, 42°32'20"W; 1013 m a.s.l.); 8 June 2022 • Female; CEPB 2306; (14°21'53"S, 42°32'20"W; 1013 m a.s.l.); 13 August 2022 • Male; CEPB 2307; (14°21'23"S, 43°32'07"W; 972 m a.s.l.); 23 November 2022 • Undetermined sex; CEPB 2309; (14 °19'46"S, 42°43'20"W; 1011 m a.s.l.); 1 September 2022 • Female; CEPB 2310; (14°19'55"S, 42°32'44"W; 1011 m a.s.l.); 8 October 2022 • Male; CEPB 2312; (14°19'56"S, 42°32'43"W; 1011 m a.s.l.); 12 October 2022 · Female; CEPB 2313; (14°22'08"S, 42°32'17"W; 9215 m a.s.l.); 6 October 2022 · Female; CEPB 2314; (14°22'08"S, 42°32'17"W; 925 m a.s.l.); 6 October 2022 · Female; CEPB 2315; (14°22'08"S, 42°32'17"W; 925 m a.s.l.); 6 October 2022 · Female; CEPB 2316; (14°19'47"S, 42°32'43"W; 1011 m a.s.l.); 1 September 2022 • Female; CEPB 2317; (14°19'55"S, 43°32'44"W; 1011 m a.s.l.); 1 October 2022 · Female; CEPB 2318; (14°19'46"S, 42°32'43"W; 1011 m a.s.l.); 1 September 2022 • Female; CEPB 2319; (14°20'37"S, 42°32'13"W; 1076 m a.s.l.); 3 August 2022 · Female; CEPB 2320; (14°20'38"S, 42°32'12"W; 1013 m a.s.l.); 2 June 2022 • Female; CEPB 2321; (14°19'53"S, 42°32'43"W; 1011 m a.s.l.); 29 September 2022 · Female; CEPB 2322; (14°14'28"S, 42°32'47"W; 842 m a.s.l.); 17 September 2022 • Female; CEPB 2323; (14°19'49"S, 42°32'17"W; 1062 m a.s.l.); 13 August 2022 · Male; CEPB 2324; (14°19'48"S, 42°32'43"W; 1011 m a.s.l.); 6 September 2022 • Female; CEPB 2325; (14°19'55"S, 42°32'44"W; 1011 m a.s.l.); 1 October 2022 • Male; CEPB 2326; (14°20'52"S, 43°32'16"W; 1053 m a.s.l.); 13 August 2022 · Female; CEPB 2328; (14°21'53"S, 42°32'22"W; 1011 m a.s.l.); 28 September 2022 • Male; CEPB 2329; (14°19'47"S, 42°32'43"W; 1011 m a.s.l.); 1 September 2022 • Male; CEPB 2330; (14°19'55"S, 43°32'44"W; 1011 m a.s.l.); 1 October 2022 • Male; CEPB 2332; (14°20'50"S, 42°32'16"W; 1057 m a.s.l.); 13 August 2022 · Female; CEPB 2333; (14°14'28"S, 42°32'47"W; 842 m a.s.l.); 17 September 2022 • Female; CEPB 2334; (14°19'46"S, 42°32'43"W; 1011 m a.s.l.); 1 September 2022 • Female; CEPB 2336; (14°15'11"S, 45°32'16"W; 987 m a.s.l.); 3 August 2022 · Female; CEPB 2337; (14°20'38"S, 45°32'12"W; 1076 m a.s.l.); 3 August 2022 · Female; CEPB 2338; (14°21'31"S, 45°32'16"W; 1010 m a.s.l.); 13 August 2022 • Female; CEPB 2339; (14°14'28"S, 42°32'47"W; 842 m a.s.l.); 17 September 2022 • Male; CEPB 2356; (14°21'27"S, 42°32'21"W; 1033 m a.s.l.); 1 November 2022 • Male; CEPB 2379; (14°21'27"S, 42°32'20"W; 1033 m a.s.l.); 26 January 2023 • Female; CEPB 2380;



Figure 1. Amphisbaena amethysta sp. nov. (Holotype, CEPB 2311) A dorsal B lateral, and C ventral views of head. Scale bar: 3 mm.



Figure 2. Tail of *Amphisbaena amethysta* sp. nov. (Holotype, CEPB 2311). Detail of the autotomic site and four precloacal pores. Scale bar: 3 mm.

(14°21'27"S, 42°32'20"W; 1033 m a.s.l.); 26 January 2023 • Female; CEPB 2381; (14°21'27"S, 42°32'20"W; 1033 m a.s.l.); 26 January 2023.

Diagnosis and comparisons with other south American amphisbaenians. *Amphisbaena amethysta* sp. nov. is a medium-sized amphisbaenid (258 mm maximum snout-vent length), and can be distinguished from its congeners by the following combination of characters (see details in Table 1): (1) snout convex in profile view, slightly compressed not keeled; (2) pectoral scales arranged in regular annuli; (3) four precloacal pores; (4) distinct cephalic shields; (5) 185–199 dorsal half-annuli; (6) 13–16 caudal annuli; (7) conspicuous autotomic site between 4th–6th caudal annuli; (8) 16–21 dorsal and ventral segments at midbody; (9) 3/3 supralabials; (10) 3/3 infralabials; and (11) smooth and rounded tail tip.

Amphisbaena amethysta sp. nov. differs from Amphisbaena acrobeles (Ribeiro, Castro-Mello & Nogueira, 2009), A. bilabialata (Stimson, 1972), A. kingi (Bell, 1833), A. anomala, Mesobaena huebneri Mertens, 1925; M. rhachicephala Hoogmoed, Pinto, Rocha & Pereira, 2009; and all Leposternon species, mainly in having the snout convex in profile view, slightly compressed not keeled (vs snout hardly compressed forming a sharp and prominent keel or snout depressed shovel-like). Differs still from A. anomala and all Leposternon species by having pectoral scales arranged in regular annuli (vs pectoral scales with an irregular form, and dermal annuli not regularly arranged).

Amphisbaena amethysta sp. nov. presents four precloacal pores, differing from all Amphisbaena species except A. acangaoba Ribeiro, Gomides & Costa, 2020, A. alba, A. albocingulata Boettger, 1885, A. angustifrons Cope, 1861, A. arda, A. arenaria, A. arenicola Perez & Borges-Martins, 2019, A. bahiana, A. bakeri Stejneger, 1904, A. barbouri Gans & Alexander, 1962, A. bedai (Vanzolini, 1991), A. bolivica Mertens, 1929, A. borellii Peracca, 1897, A. brasiliana (Gray, 1865), A. caeca Cuvier, 1829, A. camura Cope, 1862, A. carlgansi Thomas & Hedges, 1998, A. carioca Rocha, Barros-Filho & Sluys, 2023, A. carvalhoi, A. caudalis Cochron, 1928, A. cayemite Thomas & Hedges, 2006, A. cegei Montero, Sáfadez, Álvarez, 1997, A. cubana Gundlach & Peters, 1879, A. cuiabana (Strüssmann & Carvalho, 2001), A. cunhai Hoogmoed & Ávila-Pires, 1991,



Figure 3. *Amphisbaena amethysta* sp. nov. in life (not identified specimen of type series) **A** lateral view and **B** dorsal. Photograph by T.B.S.

A. darwini, A. elbakyanae Torres-Ramírez, Angarita-Sierra & Vargas-Ramírez, 2021, A. fenestrata (Cope, 1861), A. frontalis, A. gonavensis Gans & Alexander, 1962, A. gracilis Strauch, 1881, A. hastata, A. heathi, A. hogei Vanzolini, 1950, A. hoogmoedi Oliveira, Vaz-Silva, Santos-Jr, Graboski, Teixeira Jr, Dal Vechio & Ribeiro, 2018, A. hyporissor Thomas, 1965, A. innocens Weinland, 1862, A. kingi, A. kraoh (Vanzolini, 1971), A. leali Thomas & Hedges, 2006, A. lumbricalis, A. manni Barbour, 1914, A. medemi Gans & Mathers, 1977, A. metallurga, A. mongoyo, A. munoai Klappenbach, 1960, A. myersi Hoogmoed, 1989, A. nana Perez & Borges-Martins, 2019, A. nigricauda Gans, 1966, A. occidentalis Cope, 1875, A. pericensis Noble, 1921, A. plumbea Gray, 1872, A. polygrammica Werner, 1901, A. prunicolor (Cope, 1885), A. ridleyi Boulenger, 1890, A. rozei Lancini, 1963, A. sanctaeritae Vanzolini, 1994, A. saxosa (Castro-Mello, 2003), A. schmidti Gans, 1964, A. slateri Boulenger, 1907, A. slevini Schmidt, 1936, A. spurrelli Boulenger, 1915, A. steindachneri Strauch, 1881, A. supernumeraria Mott, Rodrigues & Dos Santos, 2009, A. talisiae Vanzolini, 1995, A. tyaraju, Perez & Borges-Martins, 2019, A. townsendi Stejneger, 1911, A. tragorrhectes Vanzolini, 1971, A. uroxena, A. vanzolinii Gans, 1963, A. vermicularis, and A. xera Thomas, 1966.

Amphisbaena amethysta sp. nov. differs from Amphisbaena species with four precloacal pores mainly by following combination of meristic characters (Table 1): cephalic shield distinct (vs frontals and parietals shields not distinct in A. supernumeraria, ocular and second supralabial not distinct in A. cubana); snout slightly compressed (vs hard compressed in A. kingi and rounded in all other species, except A. kraoh, A. brasiliana, A. bahiana, A. bedai, and A. saxosa); 185–199 dorsal half-annuli (vs < 170 annuli in A. cayemite and > 200 annuli in A. acangaoba, A. arda, A. arenaria, A. bahiana, A. bakeri, A. barbouri, A. bedai, A. borellii, A. brasiliana, A. caeca, A. carlgansi, A. carvalhoi, A. cayemite, A. cuiabana, A. cunhai, A. elbakyanae, A. fenestrata, A. frontalis, A. gonavensis, A. gracilis, A. hastata, A. hoogmoedi, A. kingi, A. kraoh, A. lumbricalis, A. manni, A. medemi, A. mongoyo, A. munoai, A. myersi, A. nigricauda, A. occidentalis, A. plumbea, A. polygrammica, A. rozei, A. sanctaeritae, A. saxosa, A. slevini, A. spurrelli, A. steindachneri, A. supernumeraria, A. talisiae, A. tyaraju, A. townsendi, A. uroxena, A. vanzolinii, A. vermicularis, and A. xera); 13–16 caudal annuli (vs > 16 annuli in A. albocingulata, A. arda, A. arenaria, A. arenicola, A. bedai, A. bolivica, A. borellii, A. carvalhoi, A. caudalis, A. cegei, A. cuiabana, A. cunhai, A. darwini, A. elbakyanae, A. frontalis, A. gracilis, A. hastata, A. hoogmoedi, A. leali, A. lumbricalis, A. manni, A. medemi, A. metallurga, A. mongoyo, A. munoai, A. myersi, A. nana, A. nigricauda, A. occidentalis, A. polygrammica, A. prunicolor, A. rozei, A. sanctaeritae, A. saxosa, A. schmidti, A. slateri, A. slevini, A. spurrelli, A. steindachneri, A. supernumeraria, A. talisiae, A. tyaraju, A. townsendi, A. tragorrhectes, and A. vermicularis); conspicuous autotomic site between 4th-6th caudal annuli (vs absent in A. acangaoba, A. alba, A. angustifrons, A. bakeri, A. barbouri, A. brasiliana, A. carioca, A. carlgansi, A. cayemite, A. cunhai, A. fenestrata, A. gonavensis, A. hastata, A. hoogmoedi, A. innocens, A. lumbricalis, A. occidentalis, A. ridleyi, A. saxosa, and A. uroxena; or from the 7th caudal annuli in A. albocingulata, A. arda, A. arenicola, A. carvalhoi, A. cegei, A. cuiabana, A. darwini, A. heathi, A. kingi, A. metallurga, A. mongoyo, A. myersi, A. nana, A. prunicolor, A. schmidti, A. slateri, A. steindachneri, A. supernumeraria, A. talisiae, A. tyaraju, A. townsendi, A. tragorrhectes, and A. vanzolinii), smooth and rounded tail tip



Figure 4. Variation (grey in the drawings) of cephalic shields of *Amphisbaena amethysta* sp. nov. **A** dorsal view of the head of CEPB 2280 with frontal variation **B** dorsal view of CEPB 2309 with four parietal shields, and **C** lateral view of left side of CEPB 2303 (paratype) with supralabials fused. Scale bar: 3 mm.

[vs bluntly ridged tail tip in *A. bahiana*; slightly dorsally compressed in *A. acangaoba*; with tubercles sit is depressed (compressed dorsoventrally) in *A. uroxena*; with modified conic pointed tubercles in *A. caetitensis*; and vertically keeled in A. borellii and A. steindachneri]; 16–21 dorsal segments at midbody (vs < 16 in A. albocingulata, A. arenaria, A. arenicola, A. barbouri, A. carlgansi, A. carvalhoi, A. cayemite, A. cuiabana, A. elbakyanae, A. fenestrata, A. heathi, A. hogei, A. elbakyanae, A. metallurga, A. munoai, A. nana, A. nigricauda, A. sanctaeritae, A. schmidti, A. slateri, A. slevini, A. supernumeraria, A. talisiae, A. tyaraju, A. tragorrhectes, A. uroxena, and A. vanzolinii); and > 21 segments in A. alba, A. arda, A. bolivica, A. camura, A. hoogmoedi, and A. kraoh); 16–21 ventral segments at midbody (vs < 16 in A. rozei, A. sanctaeritae, and A. tragorrhectes; and > 21 segments in A. alba, A. arda, A. bolivica, A. camura, A. cegei, A. gonavensis, A. hyporissor, A. kraoh, A. occidentalis, and A. townsendi); 3/3 supralabials (vs 2/2 in A. slevini and A. vanzolinii; and 4/4 in A. acangaoba, A. alba, A. angustifrons, A. arda, A. arenaria, A. bedai, A. camura, A. cayemite, A. occidentalis, A. plumbea, A. ridleyi, A. saxosa, A. townsendi, A. tragorrhectes, and A. vermicularis); and 3/3 infralabials (vs 2/2 in A. slevini and A. vanzolinii; and or 4/4 in A. occidentalis, A. plumbea, A. ridleyi, A. saxosa, A. townsendi, and A. tragorrhectes).

Table 1. Diagnostic characters for the *Amphisbaena* species with four precloacal pores. PC – precloacal pore, CS – cephalic shield, DA – dorsal half-annulus, CA – caudal annulus, AS – autotomic site, DS – dorsal segment, VS – ventral segment, SL – supralabial, IL – infralabial, PM – postmalar, SN – snout, DG – dorsal groove, VG – ventral groove, a – absent, cp – compressed, n/a – non-available data, p – present, rd – rounded, and sc – slightly compressed. Differences from the new species in bold.

Species	PC	CS	DA	CA	AS	DS	VS	SL	IL	PM	SN	DG	VG	Reference	
A. amethysta sp. nov.	4	distinct and paired	185-199	13-16	4-6	16-21	16-21	3	3	а	SC	а	а	present study	
A. acangaoba	4-8	distinct and paired	216-293	13–17	а	18-24	18-24	4	3	р	rd	а	а	Ribeiro et al. (2020)	
A. alba	4-10	distinct and paired	198-248	13-21	а	30-42	35-46	4	3	р	rd	а	а	Gans (1962a)	
A. albocingulata	4	distinct and paired	190-204	24-27	8-9	12-14	16-18	3	3	а	rd	а	а	Perez et al. (2012)	
A. angustifrons	3-6	distinct and paired	190-215	12-18	а	20-31	21-30	4	3	р	rd	а	а	Gans (1965a); Gans and Diefenbach (1972)	
A. arda	4	distinct and paired	242	30	8	23	23	4	3	р	rd	а	а	Rodrigues et al. (2003)	
A. arenaria	4	distinct and paired	285-307	22-23	6-7	12-14	14–16	4	3	а	rd	а	а	Teixeira Junior et al. (2016)	
A. arenicola	4	distinct and paired	199-216	20-22	8-9	12-14	16-18	3	3	Р	rd	n/a	n/a	Perez and Borges- Martins (2019)	
A. bahiana	4	distinct and paired	204-223	14-16	4-5	12-16	14-16	3	3	р	SC	р	р	Gans (1964b); Dal Vechio et al. (2018)	
A. bakeri	4	distinct and paired	239-255	14-16	а	14-16	16-17	3	3	а	rd	n/a	n/a	Gans and Alexander (1962)	
A. barbouri	4-6	distinct and paired	226-240	13-18	а	12-14	16-18	3	3	а	rd	n/a	n/a	Gans and Alexander (1962); Thomas (1966)	
A. bedai	4	distinct and paired	272-284	22-23	6	18-20	16-18	4	3	р	SC	а	а	Oliveira et al. (2018)	
A. bolivica	4-6	distinct and paired	200-231	18-26	4-5	27-36	26-36	3	3	р	rd	а	а	Montero (1996)	
A. borellii	4	distinct and paired	239-245	17-19	6-8	14-16	16-20	3	3	n/a	rd	а	а	Gans (1964b) and Oliveira et al. (2018)	
A. brasiliana	4	distinct and paired	213-229	11-15	а	18-21	18-22	3	3	р	SC	Р	а	Oliveira et al. (2018)	
A. caeca	4-6	distinct and paired	217-236	13-18	4-8	13-18	14-20	3	3	р	rd	n/a	n/a	Gans and Alexander (1962)	
A. caetitensis	4	distinct and paired	186-194	10-12	а	16	19-22	3	3	а	rd	а	а	Almeida et al. (2018)	
A. camura	4-6	distinct and paired	188-207	14–19	4-5	28-42	29-46	4	3	р	rd	а	а	Gans (1965a); Hoogmoed and Ávila- Pires (1991)	

Species	PC	CS	DA	CA	AS	DS	VS	SL	IL	PM	SN	DG	VG	Reference			
A. carioca	4	distinct and paired	186	21	а	10-12	n/a	3	3	р	rd	а	а	Rocha et al. (2023)			
A. carlgansi	4	distinct and paired	212-228	14-16	а	14	18-20	3	3	а	rd	a a		Thomas and Hedges (1998)			
A. carvalhoi	4	distinct and paired	231-245	19-22	7-8	12-14	16-18	3	3	а	rd	rd a		Gans (1965b)			
A. caudalis	4	distinct and paired	193-217	17-21	6	12-16	18-21	3	3	а	rd	n/a	n/a	Gans and Alexander (1962); Thomas and Hedges (1998)			
A. cayemite	4	distinct and paired	150-164	10-13	а	12-13	18	4	3	р	rd	n/a	n/a	Thomas and Hedges (2006)			
A. cegei	4	distinct and paired	198	22	7	21-22	22-23	3	3	а	rd	n/a	n/a	Montero et al. (1997)			
A. cubana	4-6	ocular and second SL fused	199-218	10-16	6-9	12-16	14-18	3	3	а	rd	n/a	n/a	Gans and Alexander (1962); Thomas (1966)			
A. cuiabana	4	distinct and paired	286-292	18-20	9–10	14	16	3	3	а	rd	р	а	Strüssmann and Carvalho (2001)			
A. cunhai	4	distinct and paired	226-239	25-26	а	14-16	14-18	3	3	р	rd	а	а	Hoogmoed and Ávila- Pires (1991)			
A. darwini	2-5	distinct and paired	174-195	19-25	7–10	13-19	16-23	3	3	n/a	rd	а	а	Perez et al. (2012)			
A. elbakyanae	4	distinct and paired	245-257	20-24	6-7	13-15	16-18	3	3	р	rd	р	а	Torres-Ramírez et al. (2022)			
A. fenestrata	4	distinct and paired	236-249	12-14	а	13-14	14-16	3	3	р	rd	n/a	n/a	Gans and Alexander (1962			
A. frontalis	0-4	distinct and paired	235-275	23-29	5-7	14-18	14-16	3	3	а	rd	а	а	Ribeiro-Júnior et al. (2022)			
A. gonavensis	4	distinct and paired	214-225	10-13	а	15-18	22-25	3	3	р	rd	n/a	n/a	Gans and Alexander (1962)			
A. gracilis	4	distinct and paired	224-248	21-24	6-7	13-16	14-17	3	3	р	rd	р	а	Gonzales-Sponga and Gans (1971)			
A. hastata	4	distinct and paired	266-273	40	а	18	16	3	3	а	rd	а	р	Vanzolini (1991)			
A. heathi	4	distinct and paired	183–187	n/a	7-8	12	18-20	3	3	а	rd	а	а	Gans (1965b)			
A. hogei	4	distinct and paired	177–191	15-19	4-7	10-13	14-18	3	3	р	rd	а	а	Gans (1966); Vanzolini (1950)			
A. hoogmoedi	4	distinct and paired	247-252	27	7-8	22-24	19-21	3	3	а	SC	а	а	Oliveira et al. (2018)			
A. hyporissor	4	distinct and paired	199-227	16-21	5	14-18	22-24	3	3	р	rd	n/a	n/a	Thomas (1965); Thomas and Hedges (2006)			
A. innocens	4	distinct and paired	185-220	11-14	а	13-16	18-21	3	3	а	rd	n/a	n/a	Gans and Alexander (1962); Thomas and Hedges (1998)			
A. kingi	4	distinct and paired	214-244	15-23	7	12-19	14-22	3	3	р	ср	а	а	Gans and Rhodes (1964); Oliveira et al. (2018)			
A. kraoh	4-6	distinct and paired	270-281	15	5	28	24–27	3	3	n/a	SC	n/a	n/a	Oliveira et al. (2018)			
A. leali	4	distinct and paired	188-206	17-20	6	14-16	20-22	3	3	а	rd	n/a	n/a	Thomas and Hedges			
A. lumbricalis	2-6	distinct and paired	225-247	20-26	а	12-16	16-20	3	3	n/a	rd	а	а	Vanzolini (1996)			
A. manni	4-9	distinct and paired	209-243	17-22	5-7	12-16	14-20	3	3	а	rd	n/a	n/a	Gans and Alexander (1962)			
A. medemi	4	distinct and paired	230-235	17-18	5-7	16	18	3	3	а	rd	а	р	Gans and Mather (1977)			
A. metallurga	2-4	distinct and paired	185-199	23-25	7-9	12-14	14-16	3	3	p or a	rd	а	а	Costa et al. (2019)			
A. mongoyo	4	distinct and paired	208	25	10	14	16	3	3	а	rd	а	а	Teixeira Junior et al. (2019)			
A. munoai	4	distinct and	202-218	18-23	5-9	10-14	13-18	3	3	р	rd	а	а	Perez and Borges- Martins (2019)			
A. myersi	4	distinct and	221	28	8	16	16	3	3	n/a	rd	а	а	Hoogmoed (1989)			
A. nana	4	distinct and paired	195-216	18-22	7-10	12-14	14–17	3	3	р	rd	n/a	n/a	Perez and Borges- Martins (2019)			
A. nigricauda	4	distinct and	222-226	19-24	6-9	10	16	3	3	а	rd	а	а	Gans (1966)			
A. occidentalis	4	distinct and paired	261-279	18-21	а	16-19	22–27	4	4	р	rd	р	р	Gans (1961)			

0					40	DO	1/0	01			CNI	D O	1/0	Peference		
Species	PC	CS	DA	CA	AS	DS	vs	SL	IL	РМ	SN	DG	VG	Reference		
A. pericensis	4	distinct and paired	198-218	16-19	6-8	12-16	16-20	3	3	а	rd	а	а	Gans (1963a)		
A. plumbea	4	distinct and paired	233-282	16-21	5-9	18-27	20-30	4	4	р	rd	а	а	Gans and Diefenbach (1972)		
A. polygrammica	4	distinct and paired	270	20	n/a	18	16	n/a	n/a	n/a	n/a	n/a	n/a	Vanzolini (2002)		
A. prunicolor	4	distinct and paired	181-215	18-27	7–10	10-17	14-20	3	3	р	rd	а	а	Perez et al. (2012)		
A. ridleyi	4	distinct and paired	172-192	14–17	а	16-18	20-28	4	4	р	rd	а	а	Gans (1963b)		
A. rozei	4	distinct and paired	205-209	20	6 or a	15-16	14	3	3	р	rd	а	а	Vanzolini (2002); Costa et al. (2019)		
A. sanctaeritae	4	distinct and paired	269-288	18-20	6-7	10	14	3	3	р	rd	а	а	Costa et al. (2019)		
A. saxosa	4	distinct and paired	253-272	17-21	а	18-24	16-21	4	4	р	SC	а	а	Oliveira et al. (2018)		
A. schmidti	4	distinct and paired	198-202	20-22	7-8	14	16-17	3	3	р	rd	а	а	Gans (1964a)		
A. slateri	4	distinct and paired	176-213	20-24	7-10	10-14	14-16	3	3	p or a	rd	а	а	Gans (1967); Costa et al. (2018)		
A. slevini	4	distinct and paired	204-211	23–25	5-6	10-14	10-12	2	2	а	rd	р	а	present study		
A. spurrelli	4	distinct and paired	218-222	18-20	6-7	16-18	16-18	3	3	р	rd	а	а	Gans (1962b); Costa (2020)		
A. steindachneri	4	distinct and paired	256-266	17-18	7	14-16	16	3	3	а	rd	p	p	Gans (1964b)		
A. supernumeraria	4	not distinct	333-337	22-23	10-12	14	17-18	3	3	n/a	rd	а	n/a	Mott et al. (2009)		
A. talisiae	4	distinct and paired	205-234	17-29	7	10-14	14-18	3	3	а	rd	а	а	Vanzolini (1995); Costa et al. (2019)		
A. tyaraju	4	distinct and paired	204-221	18-25	7-9	10-14	13-16	3	3	р	rd	n/a	n/a	Perez and Borges- Martins (2019)		
A. townsendi	4	distinct and paired	261-279	22-26	7-8	16-19	22–27	4	4	р	rd	n/a	n/a	Gans (1961)		
A. tragorrhectes	4	distinct and paired	196	31	12	12	12	4	4	р	rd	а	а	present study		
A. uroxena	0-4	distinct and paired	210-213	12-13	а	14	14-15	3	3	р	rd	а	р	Mott et al. (2008)		
A. vanzolinii	4	distinct and paired	225-228	n/a	7	12-13	16-17	2	2	р	rd	а	а	Gans (1963c)		
A. vermicularis	4	distinct and paired	211-254	23-34	5-7	18-26	18-25	4	3	n/a	rd	а	а	Gans and Amdur (1966)		
A. xera	4	distinct and	225-234	13 -16	5	12-16	14-16	3	3	а	rd	а	а	Thomas (1966)		

Description of the holotype. medium-sized specimen; snout-vent length 233 mm plus 0.50 mm of cloacal portion; tail length 21.24 mm, representing 9.1% of snout-vent length; midbody diameter 8.2 mm (3.5% of snout-vent length); head relatively small, 6.90 mm (~ 2.9% of snout-vent length); snout compressed in dorsal view and slightly convex in profile view, hardly keratinised, rostrum projecting forward beyond the jaw (prognathous). Anterior portion of body is slightly narrower. Rostral subtriangular, visible in dorsal and ventral view (Fig. 1), almost as high (2.21 mm) as wide (2.11 mm), in contact with nasal and first supralabial lateroposteriorly. Nasals subrectangular, aligned at the midline (1.00 mm suture) (Fig. 1A), almost as long (2.05 mm) as wide (1.99 mm), in contact with first supralabial laterally and prefrontals posteriorly, with nostrils placed near the antero-inferior angle of the nasal shield (Fig. 1B).

Prefrontals paired, relatively large (41.6% of head length), with a shorter middorsal suture (2.01 mm; 29.3% of head length), longer than the nasal middorsal suture (1.00 mm, 14.6% of head length), almost as long as frontal middorsal suture (2.10 mm, 30.6% of head length), anterior border convex, lateroposterior portion projected, in contact with second supralabial and ocular laterally, frontals posteriorly and in point contact with postocular posteriorly

(Fig. 1A). Frontals subtriangular, longer (suture length) than wide (1.58 mm), aligned at midline (2.10 mm), in narrow contact with the oculars, and in broad contact posterolaterally with the postocular and parietal. Parietals in two larger irregulars segments, wider (1.48 mm) than long (1.04 mm), intercalated by four very smalls segments; not aligned at the midline, in narrow contact with postoculars laterally, and followed by the first dorsal half-annulus. Occipitals absent (Fig. 1A). Oculars almost diamond-shape, almost as long (1.57 mm) as high (1.51 mm), in contact with prefrontals and second supralabial anteriorly, third supralabial and postocular posteriorly, and in point contact with postsupralabial. Eyes slightly visible. Postocular longer than wide, sub-pentagonal, in contact with frontal, labial, parietal and the segments of the first dorsal half-annulus, and in point contact with prefrontal. Temporal subrectangular, higher (1.55 mm) than long (0.92 mm), in contact with third supralabial anteriorly, postocular and postsupralabial laterally and first dorsal half-annulus posteriorly (Fig. 1B).

Three supralabials, irregularly polygonal; first subtrapezoid, longer (2.05 mm) than high (1.50 mm), in contact with second supralabial posteriorly; second supralabial sub-pentagonal, higher (1.76 mm) than long (1.59 mm maximum length), in contact with prefrontal, ocular and third supralabial; third supralabial subtrapezoid, almost as high (1.37 mm) as long (1.24 mm), in contact with ocular and postsupralabial. Postsupralabial subquadrangular, representing almost 50% of third supralabial high, in contact with temporal laterally and first half-annulus posteriorly (Fig. 1B). Mental longer (1.72 mm) than wide, anterior border wider (1.72 mm) than posterior (1.08 mm), in contact with the first pair of infralabials and postmental. Postmental longer (2.01 mm) than wide (1.65 mm), in contact with the first and second infralabial, narrowly with malar, and two anterior postgenials. Postgenials with five shields irregularly distributed, in contact with malars and first ventral half-annuli (Fig. 1C).

Three infralabials, first medium sized, irregular polygonal, almost as long (1.55 mm) as wide (1.56 mm), in contact with second supralabial; second the largest, sub-pentagonal, wider (2.36 mm) than long (1.81 mm maximum length), in contact with third infralabial; third infralabial smallest, almost as long (1.24 mm) as wide (1.37 mm) (Fig. 1C).

Body annuli well demarcated, first and second annuli without enlarged dorsal segments. Segments become regularly rectangular toward posterior portion of body and progressively longer than wide, and smaller in size, and larger towards midventral areas starting from the fifth half-annulus. One hundred ninety-four dorsal and 195 ventral half-annuli, three lateral half-annuli, 14 caudal annuli plus tip rounded; tail relatively long with autotomy line on the fifth annulus, 18/18 dorsal and ventral segments at midbody, respectively and 28 segments in fourth caudal annulus. Lateral sulci clearly visible from the forty-ninth annulus; dorsal and ventral sulci absent. Cloacal plate with six segments increasing in size from towards midline, eleven postcloacal segments; four precloacal pores strongly visible on the row of segments on the last ventral half-annulus; each pore placed on the posterior half of a single segment, and distributed along a continuous series of segments, but pores in the medial scales placed laterally (Fig. 2).

Intraspecific variation. The main variations in the type series for meristic and morphometric data are given in Table 2. Variation in the arrangement and contact of shields were also observed. CEPB 2280 presents frontal fused with the parietal and segments of first and second body annuli (Fig. 3A), CEPB 2309

Table 2. Variation in meristic and morphometric (mm) data in the type series of *Amphisbaena amethysta* sp. nov. S – sex, DA – dorsal half–annuli, LA – lateral half–annuli adjacent to cloacal region, VA – ventral half–annuli, CA – caudal annuli, AS – autotomic site, DS – dorsal segment in midbody, VS – ventral segment in midbody, SCA – segments of fourth caudal annulus, PRCL – precloacal segments, POCL – postcloacal segments, PGE – postgenials, PA – parietals, SVL – snout–vent length, TL – tail length, BW – body width in midbody, bs – brooked specimen, Ita – last tail annuli, n/a – non-available data, +n – mutilated tail, * – tail cicatrised, ** – tail not cicatrised, un- unidentified.

Specimens	S	DA	LA	VA	CA	AS	DS	VS	SCA	PRCL	POCL	PGE1	PA	SVL	TL	BW
CEPB 2311 (Holotype)	male	194	3	195	14	5	18	18	28	6	11	5	1/1	233	21.2	8.2
CEPB 2301 (Paratype)	female	191	3	191	5 + n	lta5**	19	17	29	7	13	5	1/1	206	3.5 + n	6.4
CEPB 2302 (Paratype)	male	194	4	192	15	5	20	18	31	7	13	5	1/1	199	17.8	7.2
CEPB 2303 (Paratype)	female	194	4	197	5 + n	lta5*	17	16	27	6	14	5	1/1	197	8.2 + n	7.0
CEPB 2308 (Paratype)	female	195	4	196	15	5	18	18	28	n/a	13	4	1/1	150	13.0	5.1
CEPB 2327 (Paratype)	female	196	3	196	14	5	20	19	31	6	13	5	1/1	205	16.5	6.0
CEPB 2331 (Paratype)	female	197	4	195	15	5	18	16	30	6	11	5	1/1	175	14.4	5.8
CEPB 2346 (Paratype)	male	195	4	195	14	5	18	18	29	6	14	5	1/1	138	11.3	4.3
CEPB 2379 (Paratype)	male	185	4	189	5 + n	lta5**	19	18	29	6	13	5	1/1	180	5.0 + n	7.0
CEPB 2381 (Paratype)	female	189	4	189	15	4	16	16	24	6	12	5	1/1	165	14.8	5.6
CEPB 2298	female	199	3	199	14	n/a	18	18	30	7	13	5	1/1	215	16.7	7.4
CEPB 2299	female	193	4	193	6+n	lta5*	18	15	29	6	13	4	1/1	186	7.6 + n	5.1
CEPB 2300	male	194	3	195	14	5	18	17	26	6	12	5	1/1	213	15.3	6.1
CEPB 2304	female	190	4	192	14	5	18	n/a	26	6	12	5	1/1	155	11.6	3.5
CEPB 2305	female	192	4	192	15	5	18	16	28	6	13	6	1/1	170	14.8	5.6
CEPB 2306	female	197	4	196	14	4	18	18	28	8	12	5	1/1	145	10.8	4.2
CEPB 2307	male	190	3	190	6 + n	lta6**	18	17	27	6	13	5	2/2	220	6.8 + n	7.9
CEPB 2309	un	196	3	197	6 + n	lta5*	19	16	n/a	6	14	6	1/2	125	7.9 + n	4.5
CEPB 2310	female	193	4	194	14	5	18	18	26	6	11	2	0/1	195	15.7	5.5
CEPB 2312	female	192	3	192	15	5	18	18	30	6	15	3	1/1	190	15.1	6.6
CEPB 2313	female	189	3	190	15	5	18	15	28	6	14	5	1/1	130	11.7	4.2
CEPB 2314	female	191	3	193	14	5	18	16	26	6	13	6	1/1	185	11.9	5.7
CEPB 2315	female	193	3	193	14	5	18	19	27	6	12	5	1/1	177	14.1	5.9
CEPB 2316	female	195	3	195	16	6	20	18	28	7	13	5	1/1	173	15.2	6.4
CEPB 2317	female	195	5	196	5 + n	lta5**	18	n/a	n/a	6	13	5	1/1	180	3.0 + n	5.3
CEPB 2318	female	196	3	195	15	5	20	18	31	6	11	5	1/1	120	16.1	4.3
CEPB 2319	female	186	4	186	5 + n	lta5**	19	18	29	6	12	5	1/1	95	2.0 + n	3.2
CEPB 2320	female	185	4	182	15	5	19	18	26	7	13	2	1/1	92	7.7	2.6
CEPB 2321	female	194	3	192	16	6	18	18	30	6	12	5	1/1	160	14.1	6.0
CEPB 2322	female	196	4	195	5 + n	lta5**	21	18	27	6	11	4	1/1	155	3.7 + n	5.3
CEPB 2323	female	194	4	193	15	5	16	16	24	6	13	2	1/1	112	8.8	3.0
CEPB 2324	male	193	3	193	16	6	19	18	31	6	13	5	1/1	162	14.6	5.7
CEPB 2325	female	193	4	190	14	5	19	18	27	6	11	5	1/1	174	14.2	6.1
CEPB 2326	male	198	3	197	16	6	17	17	28	6	10	5	1/1	195	16.4	6.4
CEPB 2328	female	192	3	194	15	6	18	18	28	6	12	5	1/1	173	15.5	6.5
CEPB 2329	male	195	4	196	15	5	19	18	30	6	13	6	1/1	170	13.0	5.7
CEPB 2330	male	194	4	193	5 + n	lta5**	20	18	30	6	13	5	1/1	178	2.6 + n	6.0
CEPB 2332	male	190 + n	4	190	15	5	18	16	28	6	15	5	1/1	bs	11.1	4.0
CEPB 2333	female	196	3	194	15	n/a	18	17	26	6	10	2	1/1	203	16.9	6.3
CEPB 2334	female	194	3	192	5 + n	5	20	18	30	6	14	6	1/1	190	7.0 + n	7.3
CEPB 2335	female	197	3	197	14	5	20	18	31	6	12	5	1/1	135	97	3.9
CEPB 2336	female	190	4	190	15	6	18	17	29	6	13	4	1/1	105	9.1	3.4
CEPB 2337	female	186	4	185	5+n	lta5**	19	18	29	6	13	5	1/1	145	37+n	4.9
CEDB 2338	female	100 ± n	4	186	12	5	19	17	29	6	10	5	1/1	a, 160	12.8	5.1
CEPB 2330	fomalo	190 + 11	4	100	16	5	10	10	20	6	12	5	1/1	~ 100	10.6	4.0
CEPB 2339	mala	192	4	193	10	5	19	10	20	7	10	2	1/1	127	16.7	4.0 E 0
CEPB 2330	famala	100	3	109	10	n/a	10	10	20	1	10	3	1/1	140	10.7	5.0
JEPD 238U	remale	192	3	195	14	5	1/	10	20	0	12	5	2/2	140	11.5	0.0
winimum		185	3	182	13	4	16	15	24	6	10	2		92	2.2	2.6
Maximum		199	5	199	16	6	21	19	32	8	15	6	n/a	233	21.2	8.2
Mean		192.9	3.6	193.0	14.8	5.0	18.4	17.4	28.3	6.2	12.6	4.7	n/a	167.3	13.4	5.5
Mode		194	4	195	15	5	18	18	28	6	13	5	n/a	190	n/a	n/a
Standard deviation		3.4	0.5	3.4	0.8	0.4	1.0	1.0	1.9	0.5	1.1	1.0	n/a	34.1	3.5	1.3

present four parietals shields (Fig. 3B), and CEPB 2303 present supralabials fused in left side (Fig. 3C).

Colour in life. Dorsum and lateral parts with dark brown coloration on the segments, which is more pronounced in the vertebral (Fig. 4A) and dorsal section of the tail regions (Fig. 4B). Pink predominates in areas where the brown colour is less pronounced. We do not have photographs of the ventral region of the live specimen.

Colour in preservative (ethylic alcohol 70%). Dorsum cream, with brown colouring on the segments in the dorsum, lateral parts, and dorsal portions of the tail portions. Dorsal, lateral, and ventral portions of head pale brown, darker than the ventral portion. Venter cream coloured.

Etymology. The specific epithet refers to the mineral amethyst that is a type of quartz and also the name of the region of the type locality "Brejinho das Ametistas", a district located south of the municipality of Caetité, state of Bahia. This region has been an amethyst mining centre since the beginning of the 20th century. Spix and Martius (1938) defined the mineral from the "Brejinho das Ametistas" mines as "the beautiful amethysts" on their trip through the "Alto Sertão" of Bahia at the beginning of the 19th century (Cotrim 2015). The region currently has an economy based on mining and energy activities focused on wind energy production. The type series was collected during the execution of environmental programs within the scope of Bahia Mineração (BAMIN), which operates in the exploration of iron ore in the "Brejinho das Ametistas" region.

Distribution and habitat. Amphisbaena amethysta sp. nov. is known from municipality of Caetité municipality, state of Bahia, Brazil (Fig. 5).



Figure 5. Geographic distribution of *Amphisbaena amethysta* sp. nov. and others *Amphisbaena* species from Espinhaço Mountain Range locality. Black arrows indicate the type localities of the species with more than one known geographic record.

The region is in the northern portion of the Espinhaço Mountain Range, has an average altitude of 1000 m a.s.l., and lies within the ecotone between two morphoclimatic domains, Caatinga and Cerrado. In the region there are patches of deciduous and semi-deciduous forests ["Floresta Estacional Decidual" and "Floresta Semidecidual Montana" sensu IBGE (2023)] associated with valleys, slopes, and gallery forests, and containing floristic elements common to the vegetation of the Caatinga, Cerrado, and Atlantic Forest morphoclimatic domains. Areas of savannah vegetation with rock outcrops, typical of the woody Caatinga, occur at higher elevations (Fig. 6).

Phylogenetic relationships. Our concatenated alignment totalled 4806 base pairs (1007 bp for 12S, 528 bp for 16S, 761 bp for *nd2*, 679 bp for *bdnf*, 574 bp for *c-mos*, and 1257 bp for *rag1*). Partition Finder identified a best-fit scheme composed of ten partitions with the GTR + G model. The resulting ML topology (Fig. 7) for the higher-level affinities was similar to those reported by previous studies



Figure 6. Caatinga site where the holotype of *Amphisbaena amethysta* sp. nov. was collected in the in the northern portion of Espinhaço Mountain Range, in state of Bahia, Brazil.



Figure 7. Maximum likelihood tree zoomed in Central and South American Amphisbaenidae resulting from the RAxML analysis based on six concatenated genes, three nuclear (BDNF, c-mos, and RAG1), and three mitochondrial (12S, 16S, and ND2) genes. Red branches denote the clade composed of the new species and its sister species. Numbers on branches represent bootstrap values RAxML (> 70%). The grey codes on the right side of the clades represent the South American subclades of Amphisbaena (G01–G09) and West Indies clades (WIC01–WIC02).

(Mott and Vieites 2009; Longrich et al. 2015; Graboski et al. 2022) (Fig. 7). *Amphisbaena amethysta* sp. nov. was recovered as a sister group of *A. caetitensis*, with 92% of bootstrap support. The clade composed by *Amphisbaena amethysta* sp. nov. and *A. caetitensis* was recovered as a sister group of *A. angustifrons*, *A. darwini*, *A. kingi*, *A. leeseri*, and *A. munoai* with low bootstrap support (21%). The genetic distance (*p-distance*) between *Amphisbaena amethysta* sp. nov. and *A. caetitensis* is 6.1% for 16S.

Discussion

Amphisbaena amethysta sp. nov. is the 73rd species of the genus with four precloacal pores, the 22nd species from Caatinga, and sixth species with a restricted distribution from this morphoclimatic domain. The recognition of *A. amethysta* as a new species is based on molecular data and on set of morphological characters that includes four precloacal pores, a slightly compressed snout, 185–199 dorsal and 182–199 ventral half-annuli, 13–16 caudal annuli, 16–21 dorsal and ventral segments in the midbody, 3/3 supralabials and infralabials, a smooth and rounded tail tip, an autotomic site between 4th and 6th caudal annuli, and by the absence of fusion of the cephalic shields, postmalar shields, and of dorsal and ventral grooves.

The new species is most closely related to A. caetitensis (from an elevation of 854 m in the municipality of Caetité, state of Bahia), a sister species with a genetic distance (p distance) of 6.1% for the 16S gene, and which differs morphologically mainly in the modified conic pointed tubercules on the tip tail and absence of an autotomic site (see diagnosis). This tail shape appears to have arisen independently in A. caetitensis and A. uroxena. Almeida et al. (2018) reported a genetic distance of the 7.65% between A. uroxena and A. caetitensis, and stated that Bayesian inference does not allow a clear resolution of the relationship between the two species. Our phylogenetic analyses corroborate the results of Almeida et al. (2018), and do not structure a clade for species with the modified conic pointed tubercules on the tip tail. We found an even lower distance between A. caetitensis and A. amethysta sp. nov. and a phylogenetic correlation between the species (Fig. 7), and recovered A. uroxena with sister species of A. mongoyo, structuring a clade with A. longingua and A. bahiana (Clade G05, Fig. 7). Additionally, with a low support, we retrieved the clade formed by Amphisbaena amethysta sp. nov. and A. caetitensis as a sister group to clade G02 (Fig. 7) formed by A. leeseri, A. darwini, A. munoai, A. angustifrons, and A. kingi. This grouping showed a relationship between the species from the Espinhaço Mountain Range and those in other areas of the Atlantic Forest and Cerrado. Additionally, it can indicate a probably convergent evolution between the species with a tuberculate tail shield (A. caetitensis and A. uroxena) and those species present on mountain within the Espinhaço Mountain Range (clade 5; Fig. 7).

Amphisbaena amethysta appears to be endemic to the northern portion of the Espinhaço Mountain Range with an average altitude of 1000 m a.s.l. Within this, its known extent of occurrence is some 38 km. This distributional pattern is similar to other five species of Amphisbaena restricted to the high-altitude areas of the Espinhaço Mountain Range in Bahia [A. bahiana, A. longinqua, A. metallurga, A. mongoyo, and A. uroxena (Costa et al. 2015; Teixeira Junior et al. 2019)], four of which are closely related phylogenetically, but show no close phylogenetic

relations with *A. amethysta* (*A. metallurga* has no molecular sample available) (Fig. 7). The other three species show no apparent relationship to those from the Espinhaço Mountain Range but also occur at similar altitudes and vegetation types: *A. kiriri* Ribeiro, Gomides & Costa, 2018 (at least 17 km from the nearest *A. bahiana* locality), *A. acangaoba* (occurring in sympatry with *A. kiriri*), and *A. ignatiana* (recorded from the lower sections of the northwestern extremity of the Espinhaço Mountain Range) (Fig. 5) (Roberto et al. 2014; Ribeiro et al. 2018b, 2020).

In the last years morphological characters were commonly used to diagnose Amphisbaena, mainly meristics (e.g. Roberto et al. 2014; Costa et al. 2015; Ribeiro et al. 2016, 2019, 2018a, b, 2020; Torres-Ramírez et al. 2021; Ribeiro-Júnior et al. 2022; Rocha et al. 2023) and morphometrics (e.g. Ribeiro et al. 2009; Oliveira et al. 2018) but also with the addition of genetic analyses (e.g., Almeida et al. 2018; Perez and Borges-Martins 2019; Ribeiro et al. 2019), but it is still unclear which characters best represent the interspecific variation of the genus. The identification of diagnostic characters depends on the recognition of intraspecific variations of different species, as well as the analysis of relatively large numbers of samples for each species, the latter being hampered by the fossorial habits of the species in this group and the consequently low frequency of encounters with them. Cryptic species of Amphisbaena are rare in bibliographic citations, but recent studies have described new species using diagnoses based on overlapping phenotypic variation and the divergence of the 16S and ND2 genes, complicating species identification using external morphology alone. To reduce the limitations in the identification of new amphisbaenid species, in addition to applying integrative taxonomy whenever possible, we consider it important to standardise the use of meristic and morphometrics characters that do not overlap and to explore new characters, such as the morphometry of the shields (Ribeiro et al. 2008, 2009, 2011, 2015, 2016, 2018a, 2019; Sindaco et al. 2014); analysing characters of internal morphology, such as osteology and gonadal morphology (Ribeiro et al. 2015, 2018a; Oliveira et al. 2018; Angiolani-Larrea et al. 2021); and studying similar species, including type series (Costa et al. 2019; Ribeiro-Júnior et al. 2022).

Amphisbaena amethysta sp. nov. varies in the number of dorsal and ventral half-annuli, caudal annuli, and dorsal and ventral segments in the midbody, the shape and number of parietal shields, and the position of the autotomic site. In addition to variations in shape and number of cephalic shields, specimens of the new species also included individuals with shield fusion, mainly in the parietal region (see discussion of variation). On the other hand, the sample did not vary the number and shape of supra and infralabials and postlabials; the presence of a malar; the shape of the rostral, prefrontal, and frontal shields; the presence and number of precloacal pores; nor in the shape of the segments of the half-annuli of the body and tail. Despite its use as an invariable character, the number of pores can vary intraspecifically. Among the other Amphisbaena species with four precloacal pores, 14 vary in the number of precloacal pores: A. acangaoba, A. alba, A. angustifrons, A. barbouri, A. bolivica, A. caeca, A. camura, A. cubana, A. darwini, A. frontalis, A. kraoh, A. lumbricalis, A. manni, and A. uroxena (Gans 1962a, 1965a; Gans and Alexander 1962; Thomas 1966; Gans and Diefenbach 1972; Hoogmoed and Ávila-Pires 1991; Montero 1996; Vanzolini 1996; Mott et al. 2008; Perez et al. 2012; Ribeiro et al. 2020; Ribeiro-Júnior et al. 2022). Additionally, sexual dimorphism in the presence or absence of pores for Amphisbaena has already been reported for A. frontalis and A. uroxena (Mott et al. 2011; Ribeiro-Júnior et al. 2022).

Mining activities cause several irreversible changes to the environment, including the loss of habitat due to the removal of vegetation and the relocation and excavation of soil during the opening and operation of new mines. Such actions have a direct impact on terrestrial and fossorial species such as amphisbaenians. In this context, applied studies, such as the evaluation of species as biomarkers for metal exposure, mainly with fossorial reptile species, are neglected (Grillitsch and Schiesari 2010; Gil-Jiménez et al. 2021). In such circumstances, the development and execution of environmental programs are critical for effective impact mitigation. Such studies can form valuable tools when conducted by specialised teams with a focus on the scientific use of collected material (Vaz-Silva 2009), and teams concerned with the technical quality of the project, which would result in more careful projects in relation to the sampling design and analytical aspects of the results, allowing greater decision-making power for effective conservation actions (Dias et al. 2019). In this case, environmental studies have made an important contribution to knowledge of the biodiversity of the area under BAMIN's control, Bahia Mineração. Finally, the identification of a new species indicates that the fossorial fauna, as well as that of other groups, in the Espinhaço Mountain Range region is far from being completely known and that it may harbour a much greater diversity of endemic taxa than has been realised so far.

Acknowledgements

We would like to thank the curators and/or collection technicians D. Frost and D. Kizirian (AMNH), G.R. Colli, M. Viana and M. Ayub (CHUNB); S. Carreira (FC-UDELAR); H.M. Chalkidis (LPHA); G. Pontes (MCT-PUCRS); J. Moura-Leite (MHNCI); M. Motte (MNHNP); P. Passo, R. Fernandes and M. Soares (MNRJ); A. Prudente, A. Travassos and F. Sarmento (MPEG); H. Zaher and A. Carvalho (MZUSP); E. Freire and T. Mott (MUFAL); A. Argolo and M. Solé (UESC); V. Ferreira (UFMS/CEUCH/AFB); and F. Curcio (UFMT) for permits, care, and access to specimens. We are grateful to D. M. Soares for photography of the holotype Amphisbaena amethysta sp. nov. and to D. Passos, one anonymous reviewer, and the editors N. Yonow and U. Garcia for suggestions that improved the quality of the manuscript. We thank BAMIN Bahia Mineração and the time of Tecsan Tecnologia e Saneamento responsible for environmental mitigation actions that culminated in the collection of specimens from the type series: Patrícia de Rossi Belufi, Andrezza Sayuri, Victoriano Hirota, Roberto Aires de Assunção, Inácio Fernandes Brito, Fabiana Soares dos Santos, Márcio Santos Borges, and Gabryelle Santos Nascimento. We thanks the National Council for Scientific and Technological Development - CNPq, through the program to support research projects and training human resources for biological taxonomy - PRO-TAX 22/2020, process number 441967/2020-5 CNPq. RG thanks Fundação de Amparo à Pesquisa do Estado de São Paulo for fellowships (FAPESP, 2012/ 24755-8 and 2016/06866-8) and IGM thanks Fundação de Amparo à Pesquisa do Estado do Pará (FAPESPA, 00000.9.000794/2023).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

RG was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2012/ 24755-8 and 2016/06866-8). IGM was supported by grants from the Fundação de Amparo à Pesquisa do Estado do Pará (FAPESPA, 00000.9.000794/2023).

Author contributions

SR, WVS, and APSJ identified the new species. WVS, and RG gathered the molecular data; SR, ECS, APJS, and IGM gathered the morphological data; all authors wrote and revised the text.

Author ORCIDs

Síria Ribeiro © https://orcid.org/0000-0002-2301-7089 Alfredo P. Santos Jr © https://orcid.org/0000-0002-2829-718X Isabelly G. Martins © https://orcid.org/0009-0002-4420-5713 Roberta Graboski © https://orcid.org/0000-0002-9123-4819 Wilian Vaz-Silva © https://orcid.org/0000-0001-6235-5331

Data availability

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

References

- Almeida JPFA, Freitas MADE, Silva MB, Valverde MCC, Rodrigues MT, Pires AM, Mott T (2018) A new four-pored *Amphisbaena* (Squamata: Amphisbaenidae) from northeastern Brazil. Zootaxa 4514(4): 553–562. https://doi.org/10.11646/zootaxa.4514.4.8
- Angiolani-Larrea F, de Fraga R, Mortati A, Ribeiro S (2021) Sex-dependent divergence in asymmetrical reproductive tract morphology between two closely-related amphisbaenian species (Squamata: Amphisbaenidae). Amphibia-Reptilia 42(3): 317–330. https://doi.org/10.1163/15685381-bja10056
- Costa HC (2020) New record and updated distribution map of the rare *Amphisbaena spurrelli* (Amphisbaenia: Amphisbaenidae). Phyllomedusa 19(1): 259–266. https://doi.org/10.11606/issn.2316-9079.v19i2p259-266
- Costa HC, Resende FC, Teixeira Jr M, Dal Vechio F, Clemente CA (2015) A new Amphisbaena (Squamata: Amphisbaenidae) from southern Espinhaço Range, southeastern Brazil. Anais da Academia Brasileira de Ciências 87(2): 891–901. https://doi. org/10.1590/0001-3765201520140088
- Costa HC, Welton LJ, Hallermann J (2018) An updated diagnosis of the rare *Amphis-baena slateri* Boulenger, 1907, based on additional specimens (Squamata, Amphisbaenia, Amphisbaenidae). Evolutionary Systematics 2(2): 125–135. https://doi.org/10.3897/evolsyst.2.28059
- Costa HC, Velasquez S, Zaher H, Garcia PCA (2019) Updated diagnosis of *Amphisbaena metallurga* and *A. sanctaeritae* and first record of *A. hiata* in Brazil (Squamata: Amphisbaenidae). South American Journal of Herpetology 14(3): 233–241. https://doi. org/10.2994/SAJH-D-17-00105.1

- Cotrim CGC (2015) "As bellas amethystas": garimpos e relações comerciais em Brejinho das Ametistas (Alto Sertão da Bahia, 1870–1930). Epígrafe 2(2): 25–46. https:// doi.org/10.11606/issn.2318-8855.v2i2p25-46
- Dal Vechio F, Teixeira Jr M, Mott T, Rodrigues MT (2018) Rediscovery of the poorly known Amphisbaena bahiana Vanzolini, 1964 (Squamata, Amphisbaenidae), with data on its phylogenetic placement, external morphology and natural history. South American Journal of Herpetology 13(3): 238–248. https://doi.org/10.2994/SAJH-D-16-00053.1
- Dias AMS, Fonseca A, Paglia AP (2019) Technical quality of fauna monitoring programs in the environmental impact assessments of large mining projects in southeastern Brazil. Science of the Total Environment 650(1): 216–223. https://doi.org/10.1016/j. scitotenv.2018.08.425
- Gans C (1961) Notes on amphisbaenids (Amphisbaenia: Reptilia). 2. Amphisbaena occidentalis Cope from the coastal plain of northern Peru. Postilla (56): 1–17.
- Gans C (1962a) Notes on amphisbaenids (Amphisbaenia: Reptilia). 5. A redefinition and a bibliography of *Amphisbaena alba* Linné. American Museum Novitates 2105: 1–31.
- Gans C (1962b) Notes on amphisbaenids (Amphisbaenia: Reptilia). 6. Redescription and range extension of *Amphisbaena spurrelli* Boulenger. Breviora 171: 1–11.
- Gans C (1963a) Notes on amphisbaenids (Amphisbaenia: Reptilia). 10. Redescription and redefinition of *Amphisbaena pericensis* Noble from the mountains of northwestern Peru. Breviora 189: 1–15.
- Gans C (1963b) Notes on amphisbaenids (Amphisbaenia: Reptilia). 9. Redescription of *Amphisbaena ridleyi* Boulenger. Copeia 1963(1): 102–107. https://doi.org/10.2307/1441276
- Gans C (1963c) Notes on amphisbaenids (Amphisbaenia, Reptilia). 8. A redescription of *Amphisbaena stejnegeri* and the description of a new species of Amphisbaena from British Guiana. American Museum Novitates 2128: 1–18.
- Gans C (1964a) Notes on amphisbaenids (Amphisbaenia: Reptilia). 11. On *Amphisbaena schmidti*, a third species of the genus from Puerto Rico (Amphisbaenia: Reptilia). Breviora 198: 1–11.
- Gans C (1964b) On the South American species of Amphisbaena with a vertically keeled tail (Amphisbaenia: Reptilia). Notes on amphisbaenids. 15. Senckenbergiana biologia (Mertens Festschrift) 45(3/5): 387–416.
- Gans C (1965a) Notes on amphisbaenids. 17. Redescription and discussion of *Amphisbaena angustifrons* Cope and *A. camura* Cope, large amphisbaenids of southern South America (Amphisbaenia: Reptilia). American Museum Novitates (2225): 1–32.
- Gans C (1965b) Notes on amphisbaenids (Amphisbaena: Reptilia). 16. On *Amphisbaena heathi* Schmidt and *A. carvalhoi* new species, small forms from the northeast of Brazil (Amphisbaenia: Reptilia). Proceedings of the California Academy of Science 31(23): 613–630.
- Gans C (1966) Studies on amphisbaenids (Amphisbaenia: Reptilia) 3. The small species from southern South America commonly identified as *Amphisbaena darwini*. Bulletin of the American Museum of Natural History 134(3): 185–260.
- Gans C (1967) Redescription of *Amphisbaena slateri* Boulenger, with comments on its range extension into Bolivia. Herpetologica 23(3): 223–227.
- Gans C, Alexander AA (1962) Studies on the amphisbaenids (Amphisbaenia; Reptilia).
 2. On the amphisbaenids of the Antilles. Bulletin of the Museum of Comparative Zoology 128(3): 65–158.
- Gans C, Amdur M (1966) Redescription of *Amphisbaena vermicularis* Wagler, with comments on its range and synonymy (Amphisbaenia: Reptilia). Notes on amphisbae-

nids 20. Proceedings of the California Academy of Science 33(5): 69–90. https://doi. org/10.2307/1441079

- Gans C, Diefenbach COC (1972) Description and geographical variation in the South American *Amphisbaena angustifrons*; the southernmost amphisbaenian in the world (Reptilia: Amphisbaenia). American Museum Novitates 2494: 1–20.
- Gans C, Mather S (1977) Amphisbaena medemi, an interesting new species from Colombia (Amphisbaenia, Reptilia), with a key to the amphisbaenians of the Americas.
 Fieldiana (Zoology) 72(2): 21–46. https://doi.org/10.5962/bhl.title.5131
- Gans C, Rhodes C (1964) Notes on amphisbaenids (Amphisbaenia: Reptilia). 13. A systematic review of *Anops* Bell, 1833. American Museum Novitates 2186: 1–25.
- Gil-Jiménez E, Lucas M, Ferrer M (2021) Metalliferous mining pollution and its impact on terrestrial and semi-terrestrial vertebrates: a review. In: Voogt P (Ed.) Reviews of environmental contamination and toxicology 256: 1–69. Springer. https://doi. org/10.1007/398_2021_65
- González-Sponga MA, Gans C (1971) *Amphisbaena gracilis* Strauch. Rediscovered (Amphisbaenia: Reptilia). Copeia 1971(4): 589–595. https://doi.org/10.2307/1442627
- Graboski R., Grazziotin FG, Mott T, Rodrigues MT (2022) The phylogenetic position of Ridley's worm lizard reveals the complex biogeographic history of New World insular amphisbaenids. Molecular Phylogenetics and Evolution 173: 107518. https://doi. org/10.1016/j.ympev.2022.107518
- Graboski R, Arredondo JC, Grazziotin FG, Guerra-Fuentes RA, Da Silva AAA, Prudente ALC, Pinto RR, Rodrigues MT, Bonatto SL, Zaher H (2023) Revealing the cryptic diversity of the widespread and poorly known South American blind snake genus *Amerotyphlops* (Typhlopidae: Scolecophidia) through integrative taxonomy. Zoological Journal of the Linnean Society 197: 719–751. https://doi.org/10.1093/zoolinnean/zlac059
- Grillitsch B, Schiesari L (2010) The ecotoxicology of metals in reptiles In: Sparling DW, Linder G, Bisho CA, Krest S (Eds) Ecotoxicology of amphibians and Reptiles, 2nd edn.: 337–448. CRC Press, New York, 944 pp. https://doi.org/10.1201/EBK1420064162-c12
- Guedes TB, Entiauspe-Neto OM, Costa HC (2023) Lista de répteis do Brasil: atualização de 2022. Herpetologia Brasileira 12(1): 56–161. https://doi.org/10.5281/zenodo.7829013
- Hoogmoed MS (1989) A new species of Amphisbaena (Amphisbaenia: Amphisbaenidae) from Suriname. Notes on the herpetofauna of Surinam XI in honour of Dr. Pieter Wagenaar Hummelinck. Foundation for Scientific Research in Surinam and the Netherlands Antilles (Amsterdam) 123: 65–73.
- Hoogmoed MS, Ávila-Pires TCS (1991) A new species of small *Amphisbaena* (Reptilia: Amphisbaenia: Amphisbaenidae) from western Amazonian Brazil. Boletim do Museu Paraense Emilio Goeldi Série Zoologia 7(1): 77–94.
- IBGE (2023) Banco de Informações Ambientais. https://www.ibge.gov.br/geociencias/ informacoes-ambientais/geologia/23382-banco-de-informacoes-ambientais.html [accessed 18 November 2023]
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kearney M, Stuart BL (2004) Repeated evolution of limblessness and digging heads in worm lizards revealed by DNA from old bones. Proceedings of the Royal Society of London. Series B: Biological Sciences 271(1549): 1677–1683. https://doi. org/10.1098/rspb.2004.2771
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2016) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and

morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772–773. https://doi.org/10.1093/molbev/msw260

- Longrich NR, Vinther J, Pyron RA, Pisani D, Gauthier JA (2015) Biogeography of worm lizards (Amphisbaenia) driven by end-Cretaceous mass extinction. Proceedings of the Royal Society B: Biological Sciences 282(1806): 21396–21401. https://doi. org/10.1098/rspb.2014.3034
- Montero R (1996) *Amphisbaena bolivica* Mertens 1929, nueva combinación (Squamata: Amphisbaenia). Cuadernos de Herpetología 9(2): 75–84.
- Montero R, Sáfadez IF, González Álvarez L (1997) On a new species of Amphisbaena (Reptilia: Amphisbaenidae) from Bolivia. Journal of Herpetology 31(2): 218–220. https://doi.org/10.2307/1565389
- Mott T, Vieites DR (2009) Molecular phylogenetics reveals extreme morphological homoplasy in Brazilian worm lizards challenging current taxonomy. Molecular Phylogenetics and Evolution 51(2): 190–200. https://doi.org/10.1016/j.ympev.2009.01.014
- Mott T, Rodrigues MT, Freitas MA, Silva TFS (2008) New species of *Amphisbaena* with a non-autotomic and dorsally tuberculate blunt tail from State of Bahia, Brazil (Squamata, Amphisbaenidae). Journal of Herpetology 42(1): 172–175. https://doi. org/10.1670/07-074R2.1
- Mott T, Rodrigues MT, Santos EM (2009) A new *Amphisbaena* with chevron-shaped anterior body annuli from state of Pernambuco: Brazil (Squamata: Amphisbaenidae). Zootaxa 2165(1): 52–58. https://doi.org/10.11646/zootaxa.2165.1.4
- Mott T, Carvalho-Neto CS, Carvalho-Filho KS (2011) *Amphisbaena miringoera* Vanzolini, 1971 (Squamata: Amphisbaenidae): New state record. Check List 7(5): 594–595. https://doi.org/10.15560/7.5.594
- Oliveira ECS, Vaz-Silva W, Santos-Jr AP, Graboski R, Teixeira Jr M, Dal Vechio F, Ribeiro S (2018) A new four-pored *Amphisbaena* Linnaeus, 1758 (Amphisbaenia, Amphisbaenidae) from Brazilian Amazon. Zootaxa 4420(4): 451–474. https://doi.org/10.11646/ zootaxa.4420.4.1
- Perez R, Borges-Martins M (2019) Integrative taxonomy of small worm lizards from Southern South America, with description of three new species (Amphisbaenia: Amphisbaenidae). Zoologischer Anzeiger 283(58): 124–141. https://doi.org/10.1016/j. jcz.2019.09.007
- Perez R, Ribeiro S, Borges-Martins M (2012) Reappraisal of the taxonomic status of *Amphisbaena prunicolor* (Cope 1885) and *Amphisbaena albocingulata* Boettger 1885 (Amphisbaenia: Amphisbaenidae). Zootaxa 3550(1): 1–25. https://doi.org/10.11646/ zootaxa.3550.1.1
- Ribeiro S, Eleutério JMP (2023) Anfisbênios dos Brasil. In: Silva-Soares T, Monjardim M, Figueiredo RG (Eds) Herpetologia para todos. Instituto Biodiversidade Neotropical, Espírito Santo, 93–105.
- Ribeiro S, Vaz-Silva W, Santos-Jr AP (2008) New pored *Leposternon* (Squamata, Amphisbaenia) from Brazilian Cerrado. Zootaxa 1930(1): 18–38. https://doi.org/10.11646/ zootaxa.1930.1.2
- Ribeiro S, Castro-Mello C, Nogueira C. (2009) New species of *Anops* Bell, 1893, (Squamata, Amphisbaenia). Journal of Herpetology 43(1): 21–28. https://doi.org/10.1670/07-299R1.1
- Ribeiro S, Nogueira C, Cintra CE, Silva Jr NJ, Zaher H (2011) Description of a new pored*Leposternon* (Squamata, Amphisbaenidae) from the Brazilian Cerrado. South American Journal of Herpetology 6(3): 177–188. https://doi.org/10.2994/057.006.0303

- Ribeiro S, Santos AP, Zaher H (2015) A new species of *Leposternon* Wagler, 1824 (Squamata, Amphisbaenia) from northeastern Argentina. Zootaxa 4034(2): 309–324. https://doi.org/10.11646/zootaxa.4034.2.4
- Ribeiro S, Gomes JO, Silva HLR, Cintra CED, Silva Jr N (2016) A new two-pored species of *Amphisbaena* (Squamata, Amphisbaenidae) from the Brazilian Cerrado, with a key to the two-pored species of *Amphisbaena*. Zootaxa 4147(2): 124–142. https://doi. org/10.11646/zootaxa.4147.2.2
- Ribeiro S, Silveira A, Santos-Jr AP (2018a) A new species of *Leposternon* (Squamata: Amphisbaenidae) from Brazilian Cerrado with a key to pored species. Journal of Herpetology 52(1): 50–58. https://doi.org/10.1670/16-125
- Ribeiro LB, Gomides SC, Costa HC (2018b) A new species of *Amphisbaena* from northeastern Brazil (Squamata: Amphisbaenidae). Journal of Herpetology 52(2): 234–241. http://doi/full/https://doi.org/10.1670/17-028
- Ribeiro S, Sá V, Santos-Jr AP, Graboski R, Zaher H, Guedes AG, Andrade SP, Vaz-Silva W (2019) A new species of the *Amphisbaena* (Squamata, Amphisbaenidae) from the Brazilian Cerrado with a key for the two-pored species. Zootaxa 4550(3): 301–320. https://doi.org/10.11646/zootaxa.4550.3.1
- Ribeiro LB, Gomides SC, Costa HC (2020) A new worm lizard species (Squamata: Amphisbaenidae: *Amphisbaena*) with non-autotomic tail, from Northeastern Brazil. Journal of Herpetology 54(1): 9–18. https://doi.org/10.1670/19-043
- Ribeiro-Júnior MA, Ribeiro S, Cintra CED, Gomes JO (2022) Amphisbaena ibijara Rodrigues, Andrade & Lima, 2003 is a junior synonym of Amphisbaena frontalis Vanzolini, 1991 (Squamata, Amphisbaenia). Journal of Herpetology 56(2): 234–240. https:// doi.org/10.1670/21-039
- Roberto IJ, Brito LBM, Ávila RW (2014) A new six-pored *Amphisbaena* (Squamata: Amphisbaenidae) from the coastal zone of northeast Brazil. Zootaxa 3753(2): 167–176. https://doi.org/10.11646/zootaxa.3753.2.6
- Rocha CFD, Barros-Filho JD, Sluys MV (2023) A new *Amphisbaena* species from a restinga habitat of Southeastern Brazil (Reptilia, Squamata, Amphisbaenidae). International Journal of Zoology and Animal Biology 6(4): 1–8. https://doi.org/10.23880/izab-16000501
- Rodrigues MT, Andrade GV, Lima JD (2003) A new species of *Amphisbaena* (Squamata, Amphisbaenidae) from state of Maranhão, Brazil. Phyllomedusa 2(1): 21–26. https://doi.org/10.11606/issn.2316-9079.v2i1p21-26
- Sindaco R, Panagiotis K, Roberto S, Petros L (2014) Taxonomic reassessment of *Blanus strauchi* (Bedriaga, 1884) (Squamata: Amphisbaenia: Blanidae), with the description of a new species from south-east Anatolia (Turkey). Zootaxa 3795(3): 311–326. https://doi.org/10.11646/zootaxa.3795.3.6
- Spix JB, Martius KFP (1938) Através da Bahia: Excerptos da obra Reise in Brasilien. Transladados a português pelos Drs. Pirajá da Silva e Paulo Wolf. Compainha Editora Nacional, São Paulo, 342 pp.
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/ bioinformatics/btu033
- Strüssmann C, Carvalho MA (2001) Two new species of *Cercolophia* Vanzolini, 1992 from the state of Mato Grosso, western Brazil (Reptilia, Amphisbaenia, Amphisbaenidae). Bollettino del Museo Regionale di Scienze Naturali di Torino 18(2): 487–505.

- Swofford DL (2003) PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Sunderland, Massachusetts: Sinauer Associates. https://paup.phylosolutions. com [accessed 08 December 2023]
- Teixeira Jr M, Dal Vechio F, Neto AM, Rodrigues MT (2014) A new two-pored Amphisbaena Linnaeus, 1758, from Western Amazonia, Brazil (Amphisbaenia: Reptilia). South American Journal of Herpetology 9(1): 62–74. https://doi.org/10.2994/SA-JH-D-14-00004.1
- Teixeira Jr M, Dal Vechio F, Rodrigues MT (2016) Diagnostic clarification, new morphological data and phylogenetic placement of *Amphisbaena arenaria* Vanzolini, 1991 (Amphisbaenia, Amphisbaenidae). Zootaxa 4205(3): 293–296. https://doi. org/10.11646/zootaxa.4205.3.9
- Teixeira Junior M, Dal Vechio F, Recoder R, Cassimiro J, Sena MA, Rodrigues MT (2019) Two new highland species of *Amphisbaena* Linnaeus, 1758 (Amphisbaenia, Amphisbaenidae) from Bahia State, Brazil. South American Journal of Herpetology 14(3): 213–232. https://doi.org/10.2994/SAJH-D-17-00097.1
- Thomas R (1965) Two new subspecies of *Amphisbaena* (Amphisbaenia, Reptilia) from the Barahona Peninsula of Hispaniola. Breviora 215: 1–14.
- Thomas R (1966) Additional notes on the amphisbaenids of Greater Puerto Rico. Breviora 249: 1–23.
- Thomas R, Hedges SB (1998) A new amphisbaenian from Cuba. Journal of Herpetology 32(1): 92–96. https://doi.org/10.2307/1565485
- Thomas R, Hedges SB (2006) Two new species of *Amphisbaena* (Reptilia: Squamata: Amphisbaenidae) from the Tiburon Peninsula of Haiti. Caribbean Journal of Science 42(2): 208–219.
- Torres-Ramírez JJ, Angarita-Sierra T, Vargas-Ramírez M (2021) A new species of *Amphisbaena* (Squamata: Amphisbaenidae) from the Orinoquian region of Colombia. Vertebrate Zoology 71(274): 55–74. https://doi.org/10.3897/vz.71.e59461
- Vanzolini PE (1950) Contribuições ao conhecimento dos largartos brasileiros da família Amphisbaenidae Gray, 1825. I. Sobre uma nova subespécie insular de *Amphisbaena darwini* D. and B., 1839. Papéis Avulsos de Zoologia 9(6): 69–78. https://doi. org/10.11606/0031-1049.1950.9p69-78
- Vanzolini PE (1991) Two further new species of *Amphisbaena* from the semi-arid northeast of Brasil (Reptilia, Amphisbaenia). Papéis Avulsos de Zoologia 37(23): 347–361. https://doi.org/10.11606/0031-1049.1990.37.p347-361
- Vanzolini PE (1992) *Cercolophia*, a new genus for the species of *Amphisbaena* with a terminal vertical keel on the tail. Papéis Avulsos de Zoologia 37(27): 401–412. https:// doi.org/10.11606/0031-1049.1990.37.p401-412
- Vanzolini PE (1995) A new species of *Amphisbaena* from the state of Mato Grosso, Brazil (Squamata, Amphisbaenidae). Papéis Avulsos de Zoologia 3(10): 217–221. https://doi.org/10.11606/0031-1049.1994.39.p217-221
- Vanzolini PE (1996) On slender species of *Amphisbaena*, with the description of a new one from northeastern Brasil (Squamata, Amphisbaenia). Papéis Avulsos de Zoologia 39(16): 293–305. https://doi.org/10.11606/0031-1049.1994.39.p293-305
- Vanzolini PE (2002) An aid to the identification of the South American species of *Amphisbaena* (Squamata, Amphisbaenidae). Papéis Avulsos de Zoologia 42(15): 351–362. https://doi.org/10.1590/S0031-10492002001500001
- Vaz-Silva W (2009) Herpetofauna im Zentralbrasilianischen Cerrado. Terraria (Munster) 17: 75–78.

Supplementary material 1

Supplementary information

Authors: Síria Ribeiro, Alfredo P. Santos Jr, Isabelly G. Martins, Elaine C. S. Oliveira, Roberta Graboski, Thiago Barbosa Da Silveira, Matheus H. M. Benício, Wilian Vaz-Silva Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1213.122265.suppl1



Short Communication

First record of *Buninotus* Maldonado Capriles and *B. palikur* Castro-Huertas, Forero & Melo from Brazil, with taxonomic notes (Hemiptera, Reduviidae, Emesinae) and an updated key to the genera of Saicini of the New World

Hélcio R. Gil-Santana¹⁰, Jader Oliveira^{2,30}

1 Laboratório de Diptera, Instituto Oswaldo Cruz, Av. Brasil, 4365, 21040-360, Rio de Janeiro, RJ, Brazil

Abstract

2 Universidade de São Paulo, Faculdade de Saúde Pública, Laboratório de Entomologia em Saúde Pública, São Paulo, SP, Brazil

3 Programa de Pós-Graduação em Ciência, Inovação e Tecnologia para a Amazônia, Universidade Federal do Acre, Rio Branco, AC, Brazil

Corresponding author: Hélcio R. Gil-Santana (helciogil@uol.com.br, helciogil@ioc.fiocruz.br)



Academic editor: Nikolay Simov Received: 10 January 2024 Accepted: 6 March 2024 Published: 25 September 2024

ZooBank: https://zoobank.org/ D3D209CA-4E2C-461D-8AA9-91676CA81D12

Citation: Gil-Santana HR, Oliveira J (2024) First record of *Buninotus* Maldonado Capriles and *B. palikur* Castro-Huertas, Forero & Melo from Brazil, with taxonomic notes (Hemiptera, Reduviidae, Emesinae) and an updated key to the genera of Saicini of the New World. ZooKeys 1213: 29–39. https://doi.org/10.3897/ zookeys.1213.118594

Copyright: ©

Hélcio R. Gil-Santana & Jader Oliveira. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Buninotus Maldonado Capriles, 1981 and *Buninotus palikur* Castro-Huertas, Forero & Melo, 2022 (Hemiptera, Reduviidae, Emesinae, Saicini) are recorded from Brazil for the first time. Taxonomic notes on *Buninotus* and its species are provided mainly based on the examination and photographs of the holotype and paratype of *Buninotus niger* Maldonado Capriles, 1981. Previous doubts on some characteristics of the genus are clarified. A hypothesis suggesting that the holotype and paratype of *B. niger* may belong to different species is presented. An updated key to the New World genera of Saicini is provided.

Key words: Heteroptera, Neotropics, Oncerotrachelus, Saicinae

Introduction

Reduviidae is one of the largest and most diverse family of predaceous Heteroptera, comprising approximately 7,000 species distributed across about 20 subfamilies worldwide (Gil-Santana et al. 2015; Schuh and Weirauch 2020; Standring et al. 2023). A new classification to the subfamily Emesinae and some closely related subfamilies was proposed by Standring et al. (2023), which resulted in Saicinae and Visayanocorinae (the latter not occuring in the New World) being considered as tribes of Emesinae. Additionally, the former emesine tribes Ploiariolini and Metapterini were treated as junior synonyms of Emesini, resulting in Emesinae sensu nov. having six tribes: Collartidini, Emesini, Leistarchini, Oncerotrachelini (as a new tribe), Saicini, and Visayanocorini.

Therefore, there are currently 10 genera of Saicini in the New World, three of which are currently monotypic (Gil-Santana et al. 2015; Castro-Huertas et al. 2023). Gil-Santana et al. (2015) provided a summary of the taxonomy of this group. Several keys to New World genera of Saicinae have been presented in

the last four decades (e.g. Maldonado Capriles 1981; Blinn 1990; Melo and Coscarón 2005; Gil-Santana et al. 2006; Weirauch and Forero 2007a; Gil-Santana and Costa 2009; Gil-Santana et al. 2015, 2020). However, due to changes in the group, now considered as a tribe, including the exclusion of the genus *Oncerotrachelus* Stål, 1868 and new information on the genera *Buninotus* Maldonado Capriles, 1981 and *Caprilesia* Gil-Santana, Marques & Costa, 2006 (Castro-Huertas et al. 2023; this work), all these keys have become outdated.

Little is known about the biology and natural history of Saicinae (Gil-Santana et al. 2010), and summaries or new data on this subject, have been provided by Gil-Santana et al. (2010), as well as Gil-Santana et al. (2015, 2020), Schuh and Weirauch (2020), and Castro-Huertas et al. (2023).

In addition to documenting the first records of *Buninotus* and *B. palikur* Castro-Huertas, Forero & Melo, 2022 from Brazil, the holotype and paratype females of *B. niger* Maldonado Capriles, 1981 were directly examined and photographed to record and clarify important characteristics and address any uncertainties.

An improved and updated key to the genera of New World genera of Saicini is presented.

Materials and methods

The female holotype and paratype of *Buninotus niger* (Figs 1–15), currently deposited in the National Museum of Natural History (**NMNH**), Smithsonian Institution, Washington, DC, USA, were directly examined and photographed (Figs 1–3, 5–15) by the second author. The photograph of Fig. 4 was taken and kindly provided by Thomas Henry. Photographs were taken using a Leica DFC450 digital camera mounted on a Leica M205 C stereomicroscope. Composite images were assembled using the Leica Application Suite v. 4.5 and the Helicon Focus v. 6.2.2 software packages.

The female of *Buninotus palikur* (Figs 16–21) from Brazil was examined and imaged by the first author. Observations were made using a stereomicroscope (Zeiss Stemi). Photographs were taken using digital cameras (Nikon D5600 with a AF-S Micro Nikkor 105 mm f/2.8G IF-ED lens and Sony DSC-W830). This specimen will be deposited in the Entomological Collection of the Museu Nacional da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (**MNRJ**).

General morphological terminology primarily follows Schuh and Weirauch (2020) and Castro-Huertas et al. (2023). The latter authors introduced a new terminology, designating cuticular processes on the ventral surface of the head and labium as "spiniform setae" and "strong setae" instead of "spines," as used by other authors. Setae without thickening were referred to as "simple setae" or simply "setae." They argued that this revised terminology would facilitate the coding of homologous characters for future phylogenetic analyses. Therefore, for the sake of uniformity, we will adhere to this approach in the present work. The visible segments of the labium are numbered as II–IV, considering that the first segment is either lost or fused to the head capsule in Reduviidae (Weirauch 2008).

When describing label data, a slash (/) separates lines, and a double slash (//) indicates different labels. Comments or translations to English of the label data are provided in square brackets ([]).

Results

Subfamily Emesinae Tribe Saicini

Buninotus Maldonado Capriles, 1981

Remarks. In 1981, Maldonado Capriles described *Buninotus* as a monotypic genus, designating *B. niger* as its type species. He outlined the following as the main characteristics of the genus: the body is mostly black, shiny, and predominantly glabrous; a subglobose posterior lobe on the head; only the first [visible] segment of the labium is spined; scutellum with a long inclined spine; mesoscutum with a short, broad, spinelike elevation. The fore coxa, femur, and tibia are spined, with the tibia curved. The forewing exhibits four closed cells.

Some characteristics recorded in Maldonado Capriles's (1981: 404, 406) description of the genus being a female, deserves to be mentioned, such as: the "tylus" [clypeus] as "ending in a sharp spine that slightly surpasses [the] apex of [the] jugae. ... Legs: forecoxa with a strong "s-spine near base on anterior side, 3 strong s-spines on rear of inner face; trochanter with four s-spines along inner-lower surface, femur ... with 5 s-spines along upper surface ...; lower inner surface with 5 s-spines of nearly equal size ...; tibia strongly curved on lateral aspect ..., four long s-spines on inner side, the basal the shortest ..."

Castro-Huertas et al. (2023) stated that *Buninotus* could be characterized by the first and second visible labial segments with a pair of spiniform setae and strong setae, respectively; the anterior lobe of pronotum with four protuberances ["humps"], a pair on each anterior and posterior region. The humeral angles project into long spines; scutellum has a long and inclined process. The forelegs exhibit coxae, femora and tibiae with long spiniform setae; protibiae are curved. The meso and metafemora each have a pair of apically located spiniform setae. Additionally, the forewing is characterized by three closed cells.

Comments. Although the venation of the wings is considered excellent for taxonomic characteristics at the generic and tribal levels in Emesinae (Wygodzinsky 1966) and has been extensively used to diagnose and/or separate supra-specific taxa, potential intra-specific variation might happen (Gil-Santana and Marques 2005) such as in the intra-specific variation in the number of cells in the forewing of *Mayemesa lapinhaensis* (Wygodzinsky, 1950) (Emesini) (Gil-Santana et al. 1999).

In regard to the number of closed cells in the forewing, there is a discrepancy between Castro-Huertas et al. (2023) statement, noting three closed cells, and the description and figure by Maldonado Capriles (1981), which indicate four closed cells. Other authors, such as Gil-Santana et al. (2020), have adhered to the original description in their key for Saicinae genera. Castro-Huertas et al. (2023: 50) justified their observation by stating that they "examined an image of the holotype of *B. niger*, and it is very difficult to see the forewing vein structure without removing the forewings from the body because of the semi-hyaline to brown coloration. Using additional specimens of both *B. niger* and *B. palikur*," they removed the forewings and found three closed cells in both species. However, it is possible that, due to the deep blackish coloration of the holotype of *B. niger*, the specimens examined by them as such may belong to a different species with a brownish general coloration (see below).

Examination of both the holotype and paratype of *B. niger* (Figs 6, 7, 14) confirms that the veins near the base of the forewing are not united, thereby not forming a closed basal cell. Consequently, the forewing has only three closed cells in all specimens of *Buninotus* examined, supporting the observations of Castro-Huertas et al. (2023).

A striking characteristic of *Buninotus* described and illustrated by Maldonado Capriles (1981: fig. 7), but in need of confirmation, is a spine on the apex of the clypeus ("tylus"). This feature was not mentioned or questioned by Castro-Huertas et al. (2023) when discussing the characteristics of the genus. According to our observations, this spine is completely absent in both the holotype and paratype (Figs 2–4, 11). It is possible that the whitish apical portion of the labrum of the holotype of *B. niger*, which projects slightly forward (Figs 2–4), may have caused confusion for Maldonado Capriles when describing the specimen. Clearly, it is definitive that there is no apical sharp spine on the clypeus, as described by Maldonado Capriles (1981).

The presence of a pair of spiniform setae and strong setae on first and second visible labial segments, respectively, is confirmed in the holotype of *B. niger* (Fig. 3) and on a specimen of *B. palikur* from Brazil examined here (Fig. 17). In the paratype of *B. niger* the setae of the second visible labial segment is not visible (Fig. 11); they may have broken off, but it was not possible ascertain if the insertion hole of these setae are present or not because the head is covered by hyphae of mold (Figs 10–12).

Some portions of the type specimens and the specimen from Brazil are broken or missing, mainly on the legs, but, taking into account the remaining portions and the previous descriptions (Maldonado Capriles 1981; Castro-Huertas et al. 2023) and the examination of these specimens, the number and location of spiniform/strong setae of fore legs could be added to characterization of the genus in more detail as follows: fore coxae with a dorsobasal and three ventral spiniform setae; fore trochanters with four spiniform setae, midventrally; fore femora with two rows of five spiniform setae, one row on anterodorsal portion and other on anteroventral region; fore tibiae with four anterodorsal spiniform setae, the most basal being shorter than the following ones.

Distribution. Brazil (new record), French Guiana and Panama.

Buninotus niger Maldonado Capriles, 1981 Figs 1–15

Type material examined. *Buninotus niger. Female holotype*: [printed label:] Panama-Chiriqui / Fortuna, 1050 m. / 8 44'N;82 15'W, / [handwritten:] ?2 [printed:] - [handwritten:] July [printed:] -197 [handwritten:] 7 / Henk Wolda // [handwritten label:] *Buninotus / niger* [in red:] TYPE / 1978 Maldonado // [printed red label:] HOLOTYPE // [printed label:] [at left side:] USNM_ENT [at right side:] QR CODE / UCR_ENT 00008023; *Female paratype*: [printed label:] Panama-Chiriqui / Fortuna, 1050 m. / 8 44'N;82 15'W, / [handwritten:] 10 [printed:] - [handwritten:] Nov [printed:] -197 [handwritten:] 6 / Henk Wolda // [handwritten:] *Buninotus / niger* / Maldonado / [printed:] det. J. Maldonado C. [handwritten:] 81 // [printed label:] Drake Colln ex / J. Maldonado C. / Coll. 1996 // [printed red faded label:] PARA-TYPE // [printed label:] USNMENT / QR CODE / 01918179 (NMNH).



Figures 1–5. *Buninotus niger* Maldonado Capriles, 1981, female holotype deposited in NMNH 1, 2 dorsal view 3–5 lateral view 2, 3 head 4 head, anterior portion of prothorax and fore coxa 5 upper portion of hind lobe of pronotum and mesoand metathorax. Scale bars: 2.0 mm (1); 0.5 mm (2–5).

Maldonado Capriles (1981) described *B. niger* based on two females from Panama (Figs 1–7, 9–14). It seems like the definition of the species coloration was primarily based on the holotype, which is deep blackish and aligns with other details recorded by Maldonado Capriles (1981) (Figs 1–7). In contrast, the paratype exhibits a general pale-brownish coloration that does not match the aforementioned description (Figs 9–14).



Figures 6–8. Buninotus niger Maldonado Capriles, 1981, female holotype deposited in NMNH 6, 7 forewings, dorsal view 6 basal two thirds 7 basal third 8 labels. Scale bars: 0.5 mm (6, 7).

Castro-Huertas (2023) examined four females of *Buninotus* from Panama, also identified them as *B. niger*, and noted a dark-brownish general coloration.

Comments. The holotype of *B. niger* exhibits a characteristic not observed in any other specimen of *Buninotus* so far: a deep blackish, piceous general coloration (Figs 1–6). In contrast, all other specimens of *Buninotus* display a general brownish coloration (Castro-Huertas et al. 2023; Figs 16–20), including the paratype of *B. niger* (Figs 9, 13). Additionally, the spiniform setae of the femur [only the left one was present when examined] are longer (Fig. 1) than those of the paratype (Fig. 9). Therefore, it is hypothesized here that the paratype of *B. niger* belongs to a different species than that of the holotype. In this case, the specimens identified by Castro-Huertas et al. (2023) as *B. niger* probably belong to this undescribed species. Finding more specimens, preferably including males, may help in confirming or disproving the hypothesis made here.

Distribution. Panama.

Buninotus palikur Castro-Huertas, Forero & Melo, 2022 Figs 16–21

Material examined. BRAZIL, Espírito Santo: Linhares, Reserva Natural Vale [Vale Natural Reserve] (RNV), 19°09'S, 40°04'W, José Simplício dos Santos leg., 25.i.2007, 1 female (MNRJ).

Comments. Buninotus palikur was described based on six females from French Guiana (Castro-Huertas et al. 2023). The female examined here (Figs 16–21), although has lost its antennae and hind legs (Fig. 16), corresponds well with the description and diagnosis of *B. palikur* provided by Castro-Huertas et al. (2023). Its general length measured 10.70 mm to the tip of the membrane; the protuberances of the fore lobe are slightly triangular (Figs 16, 18), and the forewings are brownish with pale spots (Figs 16, 20). Diverging from some characteristics recorded in the specimens described by Castro-Huertas et al.



Figures 9–15. Buninotus niger Maldonado Capriles, 1981, female paratype deposited in NMNH 9, 10 dorsal view 10, 11 head 11, 12 lateral view 11 except upper surface 12 head and anterior portion of thorax 13, 14 dorsal view 13 pronotum 14 basal half of forewings 15 labels. Scale bars: 2.0 mm (9); 1.0 mm (14) 0.5 mm (12); 0.2 mm (10, 11, 13).

(2023), the prothorax is almost uniform brownish without paler portions, the scutellar spine is curved at approximately its middle portion on lateral view (Figs 16, 18, 19), and the first spiniform setae of fore tibiae are longer (Fig. 21). However, we consider these differences as more probably intraspecific differences. If merely interindividual or geographical variations, only future examination of more specimens would allow to clarify their significance.

Distribution. Brazil (new record) and French Guiana.



Figures 16–21. *Buninotus palikur* Castro-Huertas, Forero & Melo, 2022, female specimen from Brazil 16 dorsal view 17 anterior portion of head, prothorax and some portions of the legs, lateroventral view 18, 19 lateral view 18 anterior portion of prothorax 19 spine of mesoscutum and scutellum 20, 21 dorsal view 20 hind lobe of pronotum and forewings 21 left fore tibia. Scale bars: 3.0 mm (16); 1.0 mm (20) 0.5 mm (17, 19, 21); 0.2 mm (18).

Discussion. There is a need to collect more specimens, including males of *Buninotus*, for a better understanding of the genus and its species, possibly allowing a more comprehensive study of the systematics of Saicini in general. Finding a specimen of *B. palikur* in a natural reserve in Atlantic Forest in Brazil expands the distribution of the species and the genus *Buninotus* to a broader range of biogreographical regions.

Key to the New World genera of Saicini, based on Weirauch and Forero (2007a, 2007b), Gil-Santana and Costa (2009), Gil-Santana et al. (2015, 2020), and Castro-Huertas et al. (2023)

- 1 Foreleg without spiniform setae, at most with erect setae2

- Opposed surfaces of labium and head without spiniform setae or bristles; forewing with four cells; metapleura with a tubercle near coxal cavity......

...... Saicireta Melo & Coscarón, 2005

- Process on the lower anterior angle of the prothorax subconical; second antennal segment about 3/4 as long as the first antennal segment; medial process of male pygophore a single, erect barbless spine; posterior
| | margin of seventh abdominal sternite in females sloping ventrocephalad |
|---|---|
| | Pseudosaica Blinn, 1990 |
| 4 | Humeral angles of pronotum without processes, rounded5 |
| _ | Humeral angles of pronotum with spinelike processes6 |
| 5 | Ventral portion of the head below (between) the eyes spineless; fore tibiae |
| | with a three or four (T. femorata) stronger, spiniform setae implanted on |
| | anterodorsal portion |
| - | Head with a ventral pair of spiniform setae below (between) the eyes; fore |
| | tibiae with a single or double longitudinal row of numerous short spini-
form setae on median portion of inner surface |
| | |
| 6 | Fore coxae and anterior pronotal lobe unarmed |
| | Bagriella McAtee & Malloch, 1923 |
| _ | Fore coxae spined, anterior pronotal lobe with four spines or rounded |
| | humps |
| 7 | Fore lobe of pronotum with four spinesParatagalis Monte, 1943 |
| - | Fore lobe of pronotum with four rounded protuberances |
| 8 | Fore tibiae with a row of very short spiniform setae directed mediad; only apterous females known Kiskeyana Weirauch & Forero 2007 |
| _ | Fore tibiae with three to six more or less large spiniform setae on antero- |
| | dorsal surface directed anteriad: all known females macronterous |
| q | Ventral surface of labium: first visible segment with a pair of spiniform |
| 2 | setae second segment with a pair of strong setae: third segment without |
| | setae. Forewings with three closed cells Buninotus Maldonado , 1981 |
| _ | Ventral surface of labium ⁻ first and second visible labial segments with |
| | a pair of spiniform setae, third segment with a pair of strong setae. Fore- |
| | wings with two closed cells |
| | Caprilesia Gil-Santana, Margues & Costa, 2006 |
| | |

Recently Castro-Huertas and Melo (2023) have recovered *Saicireta correntina* Melo & Coscarón, 2005 as sister species of some groups, including the clade Saicinae *sensu stricto*. However, they did not formally rule out *Saicireta* from Saicinae (Saicini), and neither did they include it in another taxonomic group. Therefore, because it has remained in Saicini, it was included in the key to this group.

Acknowledgements

The authors are grateful to Talitta Guimarães Simões (NMNH) for the support and providing the equipment to photograph the holotype and paratype of *Buninotus niger* (Figs 1–3, 5–15), to Thomas Henry (NMNH) for granting access to this specimen and kindly providing its photograph of Figure 4, and to Dr Brian Bourke for reviewing the English language. The first author is grateful to David dos Santos Martins, researcher of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER), Vitória, Espírito Santo, Brazil for donating the specimen of *Buninotus palikur* from RNV. The second author expresses gratitude to FAPESP for the financial support provided during the postdoctoral research. We are also grateful to Dimitri Forero, Pierre Moulet, and Nikolay Simov for their valuable comments and suggestions.

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

Conceptualization: HRGS. Data curation: HRGS. Formal analysis: HRGS. Investigation: HRGS, JO. Methodology: HRGS, JO. Project administration: HRGS. Software: HRGS, JO. Writing - original draft: HRGS. Writing - review and editing: JO, HRGS.

Author ORCIDs

Hélcio R. Gil-Santana ID https://orcid.org/0000-0002-0544-348X Jader Oliveira ID https://orcid.org/0000-0002-2588-1911

Data availability

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

References

- Blinn RL (1990) Pseudosaica panamaensis, a new genus and species of Assassin bug from Panama (Heteroptera: Reduviidae: Saicinae). Journal of the New York Entomological Society 98(3): 347–351.
- Castro-Huertas V, Melo MC (2023) Outside the pattern: Evolution of the genital asymmetry in Saicinae (Hemiptera: Heteroptera: Reduviidae). Journal of Morphology 284(8): e21610. https://doi.org/10.1002/jmor.21610
- Castro-Huertas V, Forero D, Melo MC (2023) New Neotropical Saicinae: new species of *Buninotus* Maldonado Capriles, *Caprilesia* Gil-Santana, Marques & Costa, and *Pseudosaica* Blinn (Hemiptera: Reduviidae). Annales de la Société entomologique de France (N.S.) 59: 45–64. https://doi.org/10.1080/00379271.2022.2147864 [Published online on 20 December 2022]
- Gil-Santana HR, Costa LAA (2009) A new species of *Paratagalis* Monte from Brazil with taxonomical notes and a key to New World genera of Saicinae (Hemiptera: Heteroptera: Reduviidae: Saicinae). Zootaxa 2197(1): 20–30. https://doi.org/10.11646/ zootaxa.2197.1.2
- Gil-Santana HR, Marques OM (2005) Primeiro registro de *Saica apicalis* Osborn & Drake para o Brasil e *Pseudosaica florida* (Barber), com notas taxonômicas e chave para os gêneros de Saicinae do Brasil (Hemiptera, Reduviidae). Revista Brasileira de Zoologia 22(2): 405–409. https://doi.org/10.1590/S0101-81752005000200015
- Gil-Santana HR, Zeraik SO, Costa LAA (1999) Sinonimização dos gêneros Amilcaria Wygodzinsky, 1950 e Mayemesa Wygodzinsky, 1945 e descrição do macho de M. lapinhaensis (Wygodzinsky, 1950), combinação nova (Hemiptera, Reduvidae, Emesinae). Boletim do Museu Nacional, Nova Série. Zoologia 400: 1–10.

- Gil-Santana HR, Marques OM, Costa LAA (2006) Caprilesia almirantiana gen. nov. and sp. nov. of Saicinae from Brazil. Revista Brasileira de Zoologia 23(2): 392–394. https:// doi.org/10.1590/S0101-81752006000200012
- Gil-Santana HR, Gouveia FBP, Zeraik SO (2010) *Tagalis evavilmae* sp. nov. (Hemiptera: Reduviidae: Saicinae), an inhabitant of birds' nests in Amazonas, Brazil, with tax-onomical notes and a key to the species of *Tagalis* Stål. Zootaxa 2721(1): 1–14. https://doi.org/10.11646/zootaxa.2721.1.1
- Gil-Santana HR, Forero D, Weirauch C (2015) Assassin bugs (Reduviidae excluding Triatominae). In: Panizzi AR, Grazia J (Eds) True bugs (Heteroptera) of the Neotropics, Entomology in Focus 2. Springer Science+Business Media, Dordrecht, 307–351. https://doi.org/10.1007/978-94-017-9861-7_12
- Gil-Santana HR, Oliveira J, Zampaulo RA (2020) *Quasitagalis afonsoi*, a new genus and a new species of Saicinae (Hemiptera, Reduviidae) inhabiting a cave in Brazil, with an updated key to the genera of Saicinae of the New World. ZooKeys 966: 9–39. https://doi.org/10.3897/zookeys.966.52930
- Maldonado Capriles J (1981) A new *Ghilianella* and a new saicine genus, *Buninotus* (Hemiptera: Reduviidae) from Panama. The Journal of Agriculture of the University of Puerto Rico 65(4): 401–407. https://doi.org/10.46429/jaupr.v65i4.7620
- Melo MC, Coscarón MC (2005) Saicireta correntina, a new genus and species of assassin bug from Argentina (Heteroptera, Reduviidae, Saicinae) with a key to the New World genera. Deutsche Entomologische Zeitschrift 52(2): 245–249. https://doi. org/10.1002/mmnd.200410017
- Schuh RT, Weirauch C (2020) True Bugs of the World (Hemiptera: Heteroptera). Classification and natural history. Second Edition. Siri Scientific Press, Manchester, UK, 767 pp. [+ 32 pls.]
- Standring S, Forero D, Weirauch C (2023) Untangling the assassin's web: Phylogeny and classification of the spider-associated Emesine complex (Hemiptera: Reduviidae). Systematic Entomology. https://doi.org/10.1111/syen.12603
- Weirauch C (2008) From four- to three- segmented labium in Reduviidae (Hemiptera: Heteroptera). Acta Entomologica Musei Nationalis Pragae 48: 331–344.
- Weirauch C, Forero D (2007a) *Kiskeya palassaina*, new genus and species of Saicinae (Heteroptera: Reduviidae) from the Dominican Republic. Zootaxa 1468(1): 57–68. https://doi.org/10.11646/zootaxa.1468.1.2
- Weirauch C, Forero D (2007b) *Kiskeyana* new replacement name for the assassin bug *Kiskeya* (Hemiptera: Heteroptera: Reduviidae) from the Dominican Republic. Zootaxa 1530(1): 68. https://doi.org/10.11646/zootaxa.1530.1.7
- Wygodzinsky P (1966) A monograph of the Emesinae (Reduviidae, Hemiptera). Bulletin of the American Museum of Natural History 133: 1–614.



Research Article

Smooth post-labial chaetae in *Homidia* (Collembola, Entomobryidae) and the description of four new species from China with the aid of DNA barcoding

Rong Zhou¹⁰, Ling Huang¹⁰, Yi-Tong Ma¹⁰

1 School of Life Sciences, Nantong University, Nantong, Jiangsu 226000, China Corresponding author: Yi-Tong Ma (mayitong@ntu.edu.cn)

Abstract

Four new species of *Homidia* are described from the Guangxi Zhuang Autonomous Region, China. *Homidia longiantenna* **sp. nov.** is characterised by its long antenna and slightly expanded post-labial chaetae; *H. guangxiensis* **sp. nov.** by the presence of smooth chaetae on the post-labium and posterior face of the ventral tube; *H. huapingensis* **sp. nov.** by the presence of smooth post-labial chaetae and pointed tenent hairs; and *H. oligoseta* **sp. nov.** by the pointed tenent hairs and fewer macrochaetae on Abdomen IV. Additions to the original description of *Homidia acutus* Jing & Ma, 2022 are also provided.

Key words: Chaetotaxy, COI, DNA sequences, Guangxi, springtails, taxonomy



Academic editor: Wanda M. Weiner Received: 24 March 2024 Accepted: 15 August 2024 Published: 25 September 2024

ZooBank: https://zoobank. org/5ABCB962-61FB-4CBC-906B-99B14014637E

Citation: Zhou R, Huang L, Ma Y-T (2024) Smooth post-labial chaetae in *Homidia* (Collembola, Entomobryidae) and the description of four new species from China with the aid of DNA barcoding. ZooKeys 1213: 41–73. https://doi.org/10.3897/ zookeys.1213.123839

Copyright: © Rong Zhou 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).

Introduction

To date, 77 species of the genus *Homidia* have been described worldwide (Bellinger et al. 1996–2024). The main characters in the taxonomy of the genus include colour pattern, body chaetotaxy, chaetae of the labial base, claw structure and dental spines. The post-labial chaetae are rarely mentioned in species descriptions because they are usually not different from the normal ciliate chaetae present in most species, except for a few expanded ones. Prior to this study, smooth post-labial chaetae had not been reported in the genus. Here, we describe four new species of *Homidia*, among which one species has slightly expanded post-labial chaetae, two have smooth post-labial chaetae and the other one lacks expanded or smooth post-labial chaetae. Additions to the original description of *Homidia acutus* Jing & Ma, 2022 are also provided.

Material and methods

Taxon sampling and specimen examination

Specimens were collected with an aspirator and stored in 99% alcohol. They were mounted on glass slides in Marc André II solution and were studied with a Leica DM2500 phase contrast microscope. Photographs were taken using a Leica DFC300 FX digital camera mounted on the microscope and enhanced

with PHOTOSHOP CS2 (Adobe Inc.). Type specimens are deposited in the School of Life Sciences Nantong University, Jiangsu, China.

The nomenclature of the dorsal macrochaetotaxy of the head and interocular chaetae follows Jordana and Baquero (2005) and Mari-Mutt (1986). Labial chaetae are designated following Gisin (1964). Post-labial chaetae follow Chen and Christiansen (1993). Labral and tergal chaetae of the body follow Szeptycki (1973, 1979).

Molecular analysis

DNA was extracted by using an Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech, Shanghai, China) following the manufacturer's standard protocols. Amplification of a 658 bp fragment of the mitochondrial COI gene was carried out using a Prime Thermal Cycler (TECHNE, Bibby Scientific Limited, Stone, Staffordshire, UK), performed in 25 µl volumes using Premix Taq polymerase system (Takara Bio, Otsu, Shiga, Japan). The primers and polymerase chain reaction (PCR) programs followed Greenslade et al. (2011). All PCR products were checked using a 1% agarose gel electrophoresis. Successful products were purified and sequenced on an ABI 3730XL DNA Analyser (Applied Biosystem, Foster City, CA, USA). All procedures were completed by Pucheng (Nanjing, China).

DNA sequences were assembled using SEQUENCHER 4.5 (Gene Codes Corp) and then deposited in GenBank (Table 1). Sequences were aligned using ClustalW implemented in MEGA 5.1 (Tamura et al. 2011) with default settings.

Species	Number of individuals	GenBank accession number	Source	
Homidia huapingensis sp. nov.	C7401	PP379450	This study	
	C7402	PP379451		
	C8201	PP379452		
	C8203	PP379453		
	C8204	PP379454		
	C8202	PP379455		
	C8303	PP379456		
Homidia longiantenna sp. nov.	C8107	PP379457	This study	
	C8103	PP379458		
	C8104	PP379459		
	C8105	PP379460		
	C8106	PP379461		
Homidia guangxiensis sp. nov.	C8302	PP379462	This study	
	C8304	PP379463		
	C8305	PP379464		
Homidia oligoseta sp. nov.	C8306	PP379465	This study	
	C8307	PP379466		
	C8308	PP379467		
	C8309	PP379468		
Homidia acutus Jing & Ma, 2022	C44-3-a	PP379469	This study	
	C29-2-a	PP379470		
	C29-1-a	PP379471		
	C2902	PP379472		
	C4404	PP379473		

 Table 1. Number of individuals, GenBank accession numbers and source of sequences of the species in this study.

Pairwise genetic distances were analysed in MEGA 5.1 employing the Kimura 2-parameter (K2P) model (Kimura 1980).

Abbreviations

Ant.	antennal segment(s);		
Th.	thoracic segment(s);	mes	mesochaeta(e);
Abd.	abdominal segment(s);	ms	specialised microchaeta(e);
mac	macrochaeta(e);	sens	specialised ordinary chaeta(e).

Results

Class Collembola Lubbock, 1873 Order Entomobryomorpha Börner, 1913 Family Entomobryidae Tömösvary, 1882

Genus Homidia Börner, 1906

Diagnosis. Moderate size, usually 1–2 mm; eyes 8+8; antennae four segmented; mucro bidentate and with a basal spine, subapical tooth much larger than apical one; dentes with spines; scales absent on body; macrochaetae on anterior part of Abd. IV arranged as an irregular "collar".

Homidia longiantenna sp. nov.

https://zoobank.org/67A3996A-CCA2-42CC-BD48-3C878435D11F Figs 1–27, Tables 2, 3

Type material. *Holotype* • \bigcirc on slide, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, 31-V-2023, 25°37'52"N, 109°54'47"E, 935.4 m asl, sample number 1281. *Paratype* • \bigcirc on slide, same data as holotype. All collected by Y-T Ma.

Description. Size. Body length up to 3.02 mm.

Coloration. Ground colour pale yellow; eye patches dark blue; scattered brown pigment present on body, especially tibiotarsus, lateral and posterior parts of Abd. IV, sometimes Ant. III–IV and Abd. II–III also with brown pigment (Fig. 1).

Head. Antenna not annulated and 0.98–1.07 times length of body. Ratio of Ant. I–IV as 1.00/1.24–1.50/1.06–1.30/2.06–2.60. Distal part of Ant. IV with many sensory chaetae and normal ciliate chaetae, apical bulb bilobed (Fig. 2). Sensory organ of Ant. III with two rod-like chaetae (Fig. 3). Sensory organ of Ant. II with 3(4) rod-like chaetae (Fig. 4). Eyes 8+8, G and H smaller than others, interocular chaetae as p, r, t mes. Dorsal chaetotaxy of head with four antennal (An), five median (M) and eight sutural (S) mac (Fig. 5). Prelabral and labral chaetae as 4/5, 5, 4, all smooth, a0, a1 longer than a2; labral papillae absent (Fig. 6). Basal chaeta on maxillary outer lobe almost as thick as apical one; sub-lobal plate with three smooth chaetae-like processes (Fig. 7). Lateral process (I. p.) of labial pap E differentiated, as thick as normal chaeta, with tip almost



Figure 1. Habitus of Homidia longiantenna sp. nov. (lateral view). Scale bar: 500 µm.



Figures 2–8. *Homidia longiantenna* sp. nov. **2** apex of Ant. IV (dorsal view) **3** distal Ant. III (ventral view) **4** distal Ant. II (ventral view) **5** dorsal head (right side) **6** prelabrum and labrum **7** maxillary palp and outer lobe (right side) **8** labial palp. Scale bars: 20 µm.





reaching apex of papilla E (Fig. 8). Labial base with $MM_1R_1ReL_1L_2$, chaeta e smooth and other ciliate, R_1 sometimes absent, R 0.60–0.73 length of M; anterior post-labial chaetae slightly expanded (Figs 9–11).

Thorax. Tergal ms formula on Th. II–Abd. V as 1, 0/1, 0, 1, 0, 0, sens as 2, 2/1, 2, 2, 23, 3 (Figs 12, 18–20). Th. II with four medio-medial (m1, m2, m2i, m2i2), three medio-sublateral (m4, m4i, m4p), 30–38 posterior mac. Th. III with about 39–41 mac (Fig. 12). Coxal macrochaetal formula as 3/4+1, 3/4+2 (Figs 13–15). Trochanteral organ with 71–76 smooth chaetae (Fig. 16). Tenent hair clavate, 0.95–0.98 length of inner edge of unguis; unguis with three inner teeth, basal pair located at 0.38–0.39 distance from base of inner edge of unguis, unpaired tooth at 0.62–0.64 distance from base; unguiculus lanceolate, outer edge slightly serrate (Fig. 17).



Figures 12-17. *Homidia longiantenna* sp. nov. **12** chaetotaxy of Th. II-III (right side) **13-15** coxal chaetotaxy of fore, middle and hind leg **16** trochanteral organ **17** hind foot complex (lateral view). Scale bars: 50 µm (**12**); 20 µm (**13-17**).

Abdomen. Range of Abd. IV length as 9.00–9.30 times as dorsal axial length of Abd. III. Abd. I with 11 (a1a, a1–3, m2i, m2–4, m4i, m4p and a5) mac. Abd. II with six (a2, a3, m3, m3e, m3ea, m3ep) central, one (m5) lateral mac. Abd. III with two (a2, m3) central, four (am6, pm6, m7a, p6) lateral mac (Fig. 18). Abd. IV with two (as, ps) normal sens, 14–20 anterior, 6–7 (A4–6, B4–6, Ae7, A4 sometimes absent) posterior and 24–26 lateral mac (Fig. 19). Abd. V with three sens (Fig. 20). Anterior face of ventral tube not seen entirely, line connecting proximal (Pr) and external-distal (Ed) mac oblique to median furrow (Fig. 21); posterior face with five or eight distal smooth and numerous ciliate chaetae (Fig. 22); lateral flap with 7–8 smooth and 19–30 ciliate chaetae (Fig. 23). Manubrial plate dorsally with 14–15 ciliate mac and 3(2) pseudopores (Fig. 24); ventrally with 33–41 ciliate chaetae on each side (Fig. 25). Dens with 54–78



Figure 18. Chaetotaxy of Abd. I-III of Homidia longiantenna sp. nov. (right side). Scale bar: 50 µm.



Figure 19. Chaetotaxy of Abd. IV of Homidia longiantenna sp. nov. (right side). Scale bar: 50 µm.



Figures 20–27. *Homidia longiantenna* sp. nov. **20** chaetotaxy of Abd. V (right side) **21** anterior face of ventral tube apically **22** posterior face of ventral tube apically **23** lateral flap of ventral tube **24** manubrial plaque (dorsal view) **25** ventro-apical part of manubrium **26** proximal section of dens (circles also representing spines) **27** mucro. Scale bars: 20 µm.

smooth inner spines (Fig. 26). Mucro bidentate with subapical tooth larger than apical one; tip of basal spine reaching apex of subapical tooth; distal smooth section of dens almost equal to mucro in length (Fig. 27).

Etymology. Named after its characteristic long antennae. **Ecology.** Found in the leaf litter.

Characters	Homidia longiantenna sp. nov.	Homidia apigmenta	Homidia jordanai	Homidia pseudofascia	Homidia wanensis
Length ratio of antenna to body	0.98-1.07	0.50	about 1.00	0.59-0.67	0.60-0.62
An irregular transverse stripe on Abd. IV posteriorly	present	absent	absent	present	present
Chaetal formula of labial base	$MM_1(R_1)ReL_1L_2$	$\begin{array}{c} M(M_1)ReL_1L_2, L_1 \And L_2 \\ \text{expanded} \end{array}$	MReL ₁ L ₂	$MM_1ReL_1L_2$	$MReL_1L_2$
Anterior post-labial chaetae	slightly expanded	strong expanded	not expanded	slightly expanded	slightly expanded
Inner teeth on unguis	3	4	4	4	4
Mac on Abd. IV anteriorly	14-20	6-9	6-9	8-11	12-13
Mac on Abd. IV posteriorly	6-7	5	3(4)	7-9	7–9
Dental spines	54-78	18-39	20-40	36-50	83

Table 2. Main differences among the new species and related species of Homidia.

Remarks. The new species is characterised by the long antennae and the slightly expanded post-labial chaetae, and can be easily distinguished from all known species of *Homidia*. It is similar to the species *H. apigmenta* Shi, Pan & Zhang, 2010, *H. pseudofascia* Pan, Zhang & Li, 2015, and *H. wanensis* Pan & Ma, 2021 in the expanded post-labial chaetae and colour pattern, but can be separated from them by the long antennae and other characters. It is also similar to *H. jordanai* Pan, Shi & Zhang, 2011 in the long antennae, but significant differences exist between them, such as the post-labial chaetotaxy and central mac on Abd. III and other characters. The detailed character comparisons are listed in Table 2.

Homidia guangxiensis sp. nov.

https://zoobank.org/D3D8DC59-3275-448D-8E92-B82F205AF05B Figs 28-56, Table 3

Type material. *Holotype* • \bigcirc on slide, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, Power Station, 2-VI-2023, 25°37'40"N, 109°54'19"E, 682.0 m asl, sample number 1283. *Paratypes* • 3, \bigcirc on slides, same data as holotype • \bigcirc on slide, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, 31-V-2023, 25°37'52"N, 109°54'47"E, 935.4 m asl, sample number 1281. All collected by Y-T Ma.

Description. Size. Body length up to 2.86 mm.

Coloration. Ground colour pale white to yellow; eye patches dark blue; brown to blue-violet pigment present on whole dorsal body, antennae, legs, ventral tube, and manubrium. Some unpigmented irregular stripes or spots present on dorsal side of body (Figs 28, 29).

Head. Antenna not annulated and 0.60–0.80 times length of body. Ratio of Ant. I–IV as 1.00/1.27–1.70/1.21–1.40/1.67–2.41. Distal part of Ant. IV with many sensory chaetae and normal ciliate chaetae, apical bulb bilobed (Fig.



Figures 28, 29. Habitus of Homidia guangxiensis sp. nov. (lateral view). Scale bars: 500 µm.

30). Sensory organ of Ant. III with two rod-like chaetae (Fig. 31). Sensory organ of Ant. II with 3–4 rod-like chaetae (Fig. 32). Eyes 8+8, G and H smaller than others, interocular chaetae as p, r, t mes. Dorsal chaetotaxy of head with four antennal (An), five median (M) and eight sutural (S) mac (Fig. 33). Prelabral and labral chaetae as 4/5, 5, 4, all smooth, a0, a1 longer than a2; labral papillae absent (Fig. 34). Basal chaeta on maxillary outer lobe slightly thicker than as apical one; sublobal plate with three smooth chaetae-like processes (Fig. 35).



Figures 30–36. *Homidia guangxiensis* sp. nov. **30** apex of Ant. IV (dorsal view) **31** distal Ant. III (ventral view) **32** distal Ant. II (ventral view) **33** dorsal head (right side) **34** prelabrum and labrum **35** maxillary palp and outer lobe (right side) **36** labial palp. Scale bars: 20 μm.



Figures 37–39. *Homidia guangxiensis* sp. nov. **37** labial and post-labial chaetotaxy (right side) **38, 39** photographs of labial and post-labial chaetotaxy (right side). Scale bars: 20 µm.



Figures 40-45. *Homidia guangxiensis* sp. nov. 40 chaetotaxy of Th. II-III (right side) 41-43 coxal chaetotaxy of fore, middle and hind leg 44 trochanteral organ 45 hind foot complex (lateral view). Scale bars: 50 µm (40); 20 µm (41-45).

Lateral process (l. p.) of labial palp E differentiated, as thick as normal chaeta, with tip almost reaching apex of papilla E (Fig. 36). Labial base with $MRel_1L_2$, M sometimes smooth, R ciliate and 0.50–0.53 length of M, chaetae e and l_1 smooth, L_2 rarely smooth; some post-labial chaetae (G_{1-4} , H_{2-4} , sometimes X and an unnamed chaeta) smooth (Figs 37–39).

Thorax. Tergal ms formula on Th. II–Abd. V as 1, 0/1, 0, 1, 0, 0, sens as 2, 2/1, 2, 2, 18–36, 3 (Figs 40, 46–48). Th. II with four medio-medial (m1, m2, m2i, m2i2), three medio-sublateral (m4, m4i, m4p), 33–39 posterior mac. Th. III with 44–49 mac (Fig. 40). Coxal macrochaetal formula as 3/4+1, 3/4+2 (Figs 41–43). Trochanteral organ with 44–71 smooth chaetae (Fig. 44). Tenent hair clavate, 0.68–0.88 length of inner edge of unguis; unguis with three inner teeth, basal pair located at 0.32–0.40 distance from base of inner edge of unguis, unpaired tooth at 0.59–0.68 distance from base; unguiculus lanceolate, outer edge slightly serrate (Fig. 45).

Abdomen. Range of Abd. IV length as 6.51–8.75 times as dorsal axial length of Abd. III. Abd. I with 11 (rarely 10) (a1–3, m2i, m2–4, m4i, m4p and a5, a1a rarely absent) mac. Abd. II with six (a2, a3, m3, m3e, m3ea, m3ep) central, one (m5) lateral mac. Abd. III with two (a2, m3) central, four (am6, pm6, m7a, p6) lateral mac (Fig. 46). Abd. IV with two (as, ps) normal sens, 8–11 anterior, five (A5–6, B4–6, Ae7) posterior and 20–23 lateral mac (Fig. 47). Abd. V with three sens (Fig. 48). Anterior face of ventral tube with 44–46 ciliate chaetae on each side, line connecting proximal (Pr) and external-distal (Ed) mac oblique to median



Figure 46. Chaetotaxy of Abd. I-III of Homidia guangxiensis sp. nov. (right side) Scale bar: 50 µm.



Figure 47. Chaetotaxy of Abd. IV of Homidia guangxiensis sp. nov. (right side). Scale bar: 50 μ m.



Figures 48–51. *Homidia guangxiensis* sp. nov. **48** chaetotaxy of Abd. V (right side) **49** anterior face of ventral tube **50** posterior face of ventral tube apically **51** photomicrograph of posterior face of ventral tube apically. Scale bars: 20 µm.



Figures 52–56. *Homidia guangxiensis* sp. nov. **52** lateral flap of ventral tube **53** manubrial plaque (dorsal view) **54** ventro-apical part of manubrium **55** proximal section of dens (circles also representing spines) **56** mucro. Scale bars: 20 µm.

furrow (Fig. 49); posterior face with 9–18 smooth and numerous ciliate chaetae (Figs 50, 51); lateral flap with 7–12 (19) smooth and 11–19 ciliate chaetae (Fig. 52). Manubrial plate dorsally with 10–14 ciliate mac and three pseudopores (Fig. 53); ventrally with (26) 40–47 ciliate chaetae on each side (Fig. 54). Dens with 24–48 smooth inner spines (Fig. 55). Mucro bidentate with subapical tooth larger than apical one; tip of basal spine reaching apex of subapical tooth; distal smooth section of dens almost equal to mucro in length (Fig. 56).

Etymology. Named after its locality: Guangxi Zhuang Autonomous Region. **Ecology.** Found in the leaf litter.

Remarks. The new species can be easily distinguished from other species of the genus by the smooth post-labial chaetae and the number and location of smooth chaetae on the posterior face of the ventral tube. Among the known *Homidia* species, except those species with expanded post-labial chaetae, the post-labial chaetae are rarely mentioned because most have normal ciliate chaetae. Smooth post-labial chaetae are discovered for the first time in the genus. In addition, the smooth chaetae on the posterior face of the ventral tube are usually located at the most distal part of the ventral tube and their number is usually less than 10 in the genus. However, the number and location of the smooth chaetae on the posterior face of the species *H. acutus* Jing & Ma, 2022, *H. pseudozhangi* Jing & Ma, 2023 and *H. zhangi* Pan & Shi, 2012 in the colour pattern, but can be separated from them by the smooth post-labial chaetae, inner teeth on unguis and other characters. The detailed character comparisons are listed in Tables 3, 4.

Homidia huapingensis sp. nov.

https://zoobank.org/164C2C44-EE5F-40AF-B682-D01ACA2BC432 Figs 57-86, Tables 3, 4

Type material. *Holotype* • \bigcirc on slide, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, 31-V-2023, 25°37'52"N, 109°54'47"E, 935.4 m asl, sample number 1281. *Paratypes* • 2 \bigcirc on slides, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Guangfu Mountain, 26-V-2023, 25°33'44"N, 109°56'16"E, 1341.0 m asl, sample number 1274 • \bigcirc on slide, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Guangfu Mountain, 29-V-2023, 25°33'25"N, 109°56'38"E, 1340.5 m asl, sample number 1279 • \bigcirc on slide, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Mountain, 31-V-2023, 25°37'52"N, 109°54'47"E, 935.4 m asl, sample number 1281 • 2 \bigcirc on slides, CHINA, Guangxi Zhuang Autonomous County, Huaping Natural Reserve, Tianping Mountain, 31-V-2023, 25°37'52"N, 109°54'47"E, 935.4 m asl, sample number 1281 • 2 \bigcirc on slides, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous Region, Guilin City, Longsheng Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, 31-V-2023, 25°37'52"N, 109°54'47"E, 935.4 m asl, sample number 1281 • 2 \bigcirc on slides, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, 2-VI-2023, 25°37'40"N, 109°54'19"E, 682.0 m asl, sample number 1283. All collected by Y-T Ma.

Description. Size. Body length up to 2.92 mm.

Coloration. Ground colour pale white to yellow; eye patches dark blue; brown to blue-violet pigment present on whole dorsal body, antennae, legs, ventral tube, and manubrium. Some unpigmented irregular stripes or spots present on dorsal side of body (Figs 57, 58).



Figures 57, 58. Habitus of Homidia huapingensis sp. nov. (lateral view). Scale bars: 500 µm.

Head. Antenna not annulated and 0.59–0.64 times length of body. Ratio of Ant. I–IV as 1.00/1.28–1.40/1.11–1.36/2.00–2.25. Distal part of Ant. IV with many sensory chaetae and normal ciliate chaetae, apical bulb bilobed (Fig. 59). Sensory organ of Ant. III with two rod-like chaetae (Fig. 60). Sensory organ of Ant. II with 4–5 rod-like chaetae (Fig. 61). Eyes 8+8, G and H smaller than others,



Figures 59–65. *Homidia huapingensis* sp. nov. **59** apex of Ant. IV (dorsal view) **60** distal Ant. III (ventral view) **61** distal Ant. II (ventral view) **62** dorsal head (right side) **63** prelabrum and labrum **64** maxillary palp and outer lobe (right side) **65** labial palp. Scale bars: 20 μm.

interocular chaetae as p, r, t mes. Dorsal chaetotaxy of head with four antennal (An), five median (M) and eight sutural (S) mac (Fig. 62). Prelabral and labral chaetae as 4/5, 5, 4, all smooth, a0, a1 longer than a2; labral papillae absent (Fig. 63). Basal chaeta on maxillary outer lobe slightly thicker than as apical one; sublobal plate with three smooth chaetae-like processes (Fig. 64). Lateral process (I. p.) of labial papil E differentiated, as thick as normal chaeta, with tip almost reaching apex of papilla E (Fig. 65). Labial base with MRel₁L₂, M rarely smooth, R ciliate and 0.50–0.69 length of M, chaetae e and l₁ smooth, L₂ ciliate; some post-labial chaetae (G₁₋₄, H₂₋₄, rarely X, X₃ and 1–2 unnamed chaetae) smooth (Figs 66–68).

Thorax. Tergal ms formula on Th. II–Abd. V as 1, 0/1, 0, 1, 0, 0, sens as 2, 2/1, 2, 2, 23–37, 3 (Figs 69, 77–79). Th. II with four medio-medial (m1, m2, m2i, m2i2), three medio-sublateral (m4, m4i, m4p), 32–42 posterior mac. Th. III with 45–57 mac (Fig. 69). Coxal macrochaetal formula as 3/4+1, 3/4+2 (Figs 70–72). Trochanteral organ with about 40–70 smooth chaetae (Fig. 73). All tenent hairs pointed and 0.53–0.68 length of inner edge of unguis; unguis with three inner teeth, basal pair located at 0.29–0.35 distance from base of inner edge of unguis, unpaired tooth at 0.59–0.62 distance from base; unguiculus lanceolate, outer edge slightly serrate (Figs 74–76).



Figures 66–68. *Homidia huapingensis* sp. nov. **66** labial and post-labial chaetotaxy (right side) **67, 68** photographs of labial and post-labial chaetotaxy (right side). Scale bars: 20 µm.

Abdomen. Range of Abd. IV length as 6.43–7.50 times as dorsal axial length of Abd. III. Abd. I with 11 (sometimes 12) (a1a, a1–3, m2i, m2–4, m4i, m4p and a5, an unnamed mac sometimes present) mac. Abd. II with six (a2, a3, m3, m3e, m3ea, m3ep) central, one (m5) lateral mac. Abd. III with two (a2, m3) central, four (am6, pm6, m7a, p6) lateral mac (Fig. 77). Abd. IV with two (as, ps) normal sens, 9–12 anterior, 5–6 (A5–6, B5–6, Ae7, A4 sometimes present) posterior and 19–23 lateral mac (Fig. 78). Abd. V with three sens (Fig. 79). Anterior face of ventral tube with 44–55 ciliate chaetae on each side, line connecting proximal (Pr) and external-distal (Ed) mac oblique to median furrow (Fig. 80);



Figures 69-76. *Homidia huapingensis* sp. nov. **69** chaetotaxy of Th. II-III (right side) **70-72** coxal chaetotaxy of fore, middle and hind leg **73** trochanteral organ **74-76** foot complex of fore, middle and hind leg (lateral view). Scale bars: 50 μm (**69**); 20 μm (**70-76**).

posterior face with 5–9 smooth and numerous ciliate chaetae (Fig. 81); lateral flap with 9–11 smooth and 16–25 ciliate chaetae (Fig. 82). Manubrial plate dorsally with 12–17 ciliate mac and 2–4 pseudopores (Fig. 83); ventrally with 39–60 ciliate chaetae on each side (Fig. 84). Dens with 37–66 smooth inner spines (Fig. 85). Mucro bidentate with subapical tooth larger than apical one; tip of basal spine reaching apex of subapical tooth; distal smooth section of dens almost equal to mucro in length (Fig. 86).



Figure 77. Chaetotaxy of Abd. I-III of Homidia huapingensis sp. nov. (right side). Scale bar: 50 µm.



Figure 78. Chaetotaxy of Abd. IV of Homidia huapingensis sp. nov. (right side). Scale bar: 50 µm.



Figures 79–86. *Homidia huapingensis* sp. nov. **79** chaetotaxy of Abd. V (right side) **80** anterior face of ventral tube apically **81** posterior face of ventral tube apically **82** lateral flap of ventral tube **83** manubrial plaque (dorsal view) **84** ventro-apical part of manubrium **85** proximal section of dens (circles also representing spines) **86** mucro. Scale bars: 20 µm.

Etymology. Named after its locality: Huaping Natural Reserve, Guangxi Guangxi Zhuang Autonomous Region.

Ecology. Found in the leaf litter.

Remarks. The new species is very similar to *H. guangxiensis* sp. nov. and *H. acutus* Jing & Ma, 2022 in the colour pattern, smooth post-labial chaetae, inner teeth on unguis and central chaetae on Abd. IV posteriorly, but can be separated from them by the tenent hair, central chaetae on Abd. IV anterior-ly and smooth chaetae on the posterior face of the ventral tube. The detailed character comparisons are listed in Tables 3, 4.

Species	Specimen number	Mac on Abd. I	Anterior mac on Abd. IV	Posterior mac on Abd. IV	Lateral mac on Abd. IV	Tip of tenent hair	Smooth post-labial mac	Smooth mac on posterior face of ventral tube
Homidia longiantenna	1281-3A	11+11	14+15	6+7	25+25	clavate	absent	?
sp. nov.	1281-3B	11+11	18+20	6+7	24+26	clavate	absent	8
Homidia guangxiensis	1281-13	10+11	10+10	5+5	20+22	clavate	present	10
sp. nov.	1283-8A	11+11	10+?	5+5	?+?	clavate	present	9
	1283-8B	11+12	9+10	5+5	23+?	clavate	present	12
	1283-9A	11+11	8+10	5+5	23+?	clavate	preent	17
	1283-9B	11+11	9+11	5+5	20+22	clavate	present	18
Homidia huapingensis	1274-2B	11+11	10+11	5+5	19+20	point	present	?
sp. nov.	1274-2C	11+12	10+11	5+6	20+20	point	present	9
	1279-6B	11+12	11+12	6+6	21+?	point	present	6
	1281-10A	11+11	9+10	5+6	19+20	point	present	9
	1281-10B	11+11	9+9	5+5	22+22	point	present	8
	1283-8C	12+?	9+10	5+6	22+23	point	present	5
Homidia oligoseta	1281-11A	11+11	3+5	4+4	16+17	point	?	7
sp. nov.	1281-11B	11+?	3+3	4+5	16+16	point	absent	7
	1281-11C	10+10	3+4	4+4	16+17	point	absent	7
	1282-4A	11+11	3+4	4+4	15+?	point	absent	5
	1282-4B	11+11	3+3	4+4	12+?	point	absent	5
	1282-5	11+11	3+3	4+4	13+?	point	absent	5
	1282-6	11+11	3+3	4+4	13+15	point	absent	6
	1283-1A	11+11	3+3	4+4	13+13	point	absent	5
	1283-1B	11+11	3+4	4+4	13+?	point	absent	5
	1283-2A	10+11	4+5	4+4	12+14	point	absent	5
	1283-2B	10+11	3+3	4+4	11+?	point	absent	?
	1283-3	10+11	3+3	4+4	13+14	point	absent	5
	1283-10A	11+11	4+4	4+4	14+?	point	absent	5
	1283-10B	11+11	4+4	4+5	13+13	point	absent	5
Homidia acutus	1229-1A	11+11	6+6	5+5	15+?	point	present	6
	1229-1B	11+?	6+6	5+5	1516	point	present	6
	1229-2A	11+11	?+?	5+5	16+?	point	present	6
	1229-2B	11+11	6+6	5+5	16+16	point	present	?

Table 3. Variation in some characters of the species described in the present paper.

*? not clearly seen.

Homidia oligoseta sp. nov.

https://zoobank.org/F75FD2C9-613D-415C-A090-F9BAC8ED6ECB Figs 87-116, Tables 3, 4

Type material. *Holotype* • \bigcirc on slide, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, 31-V-2023, 25°37'52"N, 109°54'47"E, 935.4 m asl, sample number 1281. *Paratypes* • 2 \bigcirc on slides, same data as holotype • 4 \bigcirc on slides, CHINA,

Characters	Homidia guangxiensis sp. nov.	Homidia huapingensis sp. nov.	Homidia oligoseta sp. nov.	Homidia acutus	Homidia pseudozhangi	Homidia zhangi
Medial stripe on Th. II–III	absent	absent	absent	absent	present	absent
Smooth post-labial chaetae	present	present	absent	present	absent	absent
Tenent hair	clavate	pointed	pointed	pointed	clavate	clavate
Inner teeth on unguis	3	3	3	3	4	4
Smooth chaetae on posterior face of ventral tube	9–18	5-9	5-7	6	4–5	4
Relative position of ms to sens on Abd. I	antero-external	antero-external	antero-external	antero- external	antero-external	antero- internal
Relative position of middle sens to m3 on Abd. V	postero-external	postero-external	postero-external	postero- external	antero-external	postero- external
Mac on Abd. IV anteriorly	8–11	9–12	3-4(5)	6	7-12	8-10
Mac on Abd. IV posteriorly	5	5-6	4(5)	5	6	3(4)

 Table 4. Main differences among the three new species and related species of Homidia.



Figures 87–89. Habitus of Homidia oligoseta sp. nov. (lateral view). Scale bars: 500 $\mu m.$

Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, 1-VI-2023, 25°38'01"N, 109°54'30"E, 707.8 m asl, sample number 1282 • 7^Q on slides, CHINA, Guangxi Zhuang Autonomous Region, Guilin City, Longsheng Autonomous County, Huaping Natural Reserve, Tianping Mountain, Power Station, 2-VI-2023, 25°37'40"N, 109°54'19"E, 682.0 m asl, sample number 1283. All collected by Y-T Ma.

Description. Size. Body length up to 2.21 mm.

Coloration. Ground colour pale white to yellow; eye patches dark blue; brown to blue-violet pigment present on whole dorsal body, antennae, legs, ventral tube, and manubrium; some unpigmented irregular stripes or spots present on dorsal side of body; Th. II often with less brown pigment (Figs 87–89).

Head. Antenna not annulated and 0.56-0.62 times length of body. Ratio of Ant. I–IV as 1.00/1.25-1.47/1.00-1.43/2.00-2.71. Distal part of Ant. IV with many sensory chaetae and normal ciliate chaetae, apical bulb bilobed (Fig. 90). Sensory organ of Ant. III with two rod-like chaetae (Fig. 91). Sensory organ of Ant. III with 2–3 rod-like chaetae (Fig. 92). Eyes 8+8, G and H smaller than others, interocular chaetae as p, r, t mes. Dorsal chaetotaxy of head with four antennal (An), five median (M) and eight sutural (S) mac (Fig. 93). Prelabral and labral chaetae as 4/5, 5, 4, all smooth, a0, a1 longer than a2; labral papillae absent (Fig. 94). Basal chaeta on maxillary outer lobe slightly thicker than as apical one; sublobal plate with three smooth chaetae-like processes (Fig. 95). Lateral process (I. p.) of labial palp E differentiated, as thick as normal chaeta, with tip reaching or exceeding apex of papilla E (Fig. 96). Labial base with MRel₁L₂, chaetae e and l₁ smooth, other ciliate; R 0.53–0.60 length of M. All post-labial chaetae ciliate (Figs 97, 98).

Thorax. Tergal ms formula on Th. II–Abd. V as 1, 0/1, 0, 1, 0, 0, sens as 2, 2/1, 2, 2, 39–55, 3 (Figs 99, 107–109). Th. II with four medio-medial (m1, m2, m2i, m2i2), three medio-sublateral (m4, m4i, m4p), 32-37 posterior mac. Th. III with 45–50 mac (Fig. 99). Coxal macrochaetal formula as 3(4)/4+1, 3(4)/4+2 (Figs 100–102). Trochanteral organ with 40–57 smooth chaetae (Fig. 103). All tenent hairs pointed and 0.60–0.86 length of inner edge of unguis; unguis with three inner teeth, basal pair located at 0.30–0.36 distance from base of inner edge of unguis, unpaired tooth at 0.58–0.71 distance from base; unguiculus lanceolate, outer edge slightly serrate (Figs 104–106).

Abdomen. Range of Abd. IV length as 4.75-6.25 times as dorsal axial length of Abd. III. Abd. I with 11 (10) (a1–3, m2i, m2–4, m4i, m4p and a5, a1a sometimes absent) mac. Abd. II with six (a2, a3, m3, m3e, m3ea, m3ep) central, one (m5) lateral mac. Abd. III with two (a2, m3) central, four (am6, pm6, m7a, p6) lateral mac (Fig. 107). Abd. IV with two (as, ps) normal sens, 3-4(5) anterior, 4(5) (A4–6, B4–5, A5 rarely present) posterior and 11-17 lateral mac (Fig. 108). Abd. V with three sens (Fig. 109). Anterior face of ventral tube with 19-24 ciliate chaetae on each side, line connecting proximal (Pr) and external-distal (Ed) mac oblique to median furrow (Fig. 110); posterior face with 5-7 smooth and numerous ciliate chaetae (Fig. 111); lateral flap with 6-10 smooth and 7-12 ciliate chaetae (Fig. 112). Manubrial plate dorsally with 9-12 ciliate mac and three pseudopores (Fig. 113); ventrally with 22–30 ciliate chaetae on each side (Fig. 114). Dens with 16-31 smooth inner spines (Fig. 115). Mucro bidentate with subapical tooth larger than apical one; tip of basal spine reaching apex of subapical tooth; distal smooth section of dens almost equal to mucro in length (Fig. 116).



Figures 90–96. *Homidia oligoseta* sp. nov. **90** apex of Ant. IV (dorsal view) **91** distal Ant. III (ventral view) **92** distal Ant. II (ventral view) **93** dorsal head (right side) **94** prelabrum and labrum **95** maxillary palp and outer lobe (right side) **96** labial palp. Scale bars: 20 µm.



Figures 97, 98. *Homidia oligoseta* sp. nov. **97** labial and post-labial chaetotaxy (right side) **98** photograph of labial and post-labial chaetotaxy (right side). Scale bars: 20 µm.



Figures 99–106. *Homidia oligoseta* sp. nov. **99** chaetotaxy of Th. II–III (right side) **100**, **102** coxal chaetotaxy of fore, middle and hind leg **103** trochanteral organ **104–106** foot complex of fore, middle and hind leg (lateral view). Scale bars: 50 μm (**99**); 20 μm (**100–106**).



Figure 107. Chaetotaxy of Abd. I-III of Homidia oligoseta sp. nov. (right side) Scale bar: 50 µm.



Figure 108. Chaetotaxy of Abd. IV of Homidia oligoseta sp. nov. (right side) Scale bar: 50 µm.

Etymology. The name of the new species is derived from the Latin *oligo* and *seta*, which means only a few chaetae are present on each side of the anterior part of Abd. IV.

Ecology. Found in the leaf litter.

Remarks. The new species is very similar to *H. huapingensis* sp. nov. and *H. acutus* Jing & Ma, 2022 in the colour pattern and pointed tenent hair, but can be separated from them by the chaetotaxy of Abd. IV and smooth post-labial chaetae. The detailed character comparisons are listed in Tables 3, 4.

Homidia acutus Jing & Ma, 2022

Figs 117-120

Material examined. *Holotype* and three *paratypes* • CHINA, Jiangxi Province, Pingxiang City, Luxi County, Gate of Wugong Mountain, 7-XI-2020, 27°29'27"N, 114°07'33"E, 393.0 m asl, sample number 1229, collected by Y-T Ma.

Additions to original description. Colour pattern shown in Fig. 117. Ps2 mac present on dorsal head (Fig. 118) (Ps2 is not shown in the original figure because of my carelessness). Post-labial chaetae G_{1-4} and H_{2-4} smooth, sometimes an unnamed chaeta also smooth, chaeta H_1 slightly ciliate and others normal ciliate (Figs 119, 120).



Figures 109–116. *Homidia oligoseta* sp. nov. **109** chaetotaxy of Abd. V (right side) **110** anterior face of ventral tube **111** posterior face of ventral tube apically **112** lateral flap of ventral tube **113** manubrial plaque (dorsal view) **114** ventro-apical part of manubrium **115** proximal section of dens (circles also representing spines) **116** mucro Scale bars: 20 µm.

Molecular results

Sequenced individuals in the present study had a mean K2P distance of COI sequences between 0.177–0.329. The shortest interspecific distance was 0.177 between *H. guangxiensis* sp. nov. and *H. oligoseta* sp. nov. and the greatest was 0.329 between *H. huapingensis* sp. nov. and *H. longiantenna* sp. nov. (Table 5).



Figure 117. Habitus of *Homidia acutus* Jing & Ma, 2022 (lateral view). Scale bar: 500 µm.



Figures 118–120. *Homidia acutus* Jing & Ma, 2022 **118** dorsal head (right side) **119** labial and post-labial chaetotaxy (right side) **120** photograph of labial and post-labial chaetotaxy (right side). Scale bars: 20 µm.

Species	Homidia huapingensis sp. nov.	Homidia longiantenna sp. nov.	Homidia guangxiensis sp. nov.	Homidia oligoseta sp. nov.	H. acutus
Homidia huapingensis sp. nov.	0.005-0.074				
Homidia longiantenna sp. nov.	0.305-0.329	0.000-0.004			
Homidia guangxiensis sp. nov.	0.250-0.275	0.225-0.234	0.000-0.005		
<i>Homidia oligoseta</i> sp. nov.	0.257-0.288	0.182-0.197	0.177-0.193	0.000-0.061	
Homidia acutus	0.235-0.261	0.290-0.300	0.235-0.246	0.242-0.259	0.000-0.053

Table 5. Genetic distances (mean K2-P divergence) within and between species in this study.





Therefore, the interspecific distances of COI between the four new species were more than the accepted barcoding gap recently reported for species of Entomobryidae (Zhang et al. 2018) and Tomoceridae (Yu et al. 2018). The molecular distances coincided with the morphological divergences, thus further supporting the separation of the four distinct species (Fig. 121).

Discussion

Like the chaetae on the labial base, the post-labial chaetae are also of various types. Three species (*H. acutus* Jing & Ma, 2022; *H. huapingensis* sp. nov. and *H. oligoseta* sp. nov.) have smooth post-labial chaetae; three species (*H. longiantenna* sp. nov., *H. pseudofascia* Pan, Zhang & Li, 2015 and *H. wanensis* Pan & Ma, 2021) have slightly expanded post-labial chaetae and five species (*H. apigmenta* Shi, Pan & Zhang, 2010, *H. latifolia* Chen & Li, 1999, *H. polyseta* Chen, 1998, *H. qimenensis* Yi & Chen, 1999 and *H. triangulimacula* Pan & Shi, 2015) have strongly expanded post-labial chaetae; most species have normal post-labial ciliate chaetae, or the form of the chaetae is not mentioned.

Colour pattern is a very important character in the taxonomy of Collembola, but some different species may share a very similar colour pattern, such as *H. acutus*, *H. guangxiensis* sp. nov., *H. huangxiensis* sp. nov. and *H. oligoseta* sp. nov. Therefore, it is necessary to combine colour pattern with other characters, such as the tip of the tenent hair, post-labial chaetae and smooth chaetae on the posterior face of the ventral tube, in the taxonomy of Collembola. In addition, COI sequences are useful in separating morphologically similar species.

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 funded by the National Natural Sciences Foundation of China (32070403).

Author contributions

Rong Zhou: sorting specimens and writing a manuscript. Liang Huang: analysis of molecular results. Yi-Tong Ma: collecting research materials, microscopic observations, preparing figures.

Author ORCIDs

Rong Zhou **b** https://orcid.org/0000-0002-6908-472X Ling Huang **b** https://orcid.org/0000-0002-6073-9427 Yi-Tong Ma **b** https://orcid.org/0000-0002-8660-0503

Data availability

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

References

- Bellinger PF, Christiansen KA, Janssens F (1996[-2024]) Checklist of the Collembola of the World. http://www.collembola.org [accessed 24 March 2023]
- Börner C (1906) Das System der Collembolen-nebst Beschreibung neuer Collembolen des Hamburger Naturhistorischen Museums. Mitteilungen Naturhistorishe Museum Hamburg 23: 147–187.
- Börner C (1913) Die Familien der Collembolen. Zoologischen Anzeiger 41: 315-322.
- Chen JX (1998) A new species of *Homidia* (Collembola: Entomobryidae) from Hubei Province, China. Entomotaxonomia 20(2): 97–100.
- Chen JX, Christiansen K (1993) The genus *Sinella* with special reference to *Sinella* s.s (Collembola: Entomobryidae) of China. Oriental Insects 27: 1–54. https://doi.org/10. 1080/00305316.1993.10432236
- Chen JX, Li LR (1999) A new species of *Homidia* (Collembola: Entomobryidae) from China. Entomologia Sinica 6(1): 25–28. https://doi.org/10.1111/j.1744-7917.1999. tb00006.x
- Gisin H (1964) European Collembola. VII. Revue Suisse De Zoologie 71(4): 649-678. https://doi.org/10.5962/bhl.part.75615
- Greenslade P, Stevens MI, Torricelli G, D'Haese CA (2011) An ancient Antarctic endemic genus restored: morphological and molecular support for *Gomphiocephalus hodgsoni* (Collembola: Hypogastruridae). Systematic Entomology 36: 223–240. https://doi.org/10.1111/j.1365-3113.2010.00553.x
- Jing MD, Ma YT (2022) Two new species and two new records of *Homidia* (Collembola, Entomobryide) from China. ZooKeys 1135: 181–212. https://doi.org/10.3897/zook-eys.1135.89373
- Jing MD, Ma YT (2023) Three new species of Entomobryidae (Collembola, Entomobryoidea) from China. ZooKeys 1167: 293–315. https://doi.org/10.3897/zookeys.1167.104090
- Jordana R, Baquero E (2005) A proposal of characters for taxonomic identification of *Entomobrya* species (Collembola, Entomobryomorpha), with description of a new species. Abhandlungen und Berichte des Naturkundemuseums Goerlitz 76(2): 117–134.
- Kimura M (1980) A sample method for estimating evolutionary rates of base substitution through comparative studies of nucleotide-sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581
- Lubbock J (1873) Monograph of the Collembola and Thysanura. Ray Society, London, 276 pp. https://doi.org/10.5962/bhl.title.11583
- Mari-Mutt JA (1986) Puerto Rican species of *Lepidocyrtus* and *Pseudosinella* (Collembola: Entomobryidae). Caribbean Journal of Science 22(1–2): 1–48.
- Pan ZX, Ma YT (2021) Two new species of *Homidia* (Collembola: Entomobryidae) from Eastern China. Zootaxa 4995(1): 179–194. https://doi.org/10.11646/zootaxa.4995.1.11
- Pan ZX, Shi SD (2012) Description of a new species in the genus *Homidia* (Collembola: Entomobryidae) from Dalei Mountain, Zhejiang Province. Entomotaxonomia 34(2): 96–102.
- Pan ZX, Shi SD (2015) Description of a new *Homidia* species (Collembola Entomobryidae) with labial chaetae expanded. Entomotaxonomia 37(3): 161–170.
- Pan ZX, Shi SD, Zhang F (2011) New species of *Homidia* (Collembola: Entomobryidae) from east China with description of the first instar larvae. ZooKeys 152: 21–42. https://doi.org/10.3897/zookeys.152.1455
- Pan ZX, Zhang F, Li YB (2015) Two closely related *Homidia* species (Entomobryidae, Collembola) revealed by morphological and molecular evidence. Zootaxa 3918(2): 285–294. https://doi.org/10.11646/zootaxa.3918.2.9
- Shi SD, Pan ZX, Zhang F (2010) A new species and a new record of the genus *Homidia* Börner, 1906 from East China (Collembola: Entomobryidae). Zootaxa 2351: 29–38. https://doi.org/10.11646/zootaxa.2351.1.3
- Szeptycki A (1973) North Korean Collembola. I. The genus *Homidia* Börner 1906 (Entomobryidae). Acta Zoologica Cracoviensia 31: 23–40.
- Szeptycki A (1979) Morpho-systematic studies on Collembola. IV. Chaetotaxy of the Entomobryidae and its phylogenetical significance. Polska Akademia Nauk, Kraków, 219 pp.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739. https://doi.org/10.1093/molbev/msr121
- Tömösvary O (1882) Adatok Hazánk Thysanure-Faunájához. A Mathematikai és Természettudományi Osztályok Közlönye 18: 119–130.
- Yi YD, Chen JX (1999) A new species of the genus *Homidia* (Collembola: Entomobryidae) from Anhui Province, China. Entomotaxonomia 21(4): 235–238.
- Yu DY, Qin CY, Ding YH, Hu F, Zhang F, Liu MQ (2018) Revealing species diversity of *Tomocerus ocreatus* complex (Collembola: Tomoceridae): integrative species delimitation and evaluation of taxonomic characters. Arthropod Systematics & Phylogeny 76: 147–172. https://doi.org/10.3897/asp.76.e31949
- Zhang F, Yu DY, Stevens MI, Ding YH (2018) Colouration, chaetotaxy and molecular data provide species-level resolution in a species complex of *Dicranocentrus* (Collembola: Entomobryidae). Invertebrate Systematics 32: 1298–1315. https://doi. org/10.1071/IS18019



Research Article

Checklist and key to species of stink bugs (Hemiptera, Heteroptera, Pentatomidae) of Kentucky, United States of America

Armando Falcon-Brindis^{1,2}, Raul T. Villanueva²

1 Department of Entomology, Plant Pathology and Nematology, University of Idaho, Parma Research and Extension Center, Idaho, USA

2 Department of Entomology, University of Kentucky, Research and Education Center, Kentucky, USA

Corresponding author: Raul T. Villanueva (raul.villanueva@uky.edu)

Abstract

Stink bugs (Heteroptera: Pentatomidae) have received a lot of attention as there are many economically important pest species. However, the status of species richness, distribution, and taxonomy remain overlooked and outdated in Kentucky (USA). Having such information at a regional scale is crucial to allow the development of suitable pest management and conservation programs. Here, the stink bug fauna of Kentucky was examined from museum specimens, literature, and public online repositories. Overall, 42 species in 28 genera and three subfamilies (Asopinae, Podopinae, and Pentatominae) are listed from Kentucky. Thirteen species are new records for Kentucky, 10 species are considered to be of economic importance and eight are strict predators. Pictures of species are provided along with the first key for the identification of the stink bug species of Kentucky.



Academic editor: Jader Oliveira Received: 12 March 2024 Accepted: 6 August 2024 Published: 25 September 2024

ZooBank: https://zoobank. org/97DEE483-8ECF-4B64-BB2B-41ABD7122901

Citation: Falcon-Brindis A, Villanueva RT (2024) Checklist and key to species of stink bugs (Hemiptera, Heteroptera, Pentatomidae) of Kentucky, United States of America. ZooKeys 1213: 75–93. https://doi.org/10.3897/ zookeys.1213.122843

Copyright:

© Armando Falcon-Brindis & Raul T. Villanueva. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). **Key words:** Economic importance, public databases, sampling biases, species diversity, taxonomy

Introduction

The family Pentatomidae, also called stink bugs or turtle bugs, is the third most speciose family within the suborder Heteroptera, comprising 4949 species worldwide included in nine subfamilies (Asopinae, Cyrtocorinae, Discocephalinae, Edessinae, Pentatominae, Phyllocephalinae, Podopinae, Serbaninae, and Stirotarsinae), only surpassed by Reduviidae (~6000 species) and the Miridae (>11,000) (Schaefer and Panizzi 2000; Weirauch 2008; Cassis and Schuh 2012; Rider et al. 2018; Schuh and Weirauch 2020). Although stink bugs are more diverse in the Neotropical region (>1400 species) (Grazia et al. 2015), Nearctic pentatomids are probably among the best-studied insects in terms of diversity (~300 species), life history and distribution (Henry and Froeschner 1988, 1992). However, given the complexity of the group, both taxonomic and distributional changes are still emerging (Rider and Swanson 2021; Roca-Cusachs et al. 2022; Paim et al. 2022).

Adopting a regional approach to studying the pentatomid fauna provides a more accurate view of the group and facilitates the identification of species for either scientific or educational purposes (Packauskas 2012; Paiero et al. 2013). In this regard, the revision of Pentatomoidea provided by McPherson (1982) remains the most comprehensive taxonomic work in the northeastern United States. However, it emphasizes the fauna of Illinois and does not provide habitus illustrations for most of the species (*N* = 120), thus the identification process could be challenging. Recent efforts to update the stink bug fauna at the state scale include the pentatomids of Connecticut (O'Donnell and Schaefer 2012), Kansas (Packauskas 2012), Michigan (Swanson 2012), Minnesota (Koch et al. 2014), Missouri (Sites et al. 2012), New Mexico (Bundy 2012), North Dakota (Rider 2012), Ohio (Furth 1974), Virginia (Hoffman 1971), Washington (Zack et al. 2012), and the province of Ontario, Canada (Paiero et al. 2013).

The identification of most North American stink bugs can be reasonably conducted based on external morphology (some species groups can be challenging however, see Paim et al. 2022). Stink bugs are of moderate to large size, ranging from 4 to 20 mm in length, and generally ovoid or broadly elliptical in shape (Schuh and Weirauch 2020). Phytophagous stink bugs are usually more or less round in shape (some species associated with grasses are somewhat more elongated), usually with five-segmented antennae, three-segmented tarsi, and a subtriangular scutellum (Panizzi et al. 2000). In contrast, predatory stink bugs (subfamily Asopinae) are distinguished by an incrassate rostrum, particularly the first segment, which can swing forward fully, and the posterior margins of the buccula are merged (De Clercq 2008; Schuh and Weirauch 2020).

Pentatomids are considered an economically important group as most species are plant feeders (~90%), and about 10% prey upon arthropods, including many that are considered to be pests (McPherson and McPherson 2000; Schaefer and Panizzi 2000), in many agricultural systems (Panizzi and Slansky 1985; Pezzini et al. 2019). However, there are phytophagous species acting as facultative predators, but basic aspects of the biology of such species remain unknown, especially regarding behavior, population dynamics, and host damage (McPherson 1982). Moreover, stink bugs are associated with the transmission of plant pathogens causing boll rot, yeast spot, leaf spot, and different witch broom symptoms (Mitchell et al. 2018). Monitoring and identifying stink bugs in agricultural systems is critical for pest management purposes (Koch et al. 2014, 2017; Pezzini et al. 2019), especially when invasive species resembling native pentatomids are present. Several exotic species of economic importance reported in the United States can be easily misidentified and confused with native species without the help of suitable keys, e.g., the brown marmorated stink bug Halyomorpha halys Stål, the painted bug Bagrada hilaris (Burmeister), and the southern green stink bug Nezara viridula Linnaeus (Hoebeke and Carter 2003; McPherson 2018).

Color variation in adults can be a deceiving characteristic in species identification, for example, the predaceous species *Stiretrus anchorago* Fabricius displays contrasting bicolored and unicolored forms (Waddill and Shepard 1974). Species of the genus *Banasa* Stål and *Thyanta* Stål have seasonal green or brown forms (Thomas and Yonke 1981; Rider and Chapin 1992), and species of the *Euschistus* Dallas complex are often confused by the difficult taxonomic characters and multiple forms within a species (Paim et al. 2022). In addition, identifying immature stink bugs is a challenging task as there are not many studies on immature stages (DeCoursey and Allen 1968; Herring and Ashlock 1971; Brugnera et al. 2022).

Accurate identification of stink bugs at the regional scale is crucial to allow the development of suitable pest management and conservation programs (Grazia et al. 2015). Therefore, taxonomic keys supported by relevant information are valuable resources that help to avoid ambiguity and confusion in the identification of pentatomids. The purpose of this work is to provide an updated checklist and a key to the species of Pentatomidae occurring in Kentucky.

Material and methods

The examined material was obtained from the University of Kentucky Insect Collection (UKIC), the Insect Collection of the University of Louisville (ULIC), and the University of Kentucky's Research and Education Center (UKREC) at Princeton, KY. Additionally, species occurrence records of Pentatomidae were downloaded from the Global Biodiversity Information Facility (GBIF 2023) and the Symbiota Collections of Arthropods Network (SCAN 2023). These databases contained observation records from seven different sources, namely iNaturalist, Broward College, Monte L. Bean Life Science Museum, Oregon State Arthropod Collection (OSAC), Colorado State University (CSU), North Carolina State University (NCSU), Carnegie Museum of Natural History (CMNH), The Field Museum of Natural History, Illinois Natural History Survey (INHS) Insect Museum, and Texas A&M University (TAMU). Only research-grade observations from iNaturalist were carefully considered in this work, as many identifications of pentatomids can only be made by personal examination of specimens. Species synonyms are provided in this document following the literature indicated in Table 1. See Rider (2015) for more details about synonyms and previous combination names. After standard data cleaning (Chapman 2005), only records containing location and collection date were entered into a database to produce a distribution map and to create a plot of observations across time. Duplicate observations were removed from the final database.

The species checklist was ordered alphabetically, summarizing information about the distribution, size, feeding habits, and economic importance. The taxonomic catalog used in this study follows the distribution, size, and ecology listed by McPherson (1982) and the synonyms provided by Henry and Froeschner (1988), and Rider and Swanson (2021). The economic importance of species was based on the criteria of Schaefer and Panizzi (2000). Pictures of specimens were taken using an AmScope 18MP camera mounted on a Leica S6D stereoscopic microscope. Images were stacked and cleaned with Adobe PHOTOSHOP v. 22.4.3. Images of 37 species reported in this study are presented in Plates 1–5, as not all the specimens were found in the visited collections. The distribution map and plot were computed in R v. 74.3.1. (R Core Team 2023). Species determinations were conducted following Furth (1974), Larivière (1992), Thomas and Yonke (1981), McPherson (1982), Paiero et al. (2013), Packauskas (2012), and Swanson (2012). The morphological terminology followed Schuh and Weirauch (2020) and Kment and Vilímová (2010) (Figs 1, 2). The occurrence database can be found in the Suppl. material 1.

Table 1. Checklist of species of Pentatomidae occurring in Kentucky. El = Economic importance (marked with "x"). North America (NA) refers to Canada (CAN), United States (US), and Mexico (MX). Central America (CA), South America (SA). Cardinal directions are displayed in lower cases. Size is expressed as the length in millimeters. FH = Feeding habit. Phytophagous (Ph), Predator (Pr) and facultative predator (FaP). The source column indicates the sources used to identify the species. *New record for Kentucky State.

Taxon Synonym	EI	Distribution	Size	FH	Source
Asopinae					
Apoecilus cynicus (Say, 1831)		e US	13-20	Pr	Phillips 1983
Euthyrhynchus floridanus (Linnaeus, 1767)*		e US to SA	12.0-17.0	Pr	Henry and Froeschner 1988; Thomas 1992
Perillus bioculatus (Fabricius, 1775)	х	NA	8.5-11.5	Pr	Thomas 1992
Perillus strigipes (Herrich-Schäffer, 1853)		e US	7.5-10.0	Pr	Thomas 1992
Podisus brevispinus Thomas, 1992		CAN to n US	8.0-11.0	Pr	Phillips 1983; Thomas 1992
Podisus maculiventris (Say, 1831)	х	NA	8.5-13.0	Pr	Furth 1974; Thomas 1992
Podisus serieventris (Uhler, 1871)		CAN to US	8.0-11.5	Pr	Thomas 1992; Henry and Froeschner 1988
Stiretrus anchorago (Say, 1828)		se CAN to CA	7.0-10.0	Pr	McPherson 1982; Paiero et al. 2013
Pentatominae		1			
Aelini					
Aelia americana Dallas, 1851*		w CAN and US	7.0-9.0	Ph	Henry and Froeschner 1988
Neottiglossa cavifrons Stål 1872		s US	4.0-5.2	Ph	Rider 1989
Neottiglossa sulcifrons Stål, 1872		s US	4.0-5.0	Ph	Rider 1989
Neottiglossa undata (Sav. 1832)*		s CAN and n US	4.5-6.0	Ph	Rider 1989
Cappaeini					
Halvomorpha halvs (Stål, 1855)	x	Cosmopolitan	12.0-17.0	Ph	Rider et al. 2002
Carpocorini					
Coenus delius (Sav. 1832)*		s CAN and US	8 5-10 5	Ph	Rider 1996
Cosmopenia lintheriana (Kirkaldy 1909)		NA	40-70	Ph	McDonald 1986
Euschistus politus Uhler 1897*		ellS	8 2-10 0	Ph	Henry and Froeschner 1988: Paim et al. 2022
Euschistus servus (Sav. 1832)	x	NA to CA	10.0-15.0	Ph FaP	Rolston 1974: Paim et al. 2022
Euschistus tristiamus (Sav 1832)	x	e CAN to CA	8.0-12.0	Ph FaP	Rolston 1974: Paim et al. 2022
Euschistus variolarius (Palisot de Beauvois, 1805)	x	se CAN to n MX	11 0-15 0	Ph FaP	Rolston 1974: Paim et al. 2022
Holcostethus limbolarius (Stål 1872)*	^	ΝΔ	70-90	Ph	McDonald 1974
Hymenarcys nervosa (Sav. 1832)		ellS to n MX	8 5-11 5	Ph	Rolston 1973
Monhersonarcys aegualis (Say, 1652)		e US to MX	6.0-8.5	Dh	Thomas 2012
Monoclos insortus (Say, 1822)		e CAN to p MEY	12.0-14.0	Dh EoD	Relaton 1072
Mermidea lugane (Eabricius 1775)		a CAN to no MY	5.0-7.2	FII, FaF	Polston 1972
Oobalue pugnax (Fabricius, 1775)	v	o LIS to MY	9.0-12.0	Dh EaD	Spilor 1978
Provice pupatulatus (Palicot de Reguvois, 1919)*	X		0.0-12.0	PII, FdF Dh EoD	Pider and Chapin 1997
			F E_0 0	FII, FaF	MaDanald 1076
Halvini		SE CAN LO MIX	5.5 0.0	FII	MCDOIlaid 1970
Reachymana carioca Stål 1972		o US and no MV	15 9-10 2	Dh	Larivière 1992
Brochymena guadrinustulata (Eabricius, 1775)			12.0-19.5	Ph	Henry and Freeschner 1088
biochymena quaunpustulata (rabiicius, 1773)		NA IO CA	12.0-10.0	FII	McPherson and Ahmad 2007
Parabrochymena arborea (Say, 1825)		se CAN to CA	10.0-18.0	Ph, FaP	McPherson 1982
Parabrochymena punctata punctata Van Duzee, 1909		se US	14.0-17.0	Ph	Larivière 1992
Nezarini		1			
Chinavia hilaris (Say, 1832)	х	NA	13.0-19.0	Ph, Pr	McPherson 1982
Nezara viridula (Linnaeus, 1758)*	х	Cosmopolitan	14.0-17.0	Ph	Ferrari et al. 2010
Thyanta calceata (Say, 1832)		e US	7.0-10.5	Ph	Rider and Chapin 1992
Thyanta custator accerra McAtee, 1919		s CAN to n MEX	9.0-13.0	Ph	Rider and Chapin 1992
Pentatomini		1			
Banasa calva (Say, 1832)		NA to CA	8.5-12.0	Ph	Thomas and Yonke 1981
Banasa dimidiata (Say, 1832)*		NA to CA	8.5-11.0	Ph	Thomas and Yonke 1981
Banasa euchlora (Stål, 1872)*		NA to CA	9.0-11.0	Ph	Thomas and Yonke 1981
Banasa sordida (Uhler, 1871)		s CAN to n MEX	10.0-11.5	Ph	Thomas and Yonke 1981
Procleticini					
Dendrocoris humeralis (Uhler, 1877)*		se CAN to MX	6.0-8.5	Ph, FaP	Henry and Froeschner 1988
Strachiini					
Murgantia histrionica (Hahn, 1834)	х	NA to CA	8.0-11.5	Ph	McPherson 1982
Podopinae					
Amaurochrous cinctipes (Say, 1828)*		se CAN and e US	5.0-7.5	Ph	Barber and Sailer 1953



Figure 1. Key morphological features used to identify Pentatomidae (dorsal view).



Figure 2. Key morphological features used to identify Pentatomidae (ventral view). Herbivore (**A**–**C**) and predatory stink bug (**B**–**D**).

Results

Overall, the family Pentatomidae is represented by 42 species in 28 genera and 3 subfamilies (Asopinae, Podopinae, and Pentatominae) in Kentucky. Pictures of all species are displayed in Plates 1-5. Thirteen species are new records for Kentucky, 10 species are considered to be of economic importance and seven are strict predators. Nine species are presumably facultative predators (Table 1). Chinavia hilaris (Say) and Halyomorpha halys were commonly recorded (53%) and 12 species were found as singletons and doubletons. Out of the 1837 records found in Kentucky, 41.3% were obtained from GBIF and 30.3% from SCAN, 22.3% from the UKIC, and 6.1% from the UKREC (Fig. 3). Most records were found around the Louisville and Lexington areas, and 13% of the counties did not have any records of pentatomids: Grayson (West region), Washington, Henry, Owen, Trimble, Gallatin, Montgomery (Central), Lawrence, Martin, Knott, Clay, Leslie, Owsley, Green, Adair, Russell, Cumberland (East) (Fig. 3). Historically, the number of records in Kentucky has been within the order of 20 observations per year. However, observation records remarkably started to increase in 2019 (Fig. 4).



Figure 3. Distribution and source of records of Pentatomidae across Kentucky. Hollow counties lack occurrence records. GBIF = Global Biodiversity Information Facility, SCAN = Symbiota Collections of Arthropods Network, UKIC= University of Kentucky insect collection, UKREC= University of Kentucky's Research and Education Center.





Plate 1. Asopinae and Podopinae.



Plate 2. Carpocorini.



Plate 3. Carpocorini.

PENTATOMINI



Plate 4. Pentatomini, Aelini, Procleticini, Strachiini.



Key to species of Pentatomidae of Kentucky

1	Eyes pedunculate; scutellum U-shaped, enlarged, covering hemelytral
	membrane (subfamily Podopinae) Amaurochrous cinctipes Say
-	Eyes not pedunculate; scutellum either U-shaped or triangular2
2	Rostrum thickened, always directed away from the head; first segment
	short, thick, never held to the thoracic venter nor contained between the
	bucculae (Predatory species) (subfamily Asopinae) 35
-	Rostrum not thickened; first segment slender, lying between the
	bucculae(subfamily Pentatominae) 3
3	Mandibular plates with subapical tooth; pronotum with anterolateral mar-
	gins coarsely dentate4
-	Mandibular plates without subapical tooth; pronotum smooth or crenulate
	but never strongly dentate7
4	Basal fourth of scutellum distinctly elevated above the remainder (ob-
	served in lateral view); humeri subquadrate
	Parabrochymena arborea Say
-	Basal fourth of scutellum not distinctly elevated above the remainder;
	humeri subtriangular5

5	Mandibular plates distinctly longer than clypeus and usually converging before clypeus
-	Mandibular plates equal or slightly longer than clypeus; never converging before clypeus
6	Head appearing roundly truncate anterior to subapical teeth; body greyish white to pale yellowish brown Brochymena punctata punctata Van Duzee
-	Head appearing triangular anterior to subapical teeth; body strikingly mot- tled with ivoryBrochymena cariosa Stål
7	Abdominal sternite 3 (second visible) medially armed with spine or tuber- cle
-	Abdominal sternite 3 (second visible) medially unarmed14
8	Mandibular plates longer than clypeus and converging in front of it; hu- meri often outlined in red <i>Dendrocoris humeralis</i> Uhler
-	Mandibular plates not surpassing clypeus, but if so, not converging in front of it; humeri variable9
9	Large species (> 13 mm long), color entirely green dorsally, except for a
-	few black or pale markings; spine on sternite 3 variable 10 Small species (< 13 mm long), dorsal color different; spine on sternite 3
10	Obluse
10	Chinavia hilaris Sav
-	Peritreme short, not extending laterally; spine on sternite 3 obtuse
11	Pronotum with anterior area strongly contrasting with darker color of pos- terior area 12
-	Pronotum with anterior area concolorous with posterior area13
12	Scutellum uniformly brown color, except for the pale green integument at
	apexBanasa calva Say
-	brown
13	Dorsal color green with distinct pale markings irregularly spread through- out; anterior angles of pronotum with conspicuous ivory-white spots
	Banasa euchlora Stál
-	General color brown dorsally; abdominal venter with four rows of dark
14	Color predominantly black with red. vellow, or white markings
_	Color predominantly brown or green with variable markings
15	Small species (< 7 mm); pronotum with red cross; apex of scutellum with
	2 red dots Cosmopepla lintneriana Kirkaldy
-	Large species (> 8 mm), different marking pattern 16
16	Color black and orange; humeri rounded, without spines
_	Color block appy of coutally white: hymeri with charp opined directed
_	laterally Proxys punctulatus Palisot de Beauvois
17	Scutellum equal to or longer than corium
-	Scutellum shorter than corium
18	Clypeus distinctly elevated above mandibular plates Coenus delius Say
-	Clypeus scarcely elevated above mandibular plates19

19	Prostethium with anterior margin extending beyond anterior margin of eye; costal margin of coria distinctly paler than the inner area
_	Prostethium, if present, with anterior margin not reaching anterior margin
	of eye; coria concolorous 20
20	D Dorsal surface of head and propleura with pale yellow-brown areas; cly-
	peus with median yellow line Neottiglossa undata Say
-	Head and propleura different21
2	1 Apex of head broadly rounded, dorsal surface of head deeply concave and
	covered with short inward-curving hairs
-	Apex of nead more tapering, narrowly rounded; dorsal surface of nead not
2'	Clypeus distinctly elevated above mandibular plates
~	Mcphersonarcys aegualis Sav
_	Clypeus scarcely elevated above mandibular plates
23	Base of antennal segment 5 and apex and base of segment 4 pale; venter
	of head and thorax with clusters of dark (metallic green under bright light)
	punctures
-	Antennae color without pale bands; body punctures without metallic re-
	flections24
24	4 Humeri with sharp spine directed anteriorly <i>Oebalus pugnax</i> Fabricius
-	Humeri unarmed, if spines present, then not projecting anteriorly25
2	5 Anterolateral margin of pronotum crenulate
-	Anterolateral margin of pronotum not crenulate
20	reduced or obsolete
_	Abdominal sterna immaculate if black spot present then only on male
	pvqophore
2	7 Small species (< 10 mm); anterolateral margins of pronotum weakly cren-
	ulate; posterior margin of pygophore with V-shaped excavation medially
	Euschistus politus Uhler
-	Larger species (> 10 mm); anterolateral margins of pronotum distinctly
	crenulate; posterior margin of pygophore not notched or excavated 28
28	8 Mandibular plates equal or subequal in length to clypeus; antennae with
	segment five and apical half of segment four black; humeri acute to spi-
	nose; male pygophore with a medial black spot
	Euschistus varioiarius Palisot de Beauvois
_	humeri ohtuse: pygophore without markings
20	Pronotum with margins explanate projecting forward to eve: pronotum
2	and anterior half of scutellum with pale longitudinal ridge along middle
	Menecles insertus Say
_	Without the combination above; margins of pronotum not projecting for-
	ward to eye 30
30	O Anterolateral pronotal margins arcuate; hemelytral membrane with veins
	anastomosing Hymenarcys nervosa Say
-	Anterolateral pronotal margins not arcuate; hemelytral membrane with
	veins not anastomosing31

3	1 Scutellum completely black, ivory color on lateral margins; pronotum with
	Contallum at most and with black markinger menatum without transver
-	Scutelium at most only with black markings; pronotum without transver-
~	sai stripes
32	2 Mandibular plates longer than clypeus by distance equal to at least width
	of clypeus apex Holcostethus limbolarius Stal
-	Mandibular plates equal to or slightly longer than clypeus
33	Body distinctly covered with fine pubescence Trichopepla semivittata Say
_	Body distinctly bare, without distinct pubescence
34	4 Anterolateral pronotal margins black <i>Thyanta calceata</i> Say
-	Anterolateral pronotal margins not black Thyanta custator accerra McAtee
3	5 Anterior femora armed with ventral spine or tubercle at distal third to
	fourth
-	Anterior femora unarmed38
36	6 Scutellum U-shaped, broadly rounded apically and almost reaching tip of
	abdomenStiretrus anchorago Say
-	Scutellum not U-shaped, nor rounded; never reaching tip of abdomen38
37	7 Anterior femora with tubercle obsolescent; pronotum with yellow or
	orange longitudinal stripe on midline
	Perillus strigipes Herrich-Schäffer
-	Anterior femora with stout tubercle or spine; color markings variable
	(white, yellow or red)
38	8 Rostrum reaching abdominal sternite 3; abdominal sternite 3 medially un-
	armed Euthyrhynchus floridanus Linnaeus
_	Rostrum not reaching abdominal sternite 3; abdominal sternite 3 medially
	with distinct spine
39	9 Mandibular plates longer than clypeus; large species (> 14 mm)
	Apoecilus cynicus Say
_	Mandibular plates equal than clypeus; small species (< 12 mm)
4(0 Humeri produced into outward-projecting spines; spine on sternite 3 long,
	reaching anterior margin of hind coxae
_	Humeri blunt, not produced into spines: spine on sternite 3 variable, but
	not reaching anterior margin of hind coxae 41
4	1 Hind femora almost immaculate: spine on sternite 3 short not reaching
'	hind coxae
_	Hind femora heavily covered with black spots: spine on sternite 3 reaching
	nosterior margin of hind coxae Podicus carieventris Ubler
	posterior margin or mind conde

Discussion

The taxonomy of the family Pentatomidae is known to be entangled due to the large diversity and challenging species complexes, which has led to copious attempts to solve these issues over the last decades. Overall, the taxonomy of stink bugs in North America is well developed but outdated; thus, regional keys have become relevant for understanding the distribution, conservation, and management of species in agricultural systems (Packauskas 2012; Paiero et al. 2013; Koch et al. 2014, 2017; Pezzini et al. 2019). In this work, the first list and key to species occurring in Kentucky are presented. Furthermore, pictures

of all species are provided for comparison and identification purposes. This becomes valuable to extension educators, students, and non-specialists, especially since some species can be easily confused without taxonomic training or available keys.

Our knowledge of the pentatomid fauna of Kentucky significantly increased after this work (42 species of which 13 are new records); which also provides insights into species distributions (see Table 1). *Apoecilus cynicus, Brochymena cariosa, Euschistus politus, Hymenarcys nervosa, Mcphersonarcys aequalis, Oebalus pugnax,* and *Thyanta calceata* are restricted to the eastern United States north of Mexico. Other species, such as *Perillus strigipes* Herrich-Schäffer, *Neottiglossa sulcifrons* Stål, *N. cavifrons* Stål, *Brochymena punctata punctata* Van Duzee, were recorded in Kentucky more than three decades ago (Froeschner 1988; Furth 1974; Larivière 1992, respectively); however, they were not found in the visited collections or public databases. Perhaps future works could corroborate the presence of these species in Kentucky and provide proper photographs.

It was not surprising to find the brown marmorated stink bug, Halyomorpha halys as the most commonly recorded species in Kentucky (30%), as this invasive species (originally from East Asia) is well established in the eastern United States (Lee et al. 2013). The brown marmorated stink bug is known for its impact on agriculture as it severely damages tree fruit and other crops in North America (Leskey and Nielsen 2018). Besides H. halys, we found eight stink bug species occurring in Kentucky that are considered pests in agricultural systems. All of them are widespread across North America and some (3 species) occur all the way down to Central America. Nezara viridula was not recorded in Kentucky before this work. This cosmopolitan species, referred to as the southern green stink bug, has long been considered a key pest in the tropical and subtropical regions worldwide (especially attacking Glycine max L. Merrill), between latitudes 45°N and 45°S, and it is still spreading to new areas (Todd 1989). Although formerly abundant populations have been declining in the last 15 years in warmer areas of the Americas, presumably given the combination of several factors, i.e., climate change, parasitism, competition, weed control, and cropping systems (Panizzi and Lucini 2016).

Even though many species found in Kentucky are phytophagous, 25% exhibit predaceous habits and 17.5% are strict predators (subfamily Asopinae). Predatory stink bugs play a key role in natural and agricultural habitats since they control the population of arthropods (Richman and Whitcomb 1978). There are about 300 species of Asopinae described worldwide that are generalist predators mainly feeding on slow-moving, soft-bodied insects, primarily larval forms of the Lepidoptera, Coleoptera, and Hymenoptera (De Clercq 2008). *Podisus maculiventris* was the most common predacious species recorded in Kentucky (1.8% of records). This species has been widely studied in North America since it is usually found in agricultural systems (Mukerji and LeRoux 1969; Wiedenmann and O'Neil 1991; Linder et al. 2023). In Kentucky, it is not rare to find *P. maculiventris* in field crops during the growing season, but it could be mistaken for *Euschistus* species without a trained eye.

Occurrence data of stink bugs in Kentucky provided important insights into the understanding of sampling bias and gaps. In this case, several counties in the central and eastern regions do not have records of pentatomids, namely Grayson (West region), Washington, Henry, Owen, Trimble, Gallatin, Montgomery (Central), Lawrence, Martin, Knott, Clay, Leslie, Owsley, Green, Adair, Russell, Cumberland (East). Most observations/collects were found around highly populated areas in Kentucky i.e., Louisville and Lexington (U.S. Census Bureau 2024). Insect sampling is typically influenced by cities and roads, which are known as handy locations to take samples or simply take pictures (Falcon-Brindis et al. 2021). The exponential increase in observations starting in 2019 can be attributed to the active participation of citizens on insect identification forums such as iNaturalist. In fact, 92% of the records downloaded from GBIF were research-grade observations from this open-source platform.

Conclusion

In this study, we provided an up-to-date list of stink bugs (Pentatomidae) found in Kentucky, a dichotomous key of stink bugs, and high-quality pictures of all species. Overall, the family Pentatomidae is represented by 42 species in 28 genera and three subfamilies (Asopinae, Podopinae, and Pentatominae). This study establishes a baseline of the knowledge of stink bug fauna and will leverage the integrated pest management programs needing monitoring and identification of native and exotic species. This work also summarizes the distribution, size, and economic importance of the Pentatomidae species occurring in Kentucky. Both preserved specimens and public records of stink bugs greatly contributed to the understanding of sampling efforts and biases (i.e., towards populated areas).

Acknowledgments

The authors thank Julian R. Dupuis and Eric G. Chapman at the University of Kentucky Insect Collection (UKIC) for allowing us to revise the specimens. The authors thank the anonymous reviewers for their important observations to improve the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

We acknowledge the support of the Kentucky Soybean Promotion Board, the Kentucky Corn Growers Association, the North Central Soybean Research Program (award number GR133931), and the U.S. Department of Agriculture, Hatch (award number 1014521) that provided funds to complete the studies conducted in this publication.

Author contributions

Conceptualization: AFB and RTV. Data curation: AFB. Formal Analysis: AFB. Investigation AFB. Funding Acquisition: RTV. Project Administration: RTV. Resources: RTV. Original Draft Preparation: AFB. Review and Editing: AFB and RTV.

Author ORCIDs

Armando Falcon-Brindis () https://orcid.org/0000-0003-2496-2178

Data availability

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

References

- Barber HG, Sailer RI (1953) A revision of the turtle bugs of North America (Hemiptera: Pentatomidae). Journal of the Washington Academy of Sciences 43(5): 150–162
- Brugnera R, Limberger GM, Campos LA, Grazia J (2022) The eggs and nymphs of predatory stink bugs (Hemiptera: Pentatomidae: Asopinae): what do we know? Zoology 151: 125991. https://doi.org/10.1016/j.zool.2021.125991
- Bundy CS (2012) An annotated checklist of the stink bugs (Heteroptera: Pentatomidae) of New Mexico. Great Lakes Entomologist 45(3-4): 196-209. https://doi. org/10.22543/0090-0222.2252
- Cassis G, Schuh RT (2012) Systematics, Biodiversity, Biogeography, and Host Associations of the Miridae (Insecta: Hemiptera: Heteroptera: Cimicomorpha). Annual Review of Entomology 57: 377–404. https://doi.org/10.1146/annurev-ento-121510-133533
- Chapman AD (2005) Principles and Methods of Data Cleaning: Primary Species and Species-Occurrence Data, version 1.0. Report for the Global Biodiversity Information Facility, Copenhagen. http://www.gbif.org/document/80528
- De Clercq PD (2008) Stink Bugs, Predatory (Hemiptera: Pentatomidae, Asopinae). In: Capinera JL (Ed.) Encyclopedia of Entomology. Springer, Dordrecht, 2122–2125. https://doi.org/10.1007/978-1-4020-6359-6_3115
- DeCoursey RM, Allen RC (1968) A generic key to the nymphs of the Pentatomidae of the Eastern United States (Hemiptera: Heteroptera). University of Connecticut Occasional Papers 1: 141–151.
- Falcon-Brindis A, León-Cortés JL, Montañez-Reyna M (2021) How effective are conservation areas to preserve biodiversity in Mexico? Perspectives in Ecology and Conservation 19(4): 399–410. https://doi.org/10.1016/j.pecon.2021.07.007
- Ferrari A, Schwertner CF, Grazia J (2010) Review, cladistic analysis and biogeography of *Nezara* Amyot & Serville (Hemiptera: Pentatomidae). Zootaxa 2024(1): 1–41. https://doi.org/10.11646/zootaxa.5022.1.1
- Froeschner RC (1988) Family Pentatomidae Leach, 1815. The Stink Bugs. In: Henry TJ, Froeschner RC (Eds) Catalog of the Heteroptera, or true bugs, of Canada and the continental United States. E. J. Brill, New York, 544–597. https://doi.org/10.1163/9789004590601_032
- Furth DG (1974) The stink bugs of Ohio (Hemiptera: Pentatomidae). Bulletin of the Ohio Biological Survey 5(1): 1–62.

GBIF.org (2023) GBIF Home Page. https://www.gbif.org [22 August 2023]

- Grazia J, Panizzi AR, Greve C, Schwertner CF, Campos LA, Garbelotto TA, Fernandes JAM (2015) Stink Bugs (Pentatomidae). In: Panizzi AR, Grazia J (Eds) True Bugs (Heteroptera) of the Neotropics, Springer, New York, 901 pp. https://doi. org/10.1007/978-94-017-9861-7
- Henry TJ, Froeschner RC (1988) Catalog of the Heteroptera, or True Bugs, of Canada and the Continental United States. E. J. Brill, Leiden, New York, 958 pp.

- Henry TJ, Froeschner RC (1992) Corrections and additions to the "Catalog of the Heteroptera, or true bugs, of Canada and the Continental United States." Proceedings of the Entomological Society of Washington 94(2): 263–272.
- Herring JL, Ashlock PD (1971) A Key to the Nymphs of the Families of Hemiptera (Heteroptera) of America North of Mexico. The Florida Entomologist 54(3): 207–212. https://doi.org/10.2307/3493715
- Hoebeke ER, Carter ME (2003) *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): A polyphagous plant pest from Asia newly detected in North America. Proceedings of the Entomological Society of Washington 105: 225–237.
- Hoffman RL (1971) The Insects of Virginia: No. 4. Shield bugs (Hemiptera; Scutelleroidea, Corimelaenidae, Cydnidae, Pentatomidae). Virginia Polytechnic Institute and State University, Research Division Bulletin 67, 61 pp.
- Kment P, Vilímová J (2010) Thoracic scent efferent system of Pentatomoidea (Hemiptera: Heteroptera): a review of terminology. Zootaxa 2706: 1–77. https://doi. org/10.11646/zootaxa.2706.1.1
- Koch RL, Rider DA, Tinerella PP, Rich WA (2014) Stink Bugs (Hemiptera: Heteroptera: Pentatomidae) of Minnesota: An Annotated Checklist and New State Records. The Great Lakes Entomologist 47(3–4): 171–185. https://doi.org/10.22543/0090-0222.2305
- Koch RL, Pezzini DT, Michel AP, Hunt TE (2017) Identification, biology, impacts, and management of stink bugs (Hemiptera: Heteroptera: Pentatomidae) of soybean and corn in the Midwestern United States. Journal of Integrated Pest Management 8(1): 1–14. https://doi.org/10.1093/jipm/pmx004
- Larivière MC (1992) Description of *Parabrochymena*, new genus, and redefinition and review of *Brochymena* Amyot and Audinet-Serville (Hemiptera: Pentatomidae), with considerations on natural history, chorological affinities, and evolutionary relationships. Memoirs of the Entomological Society of Canada 163: 1–75. https://doi.org/10.4039/entm124163fv
- Lee DH, Short BD, Joseph SV, Bergh JC, Leskey TC (2013) Review of the Biology, Ecology, and Management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environmental Entomology 42(4): 627–641. https://doi. org/10.1603/EN13006
- Leskey TC, Nielsen AL (2018) Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. Annual Review of Entomology 63: 599–618. https://doi.org/10.1146/annurev-ento-020117-043226
- Linder S, Jarrett BJ, Szűcs M (2023) Non-target attack of the native stink bug, *Podisus* maculiventris by Trissolcus japonicus, comes with fitness costs and trade-offs. Biological Control 177: 105107. https://doi.org/10.1016/j.biocontrol.2022.105107
- McDonald FJD (1974) Revision of the Genus *Holcostethus* in North America (Hemiptera: Pentatomidae). Journal of the New York Entomological Society 82(4): 245–258.
- McDonald FJD (1976) Revision of the genus *Trichopepla* (Hemiptera:Pentatomidae) in N. America. Journal of the New York Entomological Society 84(1): 9–22
- McDonald FJD (1986) Revision of *Cosmopepla* Stål (Hemiptera: Pentatomidae). Journal of the New York Entomological Society 94: 1–15.
- McPherson JE (1982) The Pentatomoidea (Hemiptera) of Northeastern North America with emphasis on the fauna of Illinois. Southern Illinois University Press, Carbondale, Illinois, 240 pp.
- McPherson JE (2018) Invasive stink bugs and related species (Pentatomoidea): biology, higher systematics, semiochemistry, and management. CRC Press, Taylor & Francis Group, Boca Raton, Florida, 819 pp. https://doi.org/10.1201/9781315371221

- McPherson JE, Ahmad I (2007) Redescriptions of *Brochymena* and *Parabrochymena* (Hemiptera: Heteroptera: Pentatomidae), based primarily on male genitalia, with reclassification of three species and description of New World tribe (Halyini). Annals of the Entomological Society of America 100(5): 673–682. https://doi.org/10.1603/001 3-8746(2007)100[673:ROBAPH]2.0.CO;2
- McPherson JE, McPherson RM (2000) Stinkbugs of economic importance in America north of Mexico. CRC Press, Boca Raton, 253 pp. https://doi.org/10.1201/9781420042429
- Mitchell PL, Zeilinger AR, Medrano EG, Esquivel JF (2018) Pentatomids as vectors of plant pathogens. In: McPherson JE (Ed.) Invasive stink bugs and related species (Pentatomoidea): biology, higher systematics, semiochemistry, and management. CRC Press, Taylor & Francis Group, Boca Raton, Florida, 611–640. https://doi.org/10.1201/9781315371221-13
- Mukerji MK, LeRoux EJ (1969) A quantitative study of food consumption and growth of *Podisus maculiventris* (Hemiptera: Pentatomidae). The Canadian Entomologist 101(4): 387–403. doi:https://doi.org/10.4039/Ent101387-4
- O'Donnell JE Schaefer CW (2012) Annotated checklist of the Pentatomidae (Heteroptera) of Connecticut. Great Lakes Entomologist 45(3-4): 220-234. https://doi. org/10.22543/0090-0222.2254
- Packauskas RJ (2012) The Pentatomidae, or Stink Bugs, of Kansas with a key to species (Hemiptera: Heteroptera). The Great Lakes Entomologist 45(3–4): 210–219. https://doi.org/10.22543/0090-0222.2253
- Paiero SM, Marshall SA, McPherson JE, Ma MS (2013) Stink bugs (Pentatomidae) and parent bugs (Acanthosomatidae) of Ontario and adjacent areas: A key to species and a review of the fauna. Canadian Journal of Arthropod Identification 24: 1–183. https://doi.org/10.3752/cjai.2013.24
- Paim MR, Grazia J, Rider DA, Bianchi FM (2022) Revisiting Stål's thoughts: formalizing the *ictericus* group in *Euschistus* (*Euschistus*) (Hemiptera: Heteroptera: Pentatomidae). Zootaxa 5169(6): 501–537. https://doi.org/10.11646/zootaxa.5169.6.1
- Panizzi AR, Lucini T (2016) What Happened to *Nezara viridula* (L.) in the Americas? Possible Reasons to Explain Populations Decline. Neotroprical Entomology 45: 619–628. https://doi.org/10.1007/s13744-016-0446-2
- Panizzi AR, Slansky F (1985) Review of phytophagous pentatomids (Hemiptera: Pentatomidae) associated with soybean in the Americas. Florida Entomologist 68: 184– 214. https://doi.org/10.2307/3494344
- Panizzi AR, McPherson JE, James DG, Javahery M, McPherson RM (2000) Stink Bugs (Pentatomidae). In: Schaefer CW, Panizzi AR (Eds) Heteroptera of economic importance. CRC Press, Washington, D.C., 421–474. https://doi.org/10.1201/9781420041859.ch13
- Pezzini DT, DiFonzo CD, Finke DL, Hunt TE, Knodel JJ, Krupke CH, McCornack B, Michel AP, Philips CR, Varenhorst AJ, Wright RJ, Koch RL (2019) Community composition, abundance, and phenology of stink bugs (Hemiptera: Pentatomidae) in soybean in the North Central Region of the United States. Journal of Economic Entomology 112(4): 1722–1731. https://doi.org/10.1093/jee/toz099
- Phillips KA (1983) A taxonomic revision of the Nearctic species of *Apateticus* Dallas and *Podisus* Herrich-Schaeffer (Heteroptera: Pentatomidae: Asopinae). Ph. D. Dissertation, Oregon State University, Corvallis, Oregon, 275 pp.
- R Core Team (2023) R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. https://www.R-project.org/
- Richman DB, Whitcomb WH (1978) Comparative lifecycle of four species of predatory stink bugs. The Florida Entomologist 61(3): 113–119. https://doi.org/10.2307/3494225

- Rider DA (1989) Review of the New World species of the genus *Neottiglossa* Kirby (Heteroptera: Pentatomidae). Journal of the New York Entomological Society 97: 394–408.
- Rider DA (1996) Review of the genus *Coenus* Dallas, with the description of *C. explanatus*, new species (Heteroptera: Pentatomidae). Journal of the New York Entomological Society 103: 39–47.
- Rider DA (2012) The Heteroptera (Hemiptera) of North Dakota I: Pentatomomorpha: Pentatomoidea. The Great Lakes Entomologist 45: 312–380. https://doi. org/10.22543/0090-0222.2258
- Rider DA (2015) Pentatomoidea Home Page. https://www.ndsu.edu/pubweb/~rider/ Pentatomoidea/ [12 May 2024]
- Rider DA, Chapin JB (1992) Revision of the Genus *Thyanta* Stål, 1862 (Heteroptera: Pentatomidae) II. North America, Central America, and the West Indies. Journal of the New York Entomological Society 100(1): 42–98.
- Rider DA, Swanson DR (2021) A distributional synopsis of the Pentatomidae (Heteroptera) north of Mexico, including new state and provincial records. Zootaxa 5015(1): 001–069. https://doi.org/10.11646/zootaxa.5015.1.1
- Rider DA, Zheng LY, Kerzhner IM (2002) Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera). II. Pentatominae. Zoosystematica Rossica 11(1): 135–153. https://doi.org/10.31610/zsr/2002.11.1.135
- Rider DA, Schwertner CF, Vilímová J, Rédei D, Kment P, Thomas DB (2018) Higher systematics of the Pentatomoidea. In: McPherson JE (Ed.) Invasive Stink Bugs and Related Species (Pentatomoidea) Biology, Higher Systematics, Semiochemistry, and Management. CRC Press, Boca Raton, Florida, 25–204. https://doi. org/10.1201/9781315371221-2
- Roca-Cusachs M, Schwertner CF, Kim J, Eger J, Grazia J, Jung S (2022) Opening Pandora's box: molecular phylogeny of the stink bugs (Hemiptera: Heteroptera: Pentatomidae) reveals great incongruences in the current classification. Systematic Entomology 47(1): 36–51. https://doi.org/10.1111/syen.12514
- Rolston LH (1972) The genus *Menecles* Stål (Hemiptera; Pentatomidae). Journal of the New York Entomological Society 80(4): 234–237.
- Rolston LH (1973) A review of *Hymenarcys* (Hemiptera: Pentatomidae). Journal of the New York Entomological Society 81: 111–117.
- Rolston LH (1974) Revision of the genus *Euschistus* in middle America (Hemiptera, Pentatomidae, Pentatomini). Entomologia Americana 48: 1–102.
- Rolston LH (1978) A Revision of the Genus *Mormidea* (Hemiptera: Pentatomidae). Journal of the New York Entomological Society 86(3): 161–219.
- Sailer RI (1957) *Solubea* Bergoth 1891, a synonym of *Oebalus* Stal 1862, and a note concerning the distribution of *O. ornatus* (Sailer). Proceedings of the Entomological Society of Washington 59(1): 41–42.
- SCAN (2023) Symbiota Collections of Arthropods Network: A Data Portal Built to Visualize, Manipulate, and Export Species Occurrences. https://scan-bugs.org/portal/ [3 September 2023]
- Schaefer CW, Panizzi AR (2000) Heteroptera of economic importance. CRC Press, Washington, D.C., 828 pp. https://doi.org/10.1201/9781420041859
- Schuh RT, Weirauch C (2020) True Bugs of the World (Hemiptera: Heteroptera), Classification and Natural History (Second Edition). Siri Scientific Press Monograph Series (Vol. 8). Siri Scientific Press, Manchester, 767 pp.

- Sites RW, Simpson KB, Wood DL (2012) The stink bugs (Hemiptera: Heteroptera: Pentatomidae) of Missouri. The Great Lakes Entomologist 45: 134–163. https://doi. org/10.22543/0090-0222.2249
- Swanson DR (2012) An Updated Synopsis of the Pentatomoidea (Heteroptera) of Michigan. The Great Lakes Entomologist 45(2): 263–311. https://doi.org/10.22543/0090-0222.2257
- Thomas DB (1992) Taxonomic synopsis of the Asopine Pentatomidae (Heteroptera) of the Western Hemisphere. Lanham, The Thomas Say Foundation, ESA, Monographs. 156 pp. https://doi.org/10.4182/OAC02099
- Thomas DB (2012) *Mcphersonarcys*, a new genus for *Pentatoma aequalis* Say (Heteroptera: Pentatomidae). The Great Lakes Entomologist 45(2): 127–133. https://doi. org/10.22543/0090-0222.2248
- Thomas DB, Yonke TR (1981) A Review of the Nearctic species of the genus *Banasa* Stål (Hemiptera: Pentatomidae). Journal of the Kansas Entomological Society 54(2): 233–248.
- Todd JW (1989) Ecology and behavior of *Nezara viridula*. Annual review of entomology 34(1): 273–292. https://doi.org/10.1146/annurev.en.34.010189.001421
- U. S. Census Bureau (2024) Kentucky QuickFacts. https://data.census.gov/profile/Kentucky?g=040XX00US21 [17 January 2024]
- Waddill V, Shepard M (1974) Biology of a predaceous stink bug, *Stiretrus anchorago*, (Hemiptera: Pentatomidae). Florida Entomologist 57: 249–253. https://doi. org/10.2307/3493252
- Weirauch C (2008) Cladistic analysis of Reduviidae (Heteroptera: Cimicomorpha) based on morphological characters. Systematic Entomology 33: 229–274. https://doi. org/10.1111/j.1365-3113.2007.00417.x
- Wiedenmann RN, O'Neil RJ (1991) Searching behavior and time budgets of the predator *Podisus maculiventris*. Entomologia experimentalis et applicata 60(1): 83–93. https://doi.org/10.1111/j.1570-7458.1991.tb01525.x
- Zack RS, Landolt PJ, Munyaneza JE (2012) The stink bugs (Hemiptera: Heteroptera: Pentatomidae) of Washington state. The Great Lakes Entomologist 45: 251–262. https://doi.org/10.22543/0090-0222.2256

Supplementary material 1

Occurrence database

Authors: Armando Falcon-Brindis

Data type: csv

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1213.122843.suppl1



Research Article

Old World *Micropholcus* spiders, with first records of acrocerid parasitoids in Pholcidae (Araneae)

Bernhard A. Huber¹⁰, Guanliang Meng¹⁰

1 Zoological Research Museum Alexander Koenig, LIB, Bonn, Germany Corresponding author: Bernhard A. Huber (b.huber@leibniz-lib.de)

Abstract

Micropholcus Deeleman-Reinhold & Prinsen, 1987 is one of only two Pholcidae genera known to occur both in the Old and New Worlds. However, there are major morphological and ecological differences among geographically separate groups of species, and it was mainly molecular data that have resulted in our current view of uniting all these species into a single genus. In the Old World, only four species have previously been described. Here, current knowledge about Old World *Micropholcus* is reviewed, redescribing three of the four previously known species, and describing twelve new species, originating from Saudi Arabia (*M. dhahran* Huber, **sp. nov.**, *M. harajah* Huber, **sp. nov.**, *M. alfara* Huber, **sp. nov.**, *M. abha* Huber, **sp. nov.**, *M. tanomah* Huber, **sp. nov.**, *M. shaat* Huber, **sp. nov.**, *M. maysaan* Huber, **sp. nov.**, *M. khenifra* Huber, **sp. nov.**, *M. shaat* Huber, **sp. nov.**), Morocco (*M. ghar* Huber, **sp. nov.**). We provide an exploratory species delimitation analysis based on CO1 barcodes, extensive SEM data, and first records of Acroceridae (Diptera) larvae in Pholcidae, extracted from book lungs.



Academic editor: Alireza Zamani Received: 26 July 2024 Accepted: 20 August 2024 Published: 26 September 2024

ZooBank: https://zoobank.org/ B8E6EE1A-023D-4B64-8D3A-C1843F0D8376

Citation: Huber BA, Meng G (2024) Old World *Micropholcus* spiders, with first records of acrocerid parasitoids in Pholcidae (Araneae). ZooKeys 1213: 95–182. https://doi.org/10.3897/ zookeys.1213.133178

Copyright: © Bernhard A. Huber & G. Meng. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0) **Key words:** CO1 barcode, genetic distances, Morocco, Oman, Philippines, Saudi Arabia, species delimitation, taxonomy

Table of contents

Abstract	96
Introduction	96
Materials and methods	97
Results	98
Molecular analysis	98
Taxonomy	101
Genus Micropholcus Deeleman-Reinhold & Prinsen, 1987	101
Micropholcus fauroti (Simon, 1887)	116
Micropholcus jacominae Deeleman-Reinhold & van Harten, 2001 .	117
Micropholcus dhahran Huber, sp. nov	123
Micropholcus harajah Huber, sp. nov	127
Micropholcus alfara Huber, sp. nov	131
Micropholcus abha Huber, sp. nov	135
Micropholcus tanomah Huber, sp. nov	139
Micropholcus bashayer Huber, sp. nov	142
Micropholcus maysaan Huber, sp. nov	146
Micropholcus darbat Huber, sp. nov	151
Micropholcus shaat Huber, sp. nov	155
Micropholcus agadir (Huber, 2011)	158
Micropholcus ghar Huber, sp. nov	162
Micropholcus khenifra Huber, Lecigne & Lips, sp. nov	167
Micropholcus bukidnon Huber, sp. nov	171
Discussion	176
Conclusions	178
Acknowledgements	179
References	179

Introduction

Pholcid spiders have a worldwide distribution, with the large majority of species restricted to tropical and subtropical regions (Huber and Chao 2019). A few species have followed humans around the world, but in general, distributions of most species and even genera are limited to much smaller geographic regions. Only two of the currently recognised 95 extant genera are known to be present in both the Old and New Worlds: *Pholcus* Walckenaer, 1805 and *Micropholcus* Deeleman-Reinhold & Prinsen, 1987. *Pholcus* is very species-rich and widely distributed in the Old World, but in the New World limited to a few species geographically restricted to the USA. These New World *Pholcus* species are considered to constitute a monophyletic group, and nine of the ten formally described species are known from the TGA area only (Tennessee, Georgia, Alabama), suggesting a relict distribution (Huber 2011).

Micropholcus was originally thought be an Old World genus, represented by only two species: the pantropical type species *M. fauroti* (Simon, 1887) and the putatively closely related *M. jacominae* Deeleman-Reinhold & van Harten, 2001 from the Arabian Peninsula (Yemen). Molecular data then suggested that numerous New World species running under the name *Leptopholcus* Simon, 1893 were in fact misplaced and more closely related to *M. fauroti* than to the type

species of *Leptopholcus* (Dimitrov et al. 2013). These misplaced species were formally transferred to *Micropholcus* in Huber et al. (2014), making *Micropholcus* appear much more diverse in the New than in the Old World. Geographically, New World *Micropholcus* appear largely restricted to the Greater Antilles and to semi-arid regions in Brazil (Huber et al. 2005; Huber and Wunderlich 2006; Huber et al. 2014).

In 2018, it was again molecular data that showed that Old World *Micropholcus* have a much wider distribution than suggested by the single known autochthonous species from the Arabian Peninsula. Huber et al. (2018) (based on the data in Eberle et al. 2018) transferred the Moroccan *Pholcus agadir* Huber, 2011 to *Micropholcus*, and an undescribed species from the Philippines was also placed in *Micropholcus*, as "*M*. Phi114". Here we review all the available data about Old World *Micropholcus*, describing the species from the Philippines and several additional new species from the Arabian Peninsula and Morocco, and redescribing previously published species as far as possible.

Materials and methods

Material examined

This study is based on the examination of 362 adult specimens of Old World *Micropholcus*, deposited in the following institutions: Muséum d'histoire naturelle, Genève (**MHNG**); Museum of Arthropods, College of Food and Agriculture Sciences, King Saud University, Riyadh (**KSMA**); Naturalis Biodiversity Center, Leiden (**RMNH**), and Zoologisches Forschungsmuseum Alexander Koenig, Bonn (**ZFMK**). Further material deposited in Institut Royal des Sciences Naturelles de Belgique (Brussels), Collection Carles Ribera (Barcelona), and Collection Robert Bosmans (Ghent), was examined by the first author in 2010 (Huber 2011) but not re-examined for the present study.

Taxonomy and morphology

Taxonomic descriptions follow the style of recent publications on Pholcidae (e.g., Huber 2019; based on Huber 2000). Measurements were done on a dissecting microscope with an ocular grid and are in mm unless otherwise noted; eye measurements are +/- 5 µm. Photos were made with a Canon EOS 2000D digital camera mounted on a Nikon SMZ18 stereo microscope or a Nikon Coolpix 995 digital camera mounted on a Leitz Dialux 20 compound microscope. CombineZP (https://combinezp.software.informer.com/) was used for stacking photos. Drawings are partly based on photos that were traced on a light table and later improved under a dissecting microscope, or they were directly drawn with a Leitz Dialux 20 compound microscope using a drawing tube. Cleared female genitalia were stained with chlorazol black. The number of decimals in coordinates gives a rough indication of the accuracy of the locality data: four decimals means that the collecting site is within ~ 10 m of the indicated spot; three decimals: within ~ 100 m; two decimals: within ~ 1 km. The distribution maps were generated with ArcMap 10.0. For SEM photos, specimens were dried in hexamethyldisilazane (HMDS) (Brown 1993) and photographed with a Zeiss Sigma 300 VP scanning electron microscope. Abbreviations used in the

text: **ALE** = anterior lateral eye(s); **ALS** = anterior lateral spinneret(s); **AME** = anterior median eye(s); **L/d** = length/diameter; **PLS** = posterior lateral spinnerets; **PME** = posterior median eye(s); **PMS** = posterior median spinneret(s).

Molecular analysis

We newly generated CO1 barcodes of 19 specimens of *Micropholcus* (Table 1). To this we added previously published CO1 barcodes of eight further specimens of *Micropholcus* (from Astrin et al. 2006; Dimitrov et al. 2013; Eberle et al. 2018). We also included the barcode of "*Pholcus* sp." from Dimitrov et al. (2008) because a separate phylogenetic analysis (pers. obs.; G. Meng, D. Dimitrov, L. Podsiadlowski, pers. comm. 26 July 2024) had suggested that this sequence is in fact based on a representative of *Micropholcus*. Finally, we added one outgroup species belonging to the closely related genus *Micromerys* Bradley, 1877 (from Eberle et al. 2018), and (for rooting the tree) *Artema bahla* Huber, 2019 (from Eberle et al. 2018).

One or two legs of specimens stored in non-denatured pure ethanol (~ 99%) at -20 °C were used for DNA extraction. Extracted genomic DNA is deposited at and available from the LIB Biobank, Museum Koenig, Bonn. DNA was extracted and amplified as in Huber et al. (2024a). The CO1 primers used were LCO1490-JJ and HCO2198-JJ (Astrin et al. 2016; primer versions JJ2 served as backup). PCR products were sent for bidirectional Sanger sequencing to Macrogen (Amsterdam, The Netherlands).

CO1 barcode assembly, confirmation of source, and barcode alignment was as in Huber et al. (2024a). A neighbor-joining (NJ) tree (Saitou and Nei 1987) and genetic distances among specimens were calculated using the Kimura 2-parameter model (Kimura 1980) in MEGA 11 (Tamura et al. 2021), during which pairwise deletion of gaps in the alignment was applied. The NJ tree was assessed with 5000 bootstrap replications (Felsenstein 1985). The online tool iTOL (v. 6.9) (Letunic and Bork 2021; https://itol.embl.de/) was used for tree visualisation.

The online tool ASAP Web (https://bioinfo.mnhn.fr/abi/public/asap/; Puillandre et al. 2021) was used for species delimitation using the CO1 MSA. The Kimura 2-parameter (K2P) model (Kimura 1980) was applied for genetic distance calculation. Ten delimitation schemes with best scores (the smaller the better) were kept. At every stage of the process, ASAP groups objects (either a node or a specimen) within the same distance range into a node, each node at each stage has its own probability calculated. If a node's probability falls below the specified threshold, ASAP will adjust the number of potential species, splitting any nodes with probabilities below the threshold (Puillandre et al. 2021). We used the default probability cutoff 0.01. Ten delimitation schemes with the best scores were kept.

Results

Molecular analysis

The CO1 NJ-tree (Fig. 1) is primarily intended to illustrate inter- and intraspecific distances. The exact individual values are shown in Table S1. Our sample of intraspecific distances is very low (5), and most distances are below 2%.

lable 1	י ספטטומטוווג טווטווא מווח כ							
Code	Genus and species	Vial	Country	Admin	Locality	Lat and Long	C01	Source
S497	Artema bahla	0m33	Oman	Ad Dakhiliya	W of Bahla	22.9340, 57.0840	MG268735	Eberle et al. 2018
S297	Micromerys yidin	QMB8	Australia	Queensland	Kings Plains, Cooktown	-15.4850, 145.2560	MG268724	Eberle et al. 2018
GB37	Micropholcus baoruco	DR/100-42	Dom. Rep.	Barahona	near Polo	18.1133, -71.2700	MG268886	Eberle et al. 2018
P0165	Micropholcus hispaniola	DR/100-15	Dom. Rep.	La Vega	near La Ciénaga	19.0500, -70.8833	JX023558	Dimitrov et al 2013
Mic02	Micropholcus dalei	PuR002	Puerto Rico	Rio Grande	El Yunque, Big Trees Trail	18.3087, -65.7752	KF715606	Huber et al. 2014
P0193	Micropholcus fauroti	Cam129	Cameroon	Centre Region	Yaoundé	3.8833, 11.5233	JX023574	Dimitrov et al 2013
JA66	Micropholcus fauroti	G038	Cuba	La Habana	La Habana	23.1200, -82.4200	DQ667902	Astrin et al. 2006
UH448	Micropholcus khenifra	Mor102	Morocco	Béni Mellal-Khénifra	near Sidi Ben Daoud	32.5347, -6.1285	PQ066288	NEW
S02	Micropholcus khenifra	Mor103	Morocco	Béni Mellal-Khénifra	W of El Ksiba	32.5610, -6.0515	PQ066277	NEW
UH446	Micropholcus ghar	Mor100	Morocco	Fès-Meknès	Kef el Ghar	34.4788, -4.2766	PQ066290	NEW
PUB6	Micropholcus sp.	1	Morocco	1		T	EU215669	Dimitrov et al. 2008
S321	Micropholcus agadir	Sieg11	Morocco	Souss-Massa-Draa	Agadir	30.4296, -9.6186	MG268754	Eberle et al. 2018
UH034	Micropholcus Br15-152	Br15-262	Brazil	Rio Grande do Norte	near Baraúna, Furna Feia cave	-5.0365, -37.5603	PQ066285	NEW
UH566	Micropholcus bukidnon	Phi250	Philippines	Mindanao	Barangay San Jose, Blue Water Cave	7.7060, 125.0320	PQ066291	NEW
S07	Micropholcus bukidnon	Phi250	Philippines	Mindanao	Barangay San Jose, Blue Water Cave	7.7060, 125.0320	PQ066279	NEW
GB38	Micropholcus piaui	Carv1	Brazil	Piauí	Castelo do Piauí, Parque Municipal da Pedra de Castelo	-5.2017, -41.6875	MG268905	Eberle et al. 2018
UH036	Micropholcus Br16-23	Br16-241	Brazil	Pará	Serra Pelada	-5.9310, -49.6740	PQ066286	NEW
UH354	Micropholcus evaluna?	Ven20-178	Venezuela	Miranda	El Ávila National Park, near La Julia	10.5012, -66.8111	PQ066287	NEW
GB39	Micropholcus ubajara	Carv17	Brazil	Ceará	Parque Nacional de Ubajara, Gruta de Morcego Branco	-3.8325, -40.8998	MG268847	Eberle et al. 2018
UH452	Micropholcus shaat	0m137	Oman	Dhofar	Shaat sinkhole	16.7740, 53.5870	PQ066289	NEW
S12	Micropholcus tanomah	SA100	Saudi Arabia	Asir	NW of Tanomah	19.0220, 42.1247	PQ066282	NEW
S11	Micropholcus bashayer	8496	Saudi Arabia	Asir	NW of AI Bashayer	19.8194, 41.8824	PQ066276	NEW
60S	Micropholcus maysaan	SA88	Saudi Arabia	Al Bahah	NW of Al Bahah	20.2095, 41.3700	PQ066284	NEW
S20	Micropholcus maysaan	SA140	Saudi Arabia	Mecca	NW of Maysaan	20.7717, 40.7985	PQ066278	NEW
UH565	Micropholcus darbat	0m133	Oman	Dhofar	Wadi Darbat	17.0900, 54.4500	PQ066292	NEW
S05	Micropholcus darbat	0m147	Oman	Dhofar	Ain Athoom	17.1185, 54.3667	PQ066275	NEW
S14	Micropholcus abha	SA111	Saudi Arabia	Asir	N of Abha	18.4168, 42.4646	PQ066283	NEW
S16	Micropholcus harajah	SA114	Saudi Arabia	Asir	SE of Harajah	17.8681, 43.3943	PQ066281	NEW
S15	Micropholcus alfara	SA112	Saudi Arabia	Asir	S of Al Fara	18.0487, 42.7096	PQ066280	NEW
S17	Micropholcus dhahran	SA121	Saudi Arabia	Asir	W of Dhahran Al Janub	17.7010, 43.3891	PQ066274	NEW

ZooKeys 1213: 95-182 (2024), DOI: 10.3897/zookeys.1213.133178



Figure 1. Neighbor-joining tree of analysed CO1 sequences using the Kimura 2-parameter model; numbers on the branches are bootstrap supports from 5000 replications (%). The labels North and South refer to two geographically and morphologically distinct species groups within Saudi Arabia.

Only the two sequenced specimens of *M. maysaan* sp. nov. have a higher distance, of 7.4%. This is also reflected in the ASAP analysis (Fig. 2), in which the partition with the best score splits *M. maysaan* sp. nov. into two species.

Interspecific distances within *Micropholcus* have a mean value of 19.8% (3.4%-25.7%). Only seven of the 371 distance values are at or below 10%. All of them refer to species within either the northern or southern Saudi Arabian groups. In the northern Saudi Arabian group, distances between *M. maysaan* sp. nov. and the other two species (*M. tanomah* sp. nov., *M. bashayer* sp. nov.) range from 8.0-10.0%. The distance between *M. tanomah* sp. nov. and *M. bashayer* sp. nov. is only slightly higher (10.2%). Despite these low values, the two partitions with the best scores in the ASAP analysis separate these three species.

In the southern Saudi Arabian group, *M. alfara* sp. nov. and *M. dhahran* sp. nov. are particularly close (3.4%); the distances of these two species to *M. harajah* sp. nov. are slightly higher (6.1–6.7%). Most partitions in the ASAP analysis resolve *M. alfara* sp. nov. and *M. dhahran* sp. nov. as a single species, and only the best (and the worst) resolve *M. harajah* sp. nov. as a distinct species.



Figure 2. ASAP species delimitation analysis based on CO1 data. The analysis was performed on the web server https:// bioinfo.mnhn.fr/abi/public/asap/. The Kimura 2-parameter model was applied. The ten best partitions are shown. The numbers for each column (partition) are: (1) Total number of species as identified by ASAP in the corresponding partition. (2) Score, an indicator of how good a partition is (the lower the score, the better the partition). (3) Rank of the scores. Note that the partition with the lowest (best) score splits *M. maysaan* sp. nov. into two species but joins *M. alfara* sp. nov. and *M. dhahran* sp. nov.

Taxonomy

Order Araneae Clerck, 1757 Family Pholcidae C.L. Koch, 1850

Genus Micropholcus Deeleman-Reinhold & Prinsen, 1987

Micropholcus Deeleman-Reinhold & Prinsen, 1987: 73; type species: *Pholcus fauroti* Simon, 1887.

Micropholcus – Deeleman-Reinhold and van Harten 2001: 199. Huber 2011: 24. Huber et al. 2014: 435. Mariguitaia González-Sponga, 2004: 66; type species: Mariguitaia divergentis Gonzalez-Sponga 2004. Synonymised in Huber 2009.

Diagnosis. Old World species are long-legged, eight-eyed pholcids with an oval abdomen (Figs 3, 4); New World species are more variable, sometimes without AME, sometimes with elongate to worm-shaped abdomens. Most known species (except *M. bukidnon* sp. nov.) with unique modified hair at tip of male palpal trochanter apophysis (Fig. 9A–D; see also Huber 2000: figs 105, 106; Huber and Wunderlich 2006: figs 4h, 8d; Huber 2011: fig. 95; Huber et al. 2014: figs 32, 57). Male chelicerae with frontal apophyses with modified hairs (Fig. 6), i.e., similar to putative sister genus *Cantikus* Huber but different from other close relatives (*Leptopholcus* Simon, *Pehrforsskalia* Deeleman-Reinhold & van Harten, and *Micromerys* Bradley). Procursus and bulb morphology highly variable and not diagnostic at genus level (contra Huber 2011). Females not diagnosable morphologically at genus level (highly variable and similar to closely related genera).

Note. Most parts in this general section about *Micropholcus* refer to the entire genus. The following description is limited to Old World taxa because they are relatively homogeneous, while some New World species (in particular those on the Caribbean islands) are superficially extremely different from South American (in particular Brazilian) and Old World species.

Description (Old World taxa). Male. *Measurements.* Total body length ~ 2.3–3.9. Carapace width 0.8–1.5. Diameter PME 60–100 μ m; diameter AME usually 35–55 μ m, in *M. bukidnon* sp. nov. only 15 μ m. Tibia 1 length 5.0–10.2. Tibia 1 L/d: 57–85. Leg formula 1243. Diameters of leg femora (at half length) 80–150 μ m, of leg tibiae 75–120 μ m.

Colour (in ethanol). Prosoma and legs pale ochre-yellow to grey, carapace with dark pattern, legs with darker patellae and tibia-metatarsus joints; abdomen ochre-grey to whitish, monochromous or with whitish marks. Live specimens (Figs 3, 4) similar in colour but slightly darker.

Body. Ocular area slightly raised (distinct in frontal view; Fig. 5). Carapace without thoracic groove. Clypeus unmodified. Sternum slightly wider than long, unmodified. Abdomen approximately twice as long as wide. Gonopore with four (rarely five) epiandrous spigots (Fig. 10; see also Huber 2000: fig. 123; Huber 2011: fig. 99). ALS with one strongly widened spigot, one long pointed spigot, and six cylindrical spigots (Fig. 9; see also Huber 2000: figs 158, 159; Huber 2011: fig. 100); PMS with two conical spigots; PLS without spigots.

Chelicerae. Chelicerae with pair of strong frontal apophyses provided with conical or globular, strongly sculptured modified hairs (Fig. 6; see also Huber 2011: fig. 98), usually with one or two pairs of proximal processes; without stridulatory files.

Palps. Palpal coxa unmodified. Trochanter with retrolateral-ventral apophysis usually with distinctive modified (short cylindrical) hair at tip (Fig. 9A–D), modified hair absent in *M. bukidnon* sp. nov. Femur variable in shape, often with rounded processes (usually ventrally and retrolaterally, sometimes also dorsally). Femur-patella joints shifted towards prolateral side. Tibia larger than femur, with two trichobothria. Tibia-tarsus joints shifted towards retrolateral side. Palpal tarsus with large capsulate tarsal organ (Fig. 11H, I; see also Huber 2011: fig. 97), outer diameter 30–35 µm, diameter of opening 15–20 µm; more open (almost exposed) in *M. bukidnon* sp. nov. (Fig. 11J). Procursus complex,



Figure 3. *Micropholcus* Deeleman-Reinhold & Prinsen; live specimens from Saudi Arabia **A** *M. dhahran* Huber, sp. nov., male from 'Asir, W of Dhahran Al Janub **B** *M. harajah* Huber, sp. nov., female with egg-sac from 'Asir, SE of Harajah **C, D** *M. alfara* Huber, sp. nov., male and female with egg-sac from 'Asir, S of Al Fara **E** *M. abha* Huber, sp. nov., male from 'Asir, N of Abha **F** *M. tanomah* Huber, sp. nov., male from 'Asir, NW of Tanomah **G** *M. bashayer* Huber, sp. nov., female with egg-sac from 'Asir, NW of Al Bashayer **H** *M. maysaan* Huber, sp. nov., male from Mecca, NW of Maysaan. Photographs BAH.

often with distinct dorsal hinged process (e.g., Figs 16C, 43C, 52C), in most species with transparent prolateral membranous flap densely set with teeth (Figs 7A, D, 8B). Genital bulb with distinct proximal sclerite connecting to tarsus, membranous or partly sclerotised embolus, and variably complex set of



Figure 4. *Micropholcus* Deeleman-Reinhold & Prinsen; live specimens from Oman, Morocco, and the Philippines **A**, **B** *M. darbat* Huber, sp. nov., male and female with egg-sac from Oman, Dhofar, near Qairoon Hairitti **C** *M. shaat* Huber, sp. nov., male from Oman, Dhofar, Shaat sinkhole **D**, **E** *M. agadir* (Huber), male and female with egg-sac from Morocco, Souss-Massa, Paradise Valley **F** *M. ghar* Huber, sp. nov., male from Morocco, Fès-Meknès, Kef El Ghar **G** *M. khenifra* Huber, Lecigne & Lips, sp. nov., male from Morocco, Béni Mellal-Khénifra, near Sidi Ben Daoud **H** *M. bukidnon* Huber, sp. nov., male from Philippines, Mindanao, Blue Water Cave. Photographs BAH.



Figure 5. *Micropholcus* Deeleman-Reinhold & Prinsen; SEM images of prosomata (frontal views) and tips of female palps (dorsal views) **A** *M. tanomah* Huber, sp. nov.; female **B** *M. darbat* Huber, sp. nov.; male **C, D** *M. ghar* Huber, sp. nov.; male and female **E, F** *M. bukidnon* Huber, sp. nov.; male and female; note small AME **G** *M. tanomah* Huber, sp. nov., left palp **H** *M. ghar* Huber, sp. nov., right palp. Scale bars: 100 μm (**A**–**D**); 200 μm (**E, F**); 10 μm (**G, H**).



Figure 6. *Micropholcus* Deeleman-Reinhold & Prinsen; SEM images of male chelicerae: distal apophyses with modified hairs and total view (**D**) **A**, **B** *M*. *alfara* Huber sp. nov. **C** *M*. *tanomah* Huber, sp. nov. **D**, **E** *M*. *darbat* Huber, sp. nov. **F**, **G** *M*. *ghar* Huber, sp. nov. **H** *M*. *bukidnon* Huber, sp. nov. Scale bars: 10 µm (**A–C**, **F–H**); 100 µm (**D**); 2 µm (**E**).



Figure 7. *Micropholcus* Deeleman-Reinhold & Prinsen; SEM images of male palpal structures **A**, **B** *M. alfara* Huber, sp. nov.; prolateral membranous flap on left procursus and right bulbal processes, prolateral view **C**, **D** *M. tanomah* Huber, sp. nov., tip of left procursus and prolateral membranous flap on left procursus **E** *M. tanomah* Huber, sp. nov., left procursus, dorsal view (bold arrow points at tip of procursus) **F**, **G** *M. tanomah* Huber, sp. nov., left bulbal processes, prolateral view, and embolus of same palp in slightly more distal view **H** *M. darbat* Huber, sp. nov., left procursus, retrolateral view. Abbreviations: b, genital bulb; e, embolus; hp, dorsal hinged process; mf, membranous prolateral flap; rr, retrolateral ridge; ta, tarsus; tm, transparent membrane. Scale bars: 10 μm (**A**); 100 μm (**B**, **E**, **F**, **H**); 20 μm (**C**, **D**, **G**).



Figure 8. *Micropholcus* Deeleman-Reinhold & Prinsen; SEM images of male palpal structures **A**, **B** *M*. *darbat* Huber, sp. nov.; right bulb and procursus, prolateral view (bold arrow in A points at trochanter apophysis), and prolateral membranous flap of procursus at higher magnification **C** *M*. *darbat* Huber, sp. nov., left bulbal processes **D** *M*. *ghar* Huber, sp. nov., left procursus, retrolateral view **E**, **F** *M*. *ghar* Huber, sp. nov., right bulbal processes, prolateral and prolateral-ventral views **G** *M*. *bukidnon* Huber, sp. nov., left procursus, prolateral-distal view **H** *M*. *bukidnon* Huber, sp. nov., left bulbal processes, prolateral distal view. Abbreviations: a, putative appendix; b, genital bulb; e, embolus; hp, dorsal hinged process; mf, membranous prolateral flap; pr, procursus; ta, tarsus; u, putative uncus. Scale bars: 100 μm (**A**, **D**–**H**); 10 μm (**B**); 20 μm (**C**).


Figure 9. *Micropholcus* Deeleman-Reinhold & Prinsen; SEM images of male palpal trochanter tips and of spinnerets **A***M. alfara* Huber, sp. nov. **B***M. tanomah* Huber, sp. nov. **C***M. darbat* Huber, sp. nov. **D***M. ghar* Huber, sp. nov. **E***M. bukidnon* Huber, sp. nov. **F**, **G***M. tanomah* Huber, sp. nov., male ALS, and male spinnerets and anal cone (asterisk) **H**, **I***M. ghar* Huber, sp. nov., male ALS and male spinnerets **J***M. bukidnon* Huber, sp. nov., male ALS. Scale bars: 10 μm (**A**–**F**, **H**, **J**), 100 μm (**G**), 20 μm (**I**).



Figure 10. *Micropholcus* Deeleman-Reinhold & Prinsen; SEM images of male gonopores with epiandrous spigots and of female epigyna **A**, **B** *M*. *tanomah* Huber, sp. nov. **C**, **D** *M*. *darbat* Huber, sp. nov. **E**, **F** *M*. *ghar* Huber, sp. nov. **G**, **H** *M*. *bukidnon* Huber, sp. nov. Abbreviations: aep, anterior epigynal plate; k, epigynal 'knob'; pep, posterior epigynal plate. Scale bars: 10 μm (**A**, **G**); 100 μm (**B**, **D**, **F**, **H**); 20 μm (**C**, **E**).



Figure 11. *Micropholcus* Deeleman-Reinhold & Prinsen; SEM images of epigynal knobs (**A**–**C**), trichobothria (**D**), and tarsal organs (**E**–**J**) **A** *M*. *tanomah* Huber, sp. nov. **B** *M*. *darbat* Huber, sp. nov. **C** *M*. *ghar* Huber, sp. nov. **D** *M*. *alfara* Huber, sp. nov., female left palpal tibia **E** *M*. *alfara* Huber, sp. nov., male right tarsus 2 **F** *M*. *tanomah* Huber, sp. nov., female left tarsus 2 **G**, **H** *M*. *darbat* Huber, sp. nov., female right tarsus 2 and male palpal tarsus I M. *ghar* Huber, sp. nov., male palpal tarsus **J** *M*. *bukidnon* Huber, sp. nov., male palpal tarsus. Scale bars: 20 µm (**A**); 10 µm (**B**–**D**, **H**–**J**); 2 µm (**E**–**G**).

sclerotised apophyses, sometimes likely homologues of the *Pholcus* 'appendix' and 'uncus', sometimes of uncertain homology (Figs 7B, F, 8C, E, F, H).

Legs. Without spines and curved hairs. Without slender metatarsal hairs (cf. Huber et al. 2023a). Without sexually dimorphic short vertical hairs. Chemoreceptive hairs ~ 20–25 µm long, with few side branches (Fig. 12A, H), mostly near leg tips. Retrolateral trichobothrium of tibia 1 at 5–10% of tibia length. Prolateral trichobothrium absent on tibia 1, present on tibiae 2–4. Base of trichobothria evenly rounded, without proximal ridge (cf. Fig. 11D). Legs with roundish cuticular plates (Fig. 12E; diameter ~ 6–8 µm) and rimmed pores (Fig. 12B; outer diameter 2 µm, diameter of opening 0.2 µm) apparently on all leg segments. Tarsus 1 with ~ 20–30 pseudosegments, distally usually fairly distinct. Leg tarsal organs capsulate (Figs 11E–G, 12F; diameter 12–17 µm, diameter of opening 5–7 µm). Tarsus 4 with single row of comb-hairs on prolateral side (Fig. 12G, H; see also Huber 2011: fig. 96). Main tarsal claws with ~ 10 teeth (Fig. 12 C, D).

Female. In general, very similar to males (Figs 3, 4). Legs slightly shorter than in males (male / female tibia 1 length: ~ 1.1-1.4, but sample sizes mostly small); tibia 1 length 4.3-9.1. Palpal tarsal organ smaller than in males (outer diameter: $18-25 \mu m$, diameter of opening: $6-10 \mu m$). Palpal tarsus ending distally in pointed tip and pair of strong dorsal hairs (Fig. 5G, H). Spinnerets, leg hairs, cuticular plates, rimmed pores, comb-hairs, leg tarsal organs, and tarsal claws as in male. Epigynum anterior plate usually weakly sclerotised, with rounded process ('knob'; Fig. 11A-C) in varying position; posterior epigynal plate short and indistinct. Internal genitalia often complex, highly variable, with distinct pair of pore plates.

Distribution. The type species *Micropholcus fauroti* has attained a circumtropical distribution, with most records from between 25°S and 30°N (Fig. 5A).

Micropholcus is one of only two Pholcidae genera (together with *Pholcus*) with autochthonous species in both the Old and New Worlds. New World species are mostly known from the Greater Antilles and from semi-arid regions in Brazil; the genus seems to be largely absent from the humid regions of the Amazon basin. Old World species are currently known from the Arabian Peninsula, Morocco, and the Philippines (Fig. 5B–D). Specimens have been collected from sea level to 2370 m. In Saudi Arabia, all new species described herein were collected above 1200 m. Several of the localities visited below 1000 m had suitable habitats but no *Micropholcus*.

Natural history. Old World *Micropholcus* seem to be very homogeneous with respect to their preferred microhabitats. Most species have been collected from rocks: in caves and at cave entrances, in small caverns of rock walls, and on the undersides of large boulders (Fig. 14). They share this type of microhabitat with most South American species, and with the majority of species of the putative sister genus (*Cantikus*), suggesting that this might be the plesiomorphic microhabitat. The unusual microhabitat reported for *M. jacominae* (dry plant debris in an irrigated banana plantation) needs confirmation. The synanthropic *M. fauroti* is usually found in the upper corners and edges of rooms. Caribbean species have shifted to the undersides of leaves, probably explaining their significantly different general body shape and colour (see Huber and Wunderlich 2006; Huber et al. 2014).

Old World and Brazilian *Micropholcus* spiders build fine dome-shaped webs but during the day, most species (except those deeper in caves, e.g., *M. ghar* sp. nov.) sit flat on the rock surface (Fig. 15). The webs of leaf-dwelling Caribbean species have not yet been described. Upon disturbance, the spiders show a range of



Figure 12. *Micropholcus* Deeleman-Reinhold & Prinsen, SEM images of leg structures **A** *M. tanomah* Huber, sp. nov., putative chemoreceptor **B** *M. tanomah* Huber, sp. nov., rimmed pore (arrow) on left tarsus 3 **C**, **D** *M. tanomah* Huber, sp. nov., tarsal claws of left legs 1 and 3 **E** *M. ghar* Huber, sp. nov., cuticular plate (arrow) and regular mechanoreceptor on right metatarsus 3 **F** *M. ghar* Huber, sp. nov., pseudosegmentation (and tarsal organ) of right tarsus 3 **G** *M. ghar* Huber, sp. nov., comb-hairs on male tarsus 4 **H** *M. bukidnon* Huber, sp. nov., comb-hairs on male tarsus 4. Scale bars: 2 μm (**A**); 1 μm (**B**); 20 μm (**C**); 10 μm (**D**–**H**).

reactions, from refusing to move, bouncing, walking or running away, to dropping out of the web. Egg sacs are round (in Caribbean species elongated), covered by a barely visible sparse layer of silk (Figs 3, 4), and contain up to ~ 35 eggs; egg diameters range from 0.54 to 0.71 mm. For more detailed observations on Brazilian species, see Huber et al. (2014). For detailed life history data of *M. fauroti* under lab conditions see Ahmad and Abou-Setta (2017). For anecdotal observations on further Old World species, see individual species descriptions below.

Relationships. The molecular analysis of Eberle et al. (2018) placed *Micropholcus* as sister to the South-East Asian genus *Cantikus* Huber, 2018, within a clade that included three further Old World genera: *Leptopholcus* Simon, 1893; *Pehrforsskalia* Deeleman-Reinhold & van Harten, 2001; and *Micromerys* Bradley, 1877. That analysis included ten species of *Micropholcus*: the type species *M. fauroti*, two further Old World species (*M. agadir* and *M. bukidnon* sp. nov.) and seven New World species. The monophyly of the genus received maximum support. New World species were nested within a paraphyletic Old World group.

Our NJ tree (Fig. 1) is not expected to reliably reflect phylogenetic relationships. However, some clades that receive reasonable to high support are either congruent with the results of Eberle et al. (2018) (Caribbean clade) or include geographically neighbouring species: the Moroccan clade, the southern Saudi Arabian clade, and the northern Saudi Arabian clade. The latter two are also supported by several morphological similarities each, but it is not clear which of these are synapomorphies and which not. Thus, relationships within *Micropholcus* are largely unresolved and Fig. 1 should not be misinterpreted in a phylogenetic context.

Composition. The genus now includes 30 described species: the Dominican amber fossil *M. kiskeya* (Huber & Wunderlich, 2006) and 29 extant species. Of the latter, seven occur in South America, six on the Caribbean islands, and 16 in the Old World. All Old World species are treated below except for *M. tegulifer* Barrientos, 2019 (a loan request was denied by the curator of arthropods, Museu de Ciències Naturals de Barcelona). Numerous undescribed New World species are available in collections, in particular from Brazil (L.S. Carvalho, pers. comm. 2 July 2020). At least one further undescribed species is known to occur in Morocco,



Figure 13. Known distribution of *Micropholcus fauroti* (Simon) (A) and of Old World *Micropholcus* in Mindanao (B), on the Arabian Peninsula (C) and in Morocco (D).



Figure 14. Typical habitats of *Micropholcus* Deeleman-Reinhold & Prinsen in the Old World **A** Saudi Arabia, 'Asir, SE of Harajah (type locality of *M. harajah* Huber, sp. nov.) **B** Saudi Arabia, 'Asir, S of Al Fara (type locality of *M. alfara* Huber, sp. nov.) **C** Saudi Arabia, 'Asir, N of Abha (type locality of *M. abha* Huber, sp. nov.) **D** Saudi Arabia, Mecca, NW of Maysaan (type locality of *M. maysaan* Huber, sp. nov.) **E** Oman, Dhofar, near Shaat sinkhole (type locality of *M. shaat* Huber, sp. nov.) **F** Morocco, Fès-Meknès, Kef El Ghar (type locality of *M. ghar* Huber, sp. nov.) **G** Morocco, Béni Mellal-Khénifra, Imi n'Ifri (*M. khenifra* Huber, Lecigne & Lips, sp. nov.) **H** Philippines, Mindanao, Kabyaw Cave (*M. bukidnon* Huber, sp. nov.). Photos BAH.



Figure 15. Section of cave ceiling (16 × 11 cm), showing nine adult specimens of *M. tanomah* Huber, sp. nov.; from NW of Tanomah, 'Asir, Saudi Arabia.

represented by a single male specimen deposited in the Muséum d'histoire naturelle, Genève, Switzerland ("sp. Gen377" in Fig. 13D). It resembles *M. tegulifer* but has a very different uncus. It originates from the Gorges du Dades area in the Drâa-Tafilalet Region, ~ 31.535°N, 5.918°W. Our molecular data indicate that the "*Pholcus* sp." CO1 barcode published in Dimitrov et al. (2008) is also from a *Micropholcus*, different from *M. agadir, M. ghar* sp. nov., and *M. khenifra* sp. nov. (Figs 1, 2). It could be *M. tegulifer, M.* sp. Gen377, or a different new species. Its geographic origin cannot be reconstructed, and the only available (juvenile) specimen is lost (D. Dimitrov and C. Ribera, pers. comm. 20 Mar. and 19 Apr. 2024).

Micropholcus fauroti (Simon, 1887) Figs 16–18

Notes. For synonymy, type information, and redescription, see Huber (2011). Numerous further records and an updated distribution map were published in Huber et al. (2017). Since then, new records have been published for Egypt (Ahmad and Abou-Setta 2017), Sri Lanka (Huber 2019), Venezuela (Huber and Villarreal 2020) and India (Vishnudas and Sudhikumar (2021). The map in Fig. 13A summarises all the previous records plus the new records below.

New records. COLOMBIA: La Guajira • 3 ♂♂, 5 ♀♀; Palomino; 11.2451°N, 73.5619°W; 10 m a.s.l.; in building; 17 Sep. 2022; B.A. Huber leg.; ZFMK Ar 24653. MEXICO: Guerrero • 1 ♂, 3 ♀♀; Coyuca de Benitez; 17.0075°N, 100.0893°W; 20 m a.s.l.; in building; 3 Oct. 2019; B.A. Huber leg.; ZFMK Ar 24654. Nuevo



Figure 16. *Micropholcus fauroti* (Simon, 1887); male from Mexico, Nuevo León, Santiago (ZFMK Ar 24655). Left palp in prolateral (**A**), dorsal (**B**), and retrolateral (**C**) views. Abbreviations: b, genital bulb; c, coxa; f, femur; hp, dorsal hinged process; pa, patella; pr, procursus; ta, tarsus; ti, tibia; tr, trochanter. Scale bar: 0.3 mm.

León • 1 \circlearrowright , 8 \bigcirc \bigcirc , 1 juv.; Santiago; 25.4237°N, 100.1463°W; 450 m a.s.l.; in building; 14 Oct. 2019; B.A. Huber leg.; ZFMK Ar 24655 • 5 \circlearrowright \circlearrowright , 5 \bigcirc \bigcirc , in pure ethanol (one male palp and two female abdomens transferred to ZFMK Ar 24655); same collection data as for preceding; ZFMK Mex286.

UNITED ARAB EMIRATES: **Dubai** • 2 $\Im \Im$, 1 \bigcirc ("*Micropholcus* cf. *fauroti*" in Feulner and Roobas 2016); Dubai, near Emirates Towers; 25.219°N, 55.282°E; 5 m a.s.l.; 3 Jun. 2015; G.R. Feulner leg.; ZFMK Ar 24680.

OMAN: **Ash Sharqiyah South** • 3 $33, 5 \oplus 9, 2$ juvs; Wadi Tiwi; 22.801°N, 59.240°E; on banana leaves; 60 m a.s.l.; 22 Mar. 2017; B.A. Huber leg.; ZFMK Ar 24656 • 1 3, 2 juvs, in pure ethanol; same collection data as for preceding; ZFMK Om27.

Diagnosis. Males are easily distinguished from known congeners by long and slender dorsal hinged process on procursus (Fig. 17C; similar but relatively shorter in some Saudi Arabian species, cf. Figs 24C, 40C, etc.); also by unique prolateral process on procursus (arrowed in Fig. 17A) and by unique shapes of processes of genital bulb (Fig. 17D, E). Females are distinguished by distinct U-shaped internal structure visible through epigynal plate in uncleared specimens (Fig. 18A); similar dark internal structures occur in some Saudi Arabian species (e.g., Figs 26A, 30A); also by very large pore plates and large anterior membranous element of internal genitalia (Fig. 18D).

Micropholcus jacominae Deeleman-Reinhold & van Harten, 2001 Figs 19–22

Micropholcus jacominae Deeleman-Reinhold & van Harten, 2001: 199, figs 17, 18, 21–26 (♂♀).

Material examined. YEMEN – **Al Mahwit** • 1 ♂, 1 ♀, paratypes; Khamis Bani Sa'd; 15.185°N, 43.510°E (see Note below); 490 m a.s.l.; 11 Oct. 1999; A. van Harten leg.; RMNH ARA 15019.

Note. The coordinates in Deeleman-Reinhold and van Harten (2001) (15°11'N, 43°25'E) mark a spot 10 km W of Khamis Bani Sa'd, at ~ 440 m a.s.l. (rather than 550 m as reported in the original description). We suspect that our coordinates above are closer to the actual collecting site that could only be reconstructed as being "close to Khamis Bani Sa'd, but not in the village" (A. van Harten, pers. comm. 22 Apr. 2021, 13 Mar. 2024).



Figure 17. *Micropholcus fauroti* (Simon, 1887); male from Mexico, Nuevo León, Santiago (ZFMK Ar 24655) **A–C** left procursus in prolateral, dorsal, and retrolateral views; arrow in A points at distinctive prolateral process **D**, **E** left genital bulb in prolateral and retrolateral views; bold arrows in D point at distinctive processes of unclear homology. Abbreviations: e, embolus; hp, dorsal hinged process; ps, proximal bulbal sclerite. Scale bars: 0.3 mm.



Figure 18. *Micropholcus fauroti* (Simon, 1887); female from Mexico, Nuevo León, Santiago (ZFMK Ar 24655) **A**, **B** abdomen, ventral and lateral views **C**, **D** cleared genitalia, ventral and dorsal views. Abbreviations: k, epigynal 'knob'; pp, pore plate. Scale bars: 0.5 mm (**A**, **B**); 0.2 mm (**C**, **D**).

Diagnosis. Easily distinguished from known congeners by numerous details of male palp: long ventral apophysis on trochanter (Fig. 19C; similar in some Saudi Arabian species, cf. Figs 27C, 31C); distinct dorsal process on femur (arrowed in Fig. 19C; similar only in *M. abha* sp. nov., cf. Fig. 35C); dorsal-distal hinged process on procursus short and directed towards prolateral (Fig. 20A–C); unique processes of genital bulb (Fig. 20E, F), and by female epigynum and internal genitalia (Figs 21C, 22): extensible knob in posterior position; pair of strongly curved lateral internal sclerites; long pore plates widening and only slightly converging anteriorly.



Figure 19. *Micropholcus jacominae* Deeleman-Reinhold & van Harten, 2001; male paratype from Yemen, Al Mahwit, Khamis Bani Sa'd (RMNH). Left palp in prolateral (**A**), dorsal (**B**), and retrolateral (**C**) views; arrow in C points at distinctive dorsal process on femur. Scale bar: 0.3 mm.

Redescription. Male. *Measurements.* Total body length 2.3, carapace width 0.85. Distance PME-PME 200 μ m; diameter PME 65 μ m; distance PME-ALE 20 μ m; distance AME-AME 25 μ m; diameter AME 45 μ m. Leg 1: 23.3 (5.9 + 0.4 + 5.7 + 10.2 + 1.1), tibia 2: 3.5, tibia 3: 2.1, tibia 4: 2.9; tibia 1 L/d: 76; diameters of leg femora (at half length) 0.08–0.09; of leg tibiae 0.07–0.08.

Colour (in ethanol). Prosoma and legs pale ochre-whitish, carapace with complex brown median mark similar to Saudi Arabian species (cf. Fig. 3); legs with darkened patellae and tibia-metatarsus joints; abdomen pale grey to whitish.

Body. Habitus similar to Saudi Arabian species (cf. Fig. 3). Ocular area raised (distinct in frontal view). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.50/0.40), unmodified. Abdomen oval, almost twice as long as wide.

Chelicerae. As in Fig. 21A, B; with pair of relatively long distal apophyses near laminae, each with two modified (cone-shaped) hairs; pair of proximal lateral processes weakly sclerotised and directed towards proximal; and pair of small but distinct proximal frontal processes.

Palps. As in Fig. 19; coxa unmodified; trochanter with long ventral apophysis with very indistinct retrolateral hump proximally and modified hair at tip (Fig. 20D); femur with weakly sclerotised but distinct processes retrolateral-dorsally and prolateral-ventrally; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 20A–C) proximally with sclerotised prolateral hump; at half length with prolateral sclerotised ridge transforming distally into transparent membrane, and brush of dorsal hairs; distally with retrolateral ridge, membranous ventral-prolateral flap, pointed prolateral process, and distinctive dorsal



Figure 20. *Micropholcus jacominae* Deeleman-Reinhold & van Harten, 2001; male paratype from Yemen, Al Mahwit, Khamis Bani Sa'd (RMNH) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D** tip of left trochanter apophysis **E**, **F** left genital bulb in prolateral and retrolateral views; bold arrows in E point at distinctive processes of unclear homology. Abbreviations: e, embolus; hp, dorsal hinged process; mf, membranous prolateral flap; ps, proximal bulbal sclerite; rr, retrolateral ridge; Scale bars: 0.3 mm (**A–C**, **E**, **F**); 0.05 mm (**D**).

hinged process. Genital bulb (Fig. 20E, F) with strong proximal sclerite; two sclerites of unclear homology, with two pointed processes each; and mostly semi-transparent short embolus, proximally sclerotised, distally membranous with pointed transparent extension.



Figure 21. *Micropholcus jacominae* Deeleman-Reinhold & van Harten, 2001; paratypes from Yemen, Al Mahwit, Khamis Bani Sa'd (RMNH) **A**, **B** male chelicerae, frontal and lateral views **C** cleared female genitalia, dorsal view. Abbreviations: k, epigynal 'knob'; pp, pore plates. Scale bars: 0.3 mm.



Figure 22. *Micropholcus jacominae* Deeleman-Reinhold & van Harten, 2001; female paratype from Yemen, Al Mahwit, Khamis Bani Sa'd (RMNH) **A** epigynum, ventral view **B**, **C** cleared female genitalia, ventral and dorsal views. Scale bars: 0.3 mm.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs (many hairs missing); retrolateral trichobothrium of tibia 1 at 8%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 20 pseudosegments, distally distinct.

Female. In general very similar to male but ocular area slightly less raised and triads closer together (PME-PME 170 μ m), carapace pattern more fragmented than in male. Tibia 1: 4.4. Epigynum (Fig. 22A) anterior plate oval, protruding, with membranous, possibly expandable knob in posterior position, tip directed towards posterior; lateral internal sclerites clearly visible in untreated specimens; posterior epigynal plate very short and indistinct. Internal genitalia (Figs 21C, 22B, C) with pair of long pore plates converging and widening anteriorly, pair of lateral sclerites, and transversal ventral sclerotised band; with sclerotised anterior arc continued to posterior margin.

Distribution. Known from type locality only, in western Yemen (Fig. 13C).

Natural history. Deeleman-Reinhold and van Harten (2001) report that the spiders were shaken from dry plant debris in an irrigated banana plantation. This microhabitat is unusual for *Micropholcus* on the Arabian Peninsula and needs confirmation.

Micropholcus dhahran Huber, sp. nov.

https://zoobank.org/2D3B13EE-4099-47C8-A36F-6DC154FA3742 Figs 3A, 23-26

Type material. *Holotype*. SAUDI ARABIA – '**Asir** • ♂; W of Dhahran Al Janub, 'site 2'; 17.7010°N, 43.3891°E; 2000 m a.s.l.; 24 Mar. 2024; B.A. Huber leg.; KSMA. *Paratypes*. SAUDI ARABIA – '**Asir** • 2 ♂♂, 1 ♀, and 1 cleared ♀ abdomen; same collection data as for holotype; ZFMK Ar 24657.

Other material. SAUDI ARABIA – **'Asir** • 1 \bigcirc (abdomen cleared and transferred to ZFMK Ar 24657), in pure ethanol; same collection data as for holotype; ZFMK SA121.

Diagnosis. Distinguished from known congeners by unique shapes of bulbal processes (Fig. 24D, E; similar only in *M. abha* sp. nov. but without strong retrolateral spine; without distinctive pointed prolateral sclerite as in geographically closest species, *M. harajah* sp. nov. and *M. alfara* sp. nov.); from most congeners (except *M. harajah* sp. nov. and *M. alfara* sp. nov.) by rectangular dorsal hinged process of procursus with obtuse tip and ventral terminal pointed process and small proximal spine (in other Saudi Arabian species procursus wider and more curved dorsally, without proximal spine; in *M. fauroti* relatively longer and without spine); most congeners (except for three species above and *M. jacominae* and *M. darbat* sp. nov.) also by long trochanter apophysis (Fig. 23C; longer than palpal femur). Female with pair of distinct internal crescent-shaped structures (arrows in Fig. 25C), similar only in *M. harajah* sp. nov. and *M. alfara* sp. nov., but without external pockets as in *M. harajah* sp. nov., and anterior arc wider and with different curvature than in *M. alfara* sp. nov. and *M. alfara* sp. nov.

Description. Male (holotype). *Measurements.* Total body length 2.5, carapace width 0.8. Distance PME-PME 180 μ m; diameter PME 85 μ m; distance PME-ALE 20 μ m; distance AME-AME 20 μ m; diameter AME 55 μ m.



Figure 23. *Micropholcus dhahran* Huber, sp. nov.; male from Saudi Arabia, 'Asir, W of Dhahran Al Janub (ZFMK Ar 24657). Left palp in prolateral (**A**), dorsal (**B**), and retrolateral (**C**) views. Scale bar: 0.3 mm.

Leg 1: 27.1 (6.9 + 0.4 + 6.7 + 12.0 + 1.1), tibia 2: 4.0, tibia 3: 2.1, tibia 4: 3.1; tibia 1 L/d: 84; diameters of leg femora (at half length) 0.09-0.10; of leg tibiae 0.08.

Colour (in ethanol). Carapace pale ochre-yellow with distinct brown mark, ocular area and clypeus without darker pattern; sternum monochromous whitish; legs ochre-yellow, patellae brown, tibia-metatarsus joints with indistinct brown ring, femur 1 proximally barely darkened; abdomen pale ochre-grey, dorsally and laterally with whitish internal marks.

Body. Habitus as in Fig. 3A. Ocular area slightly raised. Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.62/0.50), unmodified. Abdomen oval, approximately twice as long as wide.

Chelicerae. As in Fig. 25A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs; with pair of low proximal frontal humps; with proximal lateral processes in relatively distal position and directed towards distal.

Palps. As in Fig. 23; coxa unmodified; trochanter with long ventral apophysis with distinct proximal retrolateral hump and modified hair on distal tip; femur distally widened, with distinct ventral hump; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 24A–C) proximally with sclerotised prolateral hump; at half-length with prolateral sclerotised ridge transforming distally into transparent membrane, and brush of dorsal hairs; distally with small retrolateral ridge and strong ventral apophysis, large bifid membranous ventral-prolateral flap, and distinctive dorsal hinged process. Genital bulb (Fig. 24D, E) with strong proximal sclerite; with two sclerotised processes of unclear homology: prolateral process with dorsal row of teeth with short hair-like processes, retrolateral process originating from basis of embolus, S-shaped and distally slightly spiralling; and mostly semi-transparent short embolus with distinct pointed extension.



Figure 24. *Micropholcus dhahran* Huber, sp. nov.; male from Saudi Arabia, 'Asir, W of Dhahran Al Janub (ZFMK Ar 24657) **A–C** left procursus in prolateral, dorsal, and retrolateral views; bold arrow in C points at proximal spine on hinged process **D**, **E** left genital bulb in prolateral and retrolateral views; bold arrows in D point at distinctive processes of unclear homology. Abbreviations: e, embolus; hp, dorsal hinged process; mf, membranous prolateral flap; ps, proximal bulbal sclerite; rr, retrolateral ridge; tm, transparent membrane; Scale bars: 0.3 mm.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 7%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 20 pseudosegments, only distally distinct. *Variation* (male). Tibia 1 in two other males: 6.5, 6.7.

Female. In general very similar to male. Tibia 1 in one female: 4.3 (missing in second female). Epigynum (Fig. 26A, B) barely protruding, anterior plate oval, with indistinct knob-shaped process posteriorly; with pair of lateral and median internal dark structures visible through cuticle; posterior epigynal plate very



Figure 25. *Micropholcus dhahran* Huber, sp. nov.; from Saudi Arabia, 'Asir, W of Dhahran Al Janub (ZFMK Ar 24657) **A**, **B** male chelicerae, frontal and lateral views **C** cleared female genitalia, dorsal view; bold arrows point at crescent-shaped structures. Abbreviation: pp, pore plate. Scale bars: 0.3 mm.



Figure 26. *Micropholcus dhahran* Huber, sp. nov.; female from Saudi Arabia, 'Asir, W of Dhahran Al Janub (ZFMK Ar 24657) **A**, **B** abdomen, ventral and lateral views **C**, **D** cleared genitalia, ventral and dorsal views. Scale bars: 0.3 mm.

short and indistinct. Internal genitalia (Figs 25C, 26C, D) with pair of elongated pore plates in transversal position; with pair of lateral sclerites, median crescent-shaped structures, and large membranous anterior arc.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from type locality only, in Saudi Arabia, 'Asir Province (Fig. 13C).

Natural history. The spiders were found sitting on the undersides of large boulders, in small cave-like spaces between ground and boulder.

Micropholcus harajah Huber, sp. nov.

https://zoobank.org/249E0B68-DBB7-4601-81CF-FD7CCFD414AF Figs 3B, 27-30

Туре material. *Holotype*. SAUDI ARABIA – '**Asir** • ♂; SE of Al Harajah, 'site 1'; 17.8681°N, 43.3943°E; 2370 m a.s.l.; 22 Mar. 2024; B.A. Huber leg.; KSMA. *Paratypes*. SAUDI ARABIA – '**Asir** • 2 ♂♂, 7 ♀♀; same collection data as for holotype; ZFMK Ar 24658 and 24659.

Other material. SAUDI ARABIA – **'Asir** • 3 ♀♀, in pure ethanol; same collection data as for holotype; ZFMK SA114.

Diagnosis. Distinguished from known congeners by unique shapes of bulbal processes, in particular distinctive prolateral sclerite (arrowed in Fig. 28D; similar only in *M. alfara* sp. nov.) and by unique subdistal conical projection on hinged process of procursus (arrowed in Fig. 28C); from most congeners (except *M. alfara* sp. nov. and *M. dhahran* sp. nov.) also by rectangular hinged process of procursus with obtuse tip and small ventral terminal pointed process and small proximal spine (in other Saudi Arabian species procursus wider and more curved dorsally, without proximal spine; in *M. fauroti* relatively longer and without spine); from most congeners (except for three species above and *M. jacominae* and *M. darbat* sp. nov.) also by long trochanter apophysis (Fig. 27C; longer than palpal femur). Female with unique pair of external epigynal pockets (arrowed in Fig. 30A); with distinct internal crescent shaped structures (Fig. 29C; similar only in *M. dhahran* sp. nov. and *M. alfara* sp. nov.), crescent-shaped structures farther apart than in *M. alfara* sp. nov.

Description. Male (holotype). *Measurements.* Total body length 2.7, carapace width 0.9. Distance PME-PME 200 μ m; diameter PME 80 μ m; distance PME-ALE 20 μ m; distance AME-AME 25 μ m; diameter AME 55 μ m. Leg 1: 26.2 (6.5 + 0.5 + 6.4 + 11.6 + 1.2), tibia 2: 4.0, tibia 3: 2.3, tibia 4: 3.3; tibia 1 L/d: 75; diameters of leg femora (at half length) 0.10–0.11; of leg tibiae 0.085.

Colour (in ethanol). Carapace pale ochre-yellow with large brown median mark connected posteriorly to series of small lateral marks, ocular area slightly darkened, clypeus without darker pattern; sternum mostly whitish, posterior-ly slightly darkened; legs ochre-yellow, patellae brown, tibia-metatarsus joints with indistinct brown ring, femur 1 proximally slightly darkened; abdomen pale ochre-grey, dorsally and laterally with larger whitish internal marks.

Body. Habitus as in *M. dhahran* sp. nov. (cf. Fig. 3A). Ocular area slightly raised (more distinct in frontal view). Carapace without thoracic groove.



Figure 27. *Micropholcus harajah* Huber, sp. nov.; male from Saudi Arabia, 'Asir, SE of Harajah (ZFMK Ar 24658). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views. Scale bar: 0.3 mm.

Clypeus unmodified. Sternum wider than long (0.66/0.50), unmodified. Abdomen oval, approximately twice as long as wide.

Chelicerae. As in Fig. 29A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs; with pair of very low proximal frontal humps; with prominent pair of proximal lateral processes.

Palps. As in Fig. 27; coxa unmodified; trochanter with very long ventral apophysis with small proximal retrolateral hump and modified hair on distal tip; femur small relative to tibia, distally widened, with distinct ventral hump; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 28A-C) proximally with sclerotised prolateral hump; at half-length with prolateral-ventral sclerotised ridge, prolateral thick sclerotised ridge, and brush of dorsal hairs; distally with small retrolateral ridge and strong ventral apophysis, large membranous ventral-prolateral flap, and distinctive dorsal hinged process. Genital bulb (Fig. 28D, E) with strong proximal sclerite; two sclerotised processes of unclear homology: prolateral process with distinctive strong pointed cone directed towards bulbous part of genital bulb; retrolateral process originating from basis of embolus, heavily sclerotised with retrolateral row of four variably strong pointed processes; and mostly semi-transparent short embolus with distinct pointed process and subdistal row of transparent hair-like processes prolaterally.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 not seen in holotype, in paratype at 7%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 25 pseudosegments, distally distinct.

Variation (male). Tibia 1 in other male: 5.9; missing in third male.

Female. In general very similar to male. Tibia 1 in ten females: 4.3–5.9 (mean 4.9). Epigynum (Fig. 30A, B) variably protruding, anterior plate oval, with indistinct knob-shaped process posteriorly; with pair of lateral sclerites,



Figure 28. *Micropholcus harajah* Huber, sp. nov.; male from Saudi Arabia, 'Asir, SE of Harajah (ZFMK Ar 24658) **A–C** left procursus in prolateral, dorsal, and retrolateral views; bold arrowed in C points at distinctive subdistal projection on hinged process **D**, **E** left genital bulb in prolateral and retrolateral views; bold arrowed in D points at distinctive cone on prolateral bulbal process. Abbreviations: e, embolus; ps, proximal bulbal sclerite. Scale bars: 0.3 mm.



Figure 29. *Micropholcus harajah* Huber, sp. nov.; from Saudi Arabia, 'Asir, SE of Harajah **A**, **B** male chelicerae, frontal and lateral views (ZFMK Ar 24658) **C** cleared female genitalia, dorsal view (ZFMK Ar 24659). Scale bars: 0.3 mm.



Figure 30. *Micropholcus harajah* Huber, sp. nov.; female from Saudi Arabia, 'Asir, SE of Harajah (ZFMK Ar 24659) **A**, **B** abdomen, ventral and lateral views; arrows in A point at unique epigynal pockets **C**, **D** cleared genitalia, ventral and dorsal views. Scale bars: 0.3 mm.

each provided at posterior end with small pocket, and median internal dark structure visible through cuticle; posterior epigynal plate very short and indistinct. Internal genitalia (Figs 29C, 30C, D) with pair of elongated pore plates in transversal position; with pair of lateral sclerites, median crescent-shaped structures, and large membranous anterior arc.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from type locality only, in Saudi Arabia, 'Asir Province (Fig. 13C).

Natural history. The spiders were found sitting on the undersides of large boulders (Fig. 14A), in small cave-like spaces between boulders and between the ground and boulder. Two egg sacs contained \sim 30–35 eggs each, with an egg diameter of 0.58–0.60 mm.

Micropholcus alfara Huber, sp. nov.

https://zoobank.org/EC6070DD-193E-427D-B5A6-1354760EE20C Figs 3C, D, 6A, B, 7A, B, 9A, 11D, E, 31–34

Туре material. *Holotype*. SAUDI ARABIA – '**Asir** · ♂; S of Al Fara; 18.0487°N, 42.7096°E; 2250 m a.s.l.; 21 Mar. 2024; В.А. Huber leg.; KSMA. *Paratypes*. SAUDI ARABIA – '**Asir** · 4 ♂♂, 5 ♀♀ (1 ♂, 1 ♀ used for SEM); same collection data as for holotype; ZFMK Ar 24660 to 24661.

Other material. SAUDI ARABIA – 'Asir \cdot 1 \Diamond , 1 \bigcirc , in pure ethanol; same collection data as for holotype; ZFMK SA112.

Diagnosis. Distinguished from known congeners by unique shapes of bulbal processes, in particular distinctive prolateral sclerite (arrowed in Fig. 32D; similar only in M. harajah sp. nov., cf. Fig. 28D); from most congeners (except M. harajah sp. nov. and M. dhahran sp. nov.) also by rectangular hinged process of procursus with obtuse tip and small ventral terminal pointed process and small proximal spine (in other Saudi Arabian species procursus wider and more curved dorsally, without proximal spine; in M. fauroti relatively longer and without spine); from most congeners (except for three species above and M. jacominae and M. darbat sp. nov.) also by long trochanter apophysis (Fig. 31C; longer than palpal femur). Female with distinct internal crescent-shaped structures (Fig. 33C), similar only in M. dhahran sp. nov. and M. harajah sp. nov., but closer together; anterior arc narrow (Fig. 33C), similar only in *M. harajah* sp. nov. (cf. Fig. 29C), in *M. dhahran* sp. nov. wider and with different curvature. From M. harajah sp. nov. also distinguished by absence of subdistal conical projection on hinged process of procursus (cf. Fig. 28C) and by absence of pair of external epigynal pockets (cf. Fig. 30A).



Figure 31. *Micropholcus alfara* Huber, sp. nov.; male from Saudi Arabia, 'Asir, S of Al Fara (ZFMK Ar 24660). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views. Scale bar: 0.3 mm.



Figure 32. *Micropholcus alfara* Huber, sp. nov.; male from Saudi Arabia, 'Asir, S of Al Fara (ZFMK Ar 24660) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views; arrow in D points at distinctive cone on prolateral bulbal process. Scale bars: 0.3 mm.

Description. Male (holotype). *Measurements.* Total body length 2.6, carapace width 1.0. Distance PME-PME 185 μ m; diameter PME 80 μ m; distance PME-ALE 25 μ m; distance AME-AME 20 μ m; diameter AME 55 μ m. Leg 1: 28.4 (7.1 + 0.4 + 6.9 + 12.8 + 1.2), tibia 2: 4.1, tibia 3: 2.3, tibia 4: 3.3; tibia 1 L/d: 81; diameters of leg femora (at half length) 0.09–0.10; of leg tibiae 0.085.

Colour (in ethanol). Carapace pale ochre-yellow with large brown median mark connected posteriorly to series of small lateral marks, ocular area slightly darkened, clypeus without darker pattern; sternum whitish; legs ochre-yellow, patellae brown, tibia-metatarsus joints with indistinct brown ring, femur 1 proximally slightly darkened (very indistinct); abdomen ochre-grey, dorsally and laterally with large whitish internal marks.



Figure 33. *Micropholcus alfara* Huber, sp. nov.; from Saudi Arabia, 'Asir, S of Al Fara **A**, **B** male chelicerae, frontal and lateral views (ZFMK Ar 24660) **C** cleared female genitalia, dorsal view (ZFMK Ar 24661). Scale bars: 0.3 mm.

Body. Habitus as in Fig. 3C. Ocular area slightly raised (more distinct in frontal view). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.64/0.48), unmodified. Abdomen oval, approximately twice as long as wide.

Chelicerae. As in Fig. 33A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs (Fig. 6A, B); with pair of proximal frontal processes; with prominent pair of proximal lateral processes.

Palps. As in Fig. 31; coxa unmodified; trochanter with very long ventral apophysis with small proximal retrolateral hump and modified hair on distal tip (Fig. 9A); femur small relative to tibia, distally widened, with distinct ventral hump; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 32A-C) proximally with sclerotised prolateral hump; at half-length with prolateral-ventral sclerotised ridge, prolateral thick sclerotised ridge, and brush of dorsal hairs; distally with small retrolateral ridge and strong ventral apophysis, membranous ventral-prolateral flap (Fig. 7A), and dorsal hinged process. Genital bulb (Figs 7B, 32D, E) with strong proximal sclerite; with two sclerotised processes of unclear homology: prolateral process with strong pointed cone directed towards bulbous part of genital bulb; retrolateral process originating from basis of embolus, heavily sclerotised with retrolateral row of pointed cones of similar sizes; and mostly semi-transparent short embolus with membranous pointed extension and subdistal row of transparent hair-like processes prolaterally.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 6%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 20 pseudosegments, distally distinct.

Variation (male). Tibia 1 in five males: 6.8–7.2 (mean 7.0).

Female. In general very similar to male. Tibia 1 in five females: 4.6–5.4 (mean 5.0). Epigynum (Fig. 34A, B) variably protruding, anterior plate oval,



Figure 34. *Micropholcus alfara* Huber, sp. nov.; female from Saudi Arabia, 'Asir, S of Al Fara (ZFMK Ar 24661) **A**, **B** abdomen, ventral and lateral views **C**, **D** cleared genitalia, ventral and dorsal views. Scale bars: 0.3 mm.

with small and indistinct knob-shaped process posteriorly; with pair of lateral sclerites bent towards lateral anteriorly, without small pockets at posterior ends; and median internal dark structure visible through cuticle. Posterior epigynal plate very short and indistinct. Internal genitalia (Figs 33C, 34C, D) with pair of elongated pore plates in transversal position; with pair of lateral sclerites, median crescent-shaped structures, and large membranous anterior arc.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from type locality only, in Saudi Arabia, 'Asir Province (Fig. 13C).

Natural history. The spiders were found sitting on the undersides of large boulders (Fig. 14B), in small cave-like spaces between ground and boulder. Two egg sacs had diameters of 2.0-2.2 mm, and contained ~ 25-35 eggs each, with an egg diameter of 0.58-0.62 mm.

Micropholcus abha Huber, sp. nov.

https://zoobank.org/60AE7E1A-D17E-48E5-9318-462D97404995 Figs 3E, 35–38

Туре material. *Holotype*. SAUDI ARABIA – '**Asir** • ♂; N of Abha; 18.4168°N, 42.4646°E; 2160 m a.s.l.; 21 Mar. 2024; В.А. Huber leg.; KSMA. *Paratypes*. SAU-DI ARABIA – '**Asir** • 4 ♂♂, 1 ♀, and 1 cleared ♀ abdomen; same collection data as for holotype; ZFMK Ar 24662.

Other material. SAUDI ARABIA – 'Asir • 1 \Diamond , 3 \bigcirc \bigcirc (one abdomen transferred to ZFMK Ar 24662), in pure ethanol; same collection data as for holotype; ZFMK SA111.

Diagnosis. Distinguished from known congeners by presence of dorsal process on palpal femur (arrowed in Fig. 35C; similar only in *M. jacominae*), by bipartite tip of dorsal hinged process of procursus (arrowed in Fig. 36C), and by unique shapes of bulbal processes (Fig. 36D, E; similar to *M. dhahran* sp. nov. but with unique retrolateral pointed process); from similar congeners of southern Saudi Arabia (*M. abha* sp. nov., *M. harajah* sp. nov., *M. dhahran* sp. nov.) also by hinged process of procursus without small proximal spine; from most congeners (except for three species above and *M. jacominae* and *M. darbat* sp. nov.) also by long trochanter apophysis (Fig. 35C; longer than palpal femur). Female with unique lateral sacs in internal genitalia (arrows in Fig. 37C); with distinct anterior arc as in similar congeners from southern Saudi Arabia (*M. alfara* sp. nov., *M. harajah* sp. nov., *M. dhahran* sp. nov.) but without distinct internal crescent-shaped structures (possible homologues visible between pore plates). From *M. harajah* sp. nov. also distinguished by absence of subdistal conical projection on hinged process of procursus (cf. Fig. 28C) and by absence of pair of external epigynal pockets (cf. Fig. 30A).



Figure 35. *Micropholcus abha* Huber, sp. nov.; male from Saudi Arabia, 'Asir, N of Abha (ZFMK Ar 24662). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views; arrow in C points at distinctive dorsal process on femur. Scale bar: 0.3 mm.



Figure 36. *Micropholcus abha* Huber, sp. nov.; male from Saudi Arabia, 'Asir, N of Abha (ZFMK Ar 24662) **A–C** left procursus in prolateral, dorsal, and retrolateral views; arrow in C pointsat distinctive bipartite tip of hinged process **D**, **E** left genital bulb in prolateral and retrolateral views; arrow in E points at distinctive retrolateral pointed process. Scale bars: 0.3 mm.

Description. Male (holotype). *Measurements.* Total body length 2.4, carapace width 0.9. Distance PME-PME 195 μ m; diameter PME 70 μ m; distance PME-ALE 20 μ m; distance AME-AME 25 μ m; diameter AME 50 μ m. Leg 1: 27.1 (6.8 + 0.4 + 6.8 + 11.9 + 1.2), tibia 2: 4.1, tibia 3: 2.5, tibia 4: 3.5; tibia 1 L/d: 85; diameters of leg femora (at half length) 0.085–0.095; of leg tibiae 0.080.

Colour (in ethanol). Carapace pale ochre-yellow with large brown median mark divided medially, ocular area not darkened, clypeus slightly darkened; sternum monochromous whitish; legs pale ochre-yellow, patellae and tibia-metatarsus joints barely darkened, femur 1 proximally barely darkened; abdomen pale ochre-grey, dorsally and laterally with large whitish internal marks.



Figure 37. *Micropholcus abha* Huber, sp. nov.; from Saudi Arabia, 'Asir, N of Abha (ZFMK Ar 24662) **A**, **B** male chelicerae, frontal and lateral views **C** cleared female genitalia, dorsal view; arrows point at distinctive lateral sacs. Scale bars: 0.3 mm.

Body. Habitus as in Fig. 3E. Ocular area slightly raised (distinct in frontal view). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.66/0.54), unmodified. Abdomen oval, approximately twice as long as wide.

Chelicerae. As in Fig. 37A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs; with pair of very low proximal frontal humps; with prominent pair of proximal lateral processes.

Palps. As in Fig. 35; coxa unmodified; trochanter with very long ventral apophysis with distinct proximal bend and modified hair on distal tip; femur small relative to tibia, distally widened, with distinct ventral and dorsal humps; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 36A–C) proximally with sclerotised prolateral hump; at half-length with prolateral-ventral sclerotised ridge transforming prolaterally into transparent membrane, and brush of dorsal hairs; distally with small retrolateral ridge and strong ventral apophysis, large membranous ventral-prolateral flap, and distinctive dorsal hinged processe. Genital bulb (Fig. 36D, E) with strong proximal sclerite; with two sclerotised processes of unclear homology: prolateral process with strong pointed cone directed towards bulbous part of genital bulb and with some hair-like extensions; retrolateral process originating from basis of embolus, heavily sclerotised with strong retrolateral pointed process and flattened distal apophysis; and mostly semi-transparent short embolus with fringed membranous tip.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 6%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 20 pseudosegments, distally distinct.

Variation (male). Tibia 1 in five males (incl. holotype): 5.9–6.8 (mean 6.3); clypeus in other males not or barely darkened.

Female. In general very similar to male; sternum margins slightly darkened. Tibia 1 in two females: 4.7, 5.2 (missing in other females). Epigynum (Fig. 38A,



Figure 38. *Micropholcus abha* Huber, sp. nov.; female from Saudi Arabia, 'Asir, N of Abha (ZFMK Ar 24662) **A**, **B** abdomen, ventral and lateral views **C**, **D** cleared genitalia, ventral and dorsal views. Scale bars: 0.3 mm.

B) slightly protruding, anterior plate oval, with long but transparent and indistinct knob-shaped process posteriorly; with pair of dark brown lateral sclerites, and median internal dark structure poorly visible through cuticle; posterior epigynal plate very short and indistinct. Internal genitalia (Figs 37C, 38C, D) with pair of elongated pore plates in transversal position; with pair of lateral sclerites, pair of large distinctive lateral sacs, median ridges, and large membranous anterior arc.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from type locality only, in Saudi Arabia, 'Asir Province (Fig. 13C).

Natural history. The spiders were found in small caverns at rock outcrops in an open environment (Fig. 14C), i.e., in less sheltered microhabitats than most other species.

Micropholcus tanomah Huber, sp. nov.

https://zoobank.org/6D679588-8300-427F-89A7-6CA93102C27C Figs 3F, 5A, G, 6C, 7C-G, 9B, F, G, 10A, B, 11A, F, 12A-D, 39-42

Туре material. *Holotype.* SAUDI ARABIA – '**Asir** • ♂; NW of Tanomah; 19.0220°N, 42.1247°E; 2250 m a.s.l.; 19 Mar. 2024; B.A. Huber leg.; KSMA. *Paratypes.* SAUDI ARABIA – '**Asir** • 21 ♂♂, 24 ♀♀, 1 juv. (1 ♂, 1 ♀ used for SEM); same collection data as for holotype; ZFMK Ar 24663 to 24664.

Other material. SAUDI ARABIA – 'Asir • 3 ♀♀, 4 juvs; in pure ethanol; same collection data as for holotype; ZFMK SA100.

Diagnosis. Distinguished from similar species in the northern Saudi Arabian group (*M. bashayer* sp. nov., *M. maysaan* sp. nov.) by very slender main bulbal process (Fig. 40D; wider in other species), and by epigynal 'knob' in posterior rather than central position on epigynal plate (Fig. 42A, B); from *M. maysaan* sp. nov. also by less widened hinged process of procursus (Fig. 40C); from species of the southern Saudi Arabian group and *M. jacominae* by shorter male palpal trochanter apophysis (Fig. 39C), internal female genitalia with membranous central element rather than distinct arc (Fig. 41C), and without crescent-shaped structures.

Description. Male (holotype). *Measurements.* Total body length 3.4, carapace width 1.2. Distance PME-PME 210 μ m; diameter PME 80 μ m; distance PME-ALE 20 μ m; distance AME-AME 20 μ m; diameter AME 45 μ m. Leg 1: 28.7 (7.0 + 0.5 + 7.1 + 12.7 + 1.4), tibia 2: 4.5, tibia 3: 2.9, tibia 4: 4.0; tibia 1 L/d: 71; diameters of leg femora (at half length) 0.11–0.12; of leg tibiae 0.10.



Figure 39. *Micropholcus tanomah* Huber, sp. nov.; male from Saudi Arabia, 'Asir, NW of Tanomah (ZFMK Ar 24663). Left palp in prolateral (**A**), dorsal (**B**), and retrolateral (**C**) views. Scale bar: 0.3 mm.



Figure 40. *Micropholcus tanomah* Huber, sp. nov.; male from Saudi Arabia, 'Asir, NW of Tanomah (ZFMK Ar 24663) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views. Abbreviation: hp, dorsal hinged process. Scale bars: 0.3 mm.

Colour (in ethanol). Carapace pale ochre-yellow with distinct brown mark, ocular area not darkened, clypeus with very indistinct darker pattern; sternum monochromous whitish; legs ochre-yellow to light brown, patella dark brown, tibia-metatarsus joints with small brown ring, femur 1 ventrally proximally brown (less distinct also femur 2); abdomen pale ochre-grey, with indistinct darker internal marks.

Body. Habitus as in Fig. 3F. Ocular area slightly raised (distinct in frontal view). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.74/0.58), unmodified. Abdomen oval, approximately twice as long as wide. Gonopore with four epiandrous spigots (Fig. 10A). Spinnerets as in Fig. 9F, G.

Chelicerae. As in Fig. 41A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs (Fig. 6C); with pair of distinct proximal frontal apophyses; with pair of very low and indistinct lateral humps.

Palps. As in Fig. 39; coxa unmodified; trochanter with long ventral apophysis with distinct proximal retrolateral hump and modified hair on distal tip (Fig. 9B);



Figure 41. *Micropholcus tanomah* Huber, sp. nov.; male from Saudi Arabia, 'Asir, NW of Tanomah **A**, **B** male chelicerae, frontal and lateral views (ZFMK Ar 24663) **C** cleared female genitalia, dorsal view (ZFMK Ar 24664); bold arrow points at membranous central element. Abbreviations: k, epigynal 'knob'; pp, pore plate. Scale bars: 0.3 mm.

femur distally widened, with subdistal ventral hump; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 40A–C) proximally with sclerotised prolateral hump; at half length with prolateral sclerotised ridge transforming distally into transparent membrane, and brush of dorsal hairs; distally with small retrolateral ridge, large bifid membranous ventral-prolateral flap (Fig. 7C, D), and dorsal hinged process. Genital bulb (Fig7F, G, 40D, E) with strong proximal sclerite; with two sclerotised processes of unclear homology: prolateral process long and slender, with small, pointed branch on retrolateral side; retrolateral process; and mostly semi-transparent short embolus with membranous extensions.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 6%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 20 pseudosegments, distally distinct.

Variation (male). Tibia 1 in 21 other males: 5.1–7.3 (mean 6.5). Carapace pattern very consistent. Abdomen usually with large white marks dorsally and laterally.

Female. In general very similar to male but anterior leg femora proximally not darkened; ocular area with large median and small lateral brown marks. Tibia 1 in 24 females: 4.4–5.9 (mean 5.2). Epigynum (Figs 10B, 42A, B) protruding, anterior plate oval, mostly dark brown except medially posteriorly, with small knob-shaped process (Fig. 11A) near posterior margin; posterior epigynal plate very short and indistinct, light brown. Internal genitalia (Figs 41C, 42C, D) with pair of elongated pore plates in transversal position; with pair of lateral sclerites and complex system of membranous structures.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from type locality only, in Saudi Arabia, 'Asir Province (Fig. 13C).



Figure 42. *Micropholcus tanomah* Huber, sp. nov.; female from Saudi Arabia, 'Asir, NW of Tanomah (ZFMK Ar 24664) **A**, **B** abdomen, ventral and lateral views **C**, **D** cleared genitalia, ventral and dorsal views. Scale bars: 0.5 mm.

Natural history. The spiders were found in caverns among and under boulders, often together with a representative of *Smeringopus* Simon, 1890 (Araneae: Pholcidae). Both species sometimes occurred in very high densities. In one case, a ceiling of a cave was estimated to measure ~ 3 m^2 and to contain ~ 250 large (adult and penultimate instar) specimens (i.e., with average distances between specimens of ~ 10 cm) (Fig. 15). One egg sac had a diameter of 2.5 mm, and contained ~ 35 eggs. Egg diameters ranged from 0.69 to 0.71 mm.

Micropholcus bashayer Huber, sp. nov.

https://zoobank.org/3B85B5E5-99F7-46EB-B1D0-0380D127FC33 Figs 3G, 43-46

Туре material. *Holotype*. SAUDI ARABIA – 'Asir • ♂; NW of Al Bashayer; 19.8194°N, 41.8824°E; 1850 m a.s.l.; 19 Mar. 2024; В.А. Huber leg.; KSMA. *Paratypes*. SAUDI

Акавіа – **'Asir •** 8 ♂♂, 10 ♀♀; same collection data as for holotype; ZFMK Ar 24665 to 24666.

Other material. SAUDI ARABIA – 'Asir • 2 ♂♂, 3 ♀♀; in pure ethanol; same collection data as for holotype; ZFMK SA96.

Diagnosis. Distinguished from most similar known species (*M. maysaan* sp. nov.) by less widened dorsal hinged process of procursus (Fig. 44C), by pointed projection of bulbal process (arrowed in Fig. 44E) not directed towards bulbous part, and by darkened central area of epigynal plate (Fig. 46A; rather than anterior part, cf. Fig. 50A). From third species in northern Saudi Arabian group (*M. tanomah* sp. nov.) by wider main bulbal process (Fig. 44D), by epigynal 'knob' in central rather than posterior position on epigynal plate (Fig. 46A). From species of the southern Saudi Arabian group and *M. jacominae* by shorter male palpal trochanter apophysis (Fig. 43C), internal female genitalia with membranous central element rather than distinct arc (Fig. 45C) and without crescent-shaped structures.

Description. Male (holotype). *Measurements.* Total body length 3.0, carapace width 1.0. Distance PME-PME 195 µm; diameter PME 80 µm; distance PME-ALE 20 µm; distance AME-AME 20 µm; diameter AME 50 µm. Leg 1: 23.7 (6.0 + 0.5 + 5.9 + 10.1 + 1.2), tibia 2: 3.6, tibia 3: 2.3, tibia 4: 3.4; tibia 1 L/d: 66; diameters of leg femora (at half length) 0.10–0.11; of leg tibiae 0.09.

Colour (in ethanol). Carapace pale ochre-yellow with distinct brown mark, ocular area and clypeus also with indistinct darker pattern; sternum monochromous whitish; legs ochre-yellow to light brown, patella dark brown, tibia-meta-tarsus joints with small brown ring, femur 1 ventrally proximally brown (less distinct also femur 2); abdomen pale ochre-grey, dorsally and laterally with whitish internal marks.



Figure 43. *Micropholcus bashayer* Huber, sp. nov.; male from Saudi Arabia, 'Asir, NW of Al Bashayer (ZFMK Ar 24665). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views. Scale bar: 0.3 mm.



Figure 44. *Micropholcus bashayer* Huber, sp. nov.; male from Saudi Arabia, 'Asir, NW of Al Bashayer (ZFMK Ar 24665) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views; bold arrow in E points at retrolateral process originating from embolus. Abbreviation: e, embolus. Scale bars: 0.3 mm.

Body. Habitus as in *M. maysaan* sp. nov. (cf. Fig. 3H). Ocular area slightly raised. Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.68/0.48), unmodified. Abdomen oval, approximately twice as long as wide.

Chelicerae. As in Fig. 45A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs; with pair of distinct proximal frontal apophyses; without proximal lateral processes.

Palps. As in Fig. 43; coxa unmodified; trochanter with long ventral apophysis with distinct proximal retrolateral hump and modified hair on distal tip; femur distally widened, otherwise unmodified; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus


Figure 45. *Micropholcus bashayer* Huber, sp. nov.; from Saudi Arabia, 'Asir, NW of Al Bashayer **A**, **B** male chelicerae, frontal and lateral views (ZFMK Ar 24665) **C** cleared female genitalia, dorsal view (ZFMK Ar 24666). Scale bars: 0.3 mm.

with large tarsal organ. Procursus (Fig. 44A–C) proximally with sclerotised prolateral hump; at half length with prolateral sclerotised ridge transforming distally into transparent membrane, and brush of dorsal hairs; distally with small retrolateral ridge, large bifid membranous ventral-prolateral flap, and distinctive dorsal hinged process. Genital bulb (Fig. 44D, E) with strong proximal sclerite; with two sclerotised processes of unclear homology: prolateral process large but simple, with small, pointed side branch; retrolateral process very simple, originating from basis of embolus and directed parallel to prolateral process; and mostly semi-transparent short embolus with membranous extension.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 6%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 20 pseudosegments, distally distinct.

Variation (male). Tibia 1 in nine males (incl. holotype): 5.5–6.7 (mean 6.0). **Female.** In general, very similar to male but anterior leg femora proximally not darkened. Tibia 1 in ten females: 4.4–5.4 (mean 4.9). Epigynum (Fig. 46A, B) protruding, anterior plate oval, with knob-shaped process medially; with large brown mark slightly divided medially, anteriorly light, posteriorly with small median dark line; posterior epigynal plate very short and indistinct. Internal genitalia (Figs 45C, 46C, D) with pair of pore plates in transversal position; with pair of lateral sclerites and complex system of membranous structures.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from type locality only, in Saudi Arabia, 'Asir Province (Fig. 13C).

Natural history. The spiders were found sitting on the undersides of large boulders, in small cave-like spaces between boulder and ground. One egg sac contained approximately 30 eggs, with an egg diameter of 0.60 mm. One female had an acrocerid larva in her book lung (Fig. 76A–D).



Figure 46. *Micropholcus bashayer* Huber, sp. nov.; female from Saudi Arabia, 'Asir, NW of Al Bashayer (ZFMK Ar 24666) A, B abdomen, ventral and lateral views **C**, **D** cleared genitalia, ventral and dorsal views. Scale bars: 0.5 mm.

Micropholcus maysaan Huber, sp. nov.

https://zoobank.org/F5E999F5-8646-4083-9F4F-9B9943F2201F Figs 3H, 47-51

Туре material. *Holotype*. SAUDI ARABIA – **Месса** • ♂; NW of Maysaan; 20.7717°N, 40.7985°E; 2560 m a.s.l.; 29 Mar. 2024; B.A. Huber leg.; KSMA. *Paratypes*. SAU-DI ARABIA – **Месса** • 4 ♂♂, 5 ♀♀; same collection data as for holotype; ZFMK Ar 24667 to 24668.

Other material. SAUDI ARABIA – **Mecca** • 2 \bigcirc , 4 juvs; in pure ethanol; same collection data as for holotype; ZFMK SA140 – **Al Bahah** • 2 \eth , 6 \bigcirc ; NW of Al Bahah, 'site 2'; 20.2095°N, 41.3700°E; 2250 m a.s.l.; 16 Mar. 2024; B.A. Huber leg.; ZFMK Ar 24669 • 1 \circlearrowright , 4 \bigcirc ; in pure ethanol; same collection data as for preceding; ZFMK SA88 • 1 \circlearrowright ; S of Al Bahah, 'site 2'; 19.9896°N, 41.4373°E; 1250 m a.s.l.; 17 Mar. 2024; B.A. Huber leg.; ZFMK Ar 24670.



Figure 47. *Micropholcus maysaan* Huber, sp. nov.; male from Saudi Arabia, Mecca, NW of Maysaan (ZFMK Ar 24667). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views. Scale bar: 0.3 mm.

Diagnosis. Distinguished from other species in northern Saudi Arabian group (*M. bashayer* sp. nov., *M. tanomah* sp. nov.) by strongly widened dorsal hinged process of procursus (Fig. 48C), by pointed process originating from embolus directed towards bulbous part (arrowed in Fig. 48E); from *M. bashayer* sp. nov. also by darkened anterior area of epigynal plate (Fig. 50A; rather than central area, cf. Fig. 46A); from *M. tanomah* sp. nov. also by wider main bulbal process (Fig. 48D), and by epigynal 'knob' in central rather than posterior position on epigynal plate (Figs 50A, B, 51A, B). From species of the southern Saudi Arabian group and *M. jacominae* by shorter male palpal trochanter apophysis (Fig. 47C), internal female genitalia with membranous central element rather than distinct arc (Fig. 49C), and without crescent-shaped structures.

Description. Male (holotype). *Measurements.* Total body length 3.1, carapace width 1.1. Distance PME-PME 200 μ m; diameter PME 90 μ m; distance PME-ALE 30 μ m; distance AME-AME 40 μ m; diameter AME 50 μ m. Leg 1: 26.3 (6.6 + 0.5 + 6.6 + 11.3 + 1.3), tibia 2: 4.2, tibia 3: 2.7, tibia 4: 3.7; tibia 1 L/d: 69; diameters of leg femora (at half length) 0.10–0.11; of leg tibiae 0.09–0.10.

Colour (in ethanol). Carapace pale ochre-yellow with distinct brown mark, clypeus also with indistinct darker pattern; sternum monochromous whitish; legs ochre-yellow to light brown, patella dark brown, tibia-metatarsus joints with small brown ring, femur 1 ventrally proximally brown (less distinct also femur 2); abdomen pale ochre-grey, dorsally and laterally with whitish internal marks.

Body. Habitus as in Fig. 3H. Ocular area slightly raised. Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.72/0.58), unmodified. Abdomen oval, approximately twice as long as wide.



Figure 48. *Micropholcus maysaan* Huber, sp. nov.; male from Saudi Arabia, Mecca, NW of Maysaan (ZFMK Ar 24667) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views; bold arrow in E points at retrolateral process originating from embolus. Abbreviation: e, embolus. Scale bars: 0.3 mm.

Chelicerae. As in Fig. 49A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs; with pair of distinct proximal frontal apophyses; without proximal lateral processes.

Palps. As in Fig. 47; coxa unmodified; trochanter with long ventral apophysis with distinct proximal retrolateral hump and modified hair on distal tip; femur distally widened, otherwise unmodified; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 48A–C) proximally with sclerotised prolateral hump; at half length with prolateral sclerotised ridge transforming distally into transparent membrane, and brush of dorsal hairs; distally with small retrolateral ridge, large bifid membranous ventral-prolateral flap, and distinctively widened dorsal hinged process. Genital bulb (Fig. 48D, E) with strong proximal sclerite;



Figure 49. *Micropholcus maysaan* Huber, sp. nov.; male from Saudi Arabia, Mecca, NW of Maysaan (ZFMK Ar 24667), female from Saudi Arabia, Al Bahah, NW of Al Bahah (ZFMK Ar 24669) **A**, **B** male chelicerae, frontal and lateral views **C** cleared female genitalia, dorsal view. Scale bars: 0.3 mm.



Figure 50. *Micropholcus maysaan* Huber, sp. nov.; female from Saudi Arabia, Mecca, NW of Maysaan (ZFMK Ar 24668) A, B abdomen, ventral and lateral views C, D cleared genitalia, ventral and dorsal views. Scale bars. 0.5 mm.





with two sclerotised processes of unclear homology: prolateral process large but simple, with small, pointed side branch; retrolateral process very simple, originating from basis of embolus and pointing towards globular part of bulb; and mostly semi-transparent short embolus with membranous extensions.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 6%; prolateral trichobothrium absent on tibia 1; tarsus 1 with ~ 20 pseudosegments, distally distinct.

Variation (male). Tibia 1 in eight males (incl. holotype): 5.1–7.1 (mean 6.1). Males from NW of Maysaan and from NW of Al Bahah appear essentially identical. In the single male from S of Al Bahah, the shape of the dorsal hinged process of the procursus is slightly different: its widest point is at one third of its length rather than at half-length. Also, the retrolateral bulbal process in this specimen is (in prolateral view) less strongly protruding from behind the prolateral process.

Female. In general, very similar to male but anterior leg femora proximally not darkened. Tibia 1 in ten females: 4.6–5.5 (mean 4.9). Epigynum (Fig. 50A, B) protruding, anterior plate oval, with prominent knob-shaped process medially;

posterior epigynal plate very short and indistinct. Internal genitalia (Figs 49C, 50C, D, 51C, D) with pair of oval pore plates in transversal position; with pair of lateral sclerites and complex system of membranous structures. Females from NW of Maysaan and from NW of Al Bahah appear essentially identical. No female is available from S of Al Bahah.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from three localities in Saudi Arabia, in Mecca and Al Bahah Provinces (Fig. 13C).

Natural history. At the type locality, the spiders were found in small caverns under large boulders on a hill (Fig. 14D), where they were tightly pressed against the rock on the ceiling. Upon disturbance, they were very reluctant to move; only when the sparse sheet of silk covering the spider was removed, they started to run away over the rock. NW of Al Bahah most specimens were collected from a small hole under a flat rock; distances between specimens ranged from ~ 10–20 cm. One egg sac contained ~ 12 eggs, with an egg diameter of 0.62 mm.

Micropholcus darbat Huber, sp. nov.

https://zoobank.org/A7E37832-2C7E-43AA-B85F-DDFCCBA26009 Figs 4A, B, 5B, 6D, E, 7H, 8A-C, 9C, 10C, D, 11B, G, H, 52-55

Micropholcus sp. n. Om74 - Huber and Eberle 2021, Suppl. material 1.

Type material. *Holotype.* OMAN – **Dhofar** • ♂; Wadi Darbat; between 17.086°N, 54.444°E and 17.095°N, 54.452°E; 200–230 m a.s.l., 23 Feb. 2018; B.A. Huber leg.; ZFMK Ar 24671.

Other material. OMAN – **Dhofar** • 9 \Im , 7 \Im , 1 juv. (1 \Im , 1 \Im used for SEM); same collection data as for holotype; ZFMK Ar 24672, 24699 • 1 \Im , 3 \Im , in pure ethanol; same collection data as for holotype; ZFMK Om133 • 2 \Im , 2 \Im ; Ain Athoom; 17.1185°N, 54.3667°E; 280 m a.s.l.; in small cave; 28 Feb. 2018; B.A. Huber leg.; ZFMK Ar 24673 • 1 \Im , 1 \Im , in pure ethanol; same collection data as for preceding; ZFMK Om147 • 3 \Im , 1 \Im ; near Qairoon Hairitti; 17.2600°N, 54.0808°E; 845 m a.s.l.; in small cave; 27 Feb. 2018; B.A. Huber leg.; ZFMK Ar 24674 • 2 \Im , in pure ethanol; same collection data as for preceding; ZFMK Om147 • 3 \Im , 1 \Im ; near Qairoon Hairitti; 17.2600°N, 54.0808°E; 845 m a.s.l.; in small cave; 27 Feb. 2018; B.A. Huber leg.; ZFMK Ar 24674 • 2 \Im , in pure ethanol; same collection data as for preceding; ZFMK Om146 • 3 \Im , 5 \Im , 1 juv.; Wadi Nahiz; 17.140°N, 54.123°E; 140 m a.s.l.; in small caverns; 26 Feb. 2018; B.A. Huber leg.; ZFMK Ar 24675 • 1 \Im , 3 juvs, in pure ethanol; same collection data as for preceding; ZFMK Om142.

Diagnosis. Males are easily distinguished from known congeners by shape of procursus with distinctive dorsal hinged process split into two branches (Fig. 53C); also by shapes of bulbal processes (Fig. 53D, E; prolateral apophysis simple, with small proximal prolateral hump) and cheliceral processes (Fig. 54A, B; proximal frontal processes very low and indistinct); from geographically close *M. shaat* sp. nov. also by longer trochanter apophysis without distinct proximal process (Fig. 52C). Females differ from known congeners by pair of internal pockets visible also in uncleared specimens (arrows in Fig. 55); from geographically close *M. shaat* sp. nov. also by epigynum without median sclerotised band (Fig. 55A), pore plates oval and converging anteriorly (Fig. 55C), and internal genitalia without large membranous sac.



Figure 52. *Micropholcus darbat* Huber, sp. nov.; male from Oman, Dhofar, Wadi Darbat (ZFMK Ar 24672). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views. Scale bar: 0.3 mm.

Description. Male (holotype). *Measurements.* Total body length 3.2, carapace width 1.2. Distance PME-PME 250 μ m; diameter PME 90 μ m; distance PME-ALE 20 μ m; distance AME-AME 15 μ m; diameter AME 55 μ m. Leg 1: 36.5 (9.1 + 0.6 + 9.1 + 16.1 + 1.6), tibia 2: 5.9, tibia 3: 3.8, tibia 4: 5.1; tibia 1 L/d: 83; diameters of leg femora (at half length) 0.12–0.13; of leg tibiae 0.11.

Colour (in ethanol). Prosoma and legs pale ochre-yellow, carapace with brown median mark; legs with darkened patellae and tibia-metatarsus joints; abdomen pale grey to whitish.

Body. Habitus as in Fig. 4A. Ocular area raised (distinct in frontal view; Fig. 5B). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.75/0.60), unmodified. Abdomen oval, approximately twice as long as wide. Gonopore with four epiandrous spigots (Fig. 10C).

Chelicerae. As in Figs 6D, 54A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs (Fig. 6E); pair of proximal lateral processes weakly sclerotised and directed towards distal; and pair of very low proximal frontal humps.

Palps. As in Fig. 52; coxa unmodified; trochanter with long ventral apophysis with retrolateral hump at basis and modified hair at tip (Fig. 9C); femur proximally with low dorsal hump, distally with weakly sclerotised rounded process on prolateralal-ventral side; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Figs 7H, 53A–C) proximally with sclerotised prolateral hump; at half length with prolateral sclerotised ridge transforming distally into transparent membrane, and dense brush of dorsal hairs; distally with small retrolateral ridge, ventral apophysis directed towards prolateral, membranous ventral-prolateral flap (Fig. 8A, B), and distinctive dorsal hinged process split into two branches. Genital bulb (Figs 8C, 53D, E) with strong proximal sclerite, prolateral sclerite simple with small proximal prolateral prolateral hump, simple retrolateral sclerite, and mostly semi-transparent short embolus.



Figure 53. *Micropholcus darbat* Huber, sp. nov.; male from Oman, Dhofar, Wadi Darbat (ZFMK Ar 24672) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views; bold arrow in D points at proximal hump on prolateral bulbal process. Abbreviations: e, embolus; hp, dorsal hinged process; ps, proximal bulbal sclerite. Scale bars: 0.3 mm.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs (most hairs missing in holotype but confirmed in males from near Qairoon Hairitti); retrolateral trichobothrium of tibia 1 at 6%; prolateral trichobo-thrium absent on tibia 1; tarsus 1 with > 30 pseudosegments, distally distinct.

Variation (male). Tibia 1 in 16 males (incl. holotype): 6.9-9.2 (mean 8.2). Distance between eye triads $190-250 \mu m$. Some males with white marks dorsally on abdomen.



Figure 54. *Micropholcus darbat* Huber, sp. nov.; from Oman, Dhofar, Wadi Darbat (ZFMK Ar 24672) **A**, **B** male chelicerae, frontal and lateral views **C** cleared female genitalia, dorsal view. Scale bars: 0.3 mm.



Figure 55. *Micropholcus darbat* Huber, sp. nov.; female from Oman, Dhofar, Wadi Darbat (ZFMK Ar 24672) **A** epigynum, ventral view **B**, **C** cleared female genitalia, ventral and dorsal views. Arrows point at distinctive internal pockets. Scale bars: 0.3 mm.

Female. In general very similar to male but abdomen often much wider, ocular area slightly less raised and triads closer together (PME-PME 180–190 μ m). Tibia 1 in 13 females: 5.7–7.1 (mean 6.3). Epigynum (Figs 10D, 55A) anterior plate oval, protruding, with membranous knob (Fig. 11B) in posterior position and slightly directed towards anterior; lateral internal sclerites clearly visible in untreated specimens; posterior epigynal plate very short and indistinct. Internal genitalia (Fig. 54C, 55B, C) with pair of oval pore plates converging anteriorly, pair of lateral sclerites and pair of ventral pockets (arrows in Fig. 55); with sclerotised anterior arc.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from several localities in Dhofar, western Oman (Fig. 13C).

Natural history. In Wadi Darbat and Wadi Nahiz, the spiders were abundant on the vertical rocks and rock shelters lining the valleys. They were tightly pressed against the rock surface, making them difficult to spot. Upon disturbance, they ran away or dropped to the ground. Near Qairoon Hairitti, the spiders were collected in a small and shallow cave. At Ain Athoom, most specimens were found in a small cave, but juveniles were also found under rocks in the neighbouring area. Two egg sacs contained 21 and 27 eggs, respectively, with an egg diameter of 0.59 mm (Huber and Eberle 2021). One male had an acrocerid larva in his book lung (Fig. 76E, F).

Micropholcus shaat Huber, sp. nov.

https://zoobank.org/03BCF62A-51BC-46EF-9CA1-FC2B8720C36C Figs 4C, 56-59

Type material. *Holotype.* OMAN – **Dhofar** • ♂; Shaat sinkhole, in wadis leading to sinkhole; 16.774°N, 53.587°E; 850 m a.s.l.; 25 Feb. 2018; B.A. Huber leg.; ZFMK Ar 24676.

Diagnosis. Males are easily distinguished from known congeners by several details of male palp (Figs 56, 57; proximal process on trochanter; slender femur without distinct processes; procursus with simple dorsal hinged process; prolateral sclerite on genital bulb simple and slender). Females are easily distinguished from known congeners by anterior position of epigynal 'knob' (Fig. 58C), by distinctive sclerotised band medially on epigynal plate (Fig. 59A), and by presence of large membranous sac in internal genitalia (Fig. 59D); from geographically close *M. darbat* sp. nov. also by larger and wider pore plates (Fig. 58C).

Description. Male (holotype). *Measurements.* Total body length 2.6, carapace width 0.9. Distance PME-PME 260 μ m; diameter PME 85 μ m; distance PME-ALE 15 μ m; distance AME-AME 20 μ m; diameter AME 45 μ m. Leg 1: 27.3 (6.8 + 0.5 + 6.9 + 11.7 + 1.4), tibia 2: 4.3, tibia 3: 2.6, tibia 4: 3.6; tibia 1 L/d: 81; diameters of leg femora (at half length) 0.09–0.10; of leg tibiae 0.08–0.09.

Colour (in ethanol). Prosoma and legs ochre-yellow, carapace with brown median mark; leg femora 1 and 2 proximally darkened; legs with darkened patellae and tibia-metatarsus joints; abdomen pale ochre-grey.



Figure 56. *Micropholcus shaat* Huber, sp. nov.; male from Oman, Dhofar, Shaat sinkhole (ZFMK Ar 24677). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views; arrow in C points at distinctive process proximally on trochanter apophysis. Scale bar: 0.3 mm.

Body. Habitus as in Fig. 4C. Ocular area raised (distinct in frontal view). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.65/0.50), unmodified. Abdomen oval, approximately twice as long as wide.

Chelicerae. As in Fig. 58A, B; with pair of distal apophyses near laminae, each with two cone-shaped hairs; pair of lateral, indistinct, weakly sclerotised humps; and pair of distinct proximal frontal apophyses.

Palps. As in Fig. 56; coxa unmodified; trochanter with long ventral apophysis with distinct proximal process directed towards coxa; femur slender, distally widened, with indistinct ventral hump at half length; femur-patella joints shifted toward prolateral side; tibia-tarsus joints shifted toward retrolateral side; tarsus with large tarsal organ. Procursus (Fig. 57A–C) proximally with sclerotised prolateral hump; at half length with prolateral sclerotised ridge transforming distally into transparent membrane, and brush of dorsal hairs; distally with small retrolateral ridge, bifid membranous ventral-prolateral flap, and distinctive dorsal hinged process. Genital bulb (Fig. 57D, E) with strong proximal sclerite, prolateral process simple and slender, and short embolus proximally sclerotised, distally with transparent extensions.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 6%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 25 pseudosegments, distally distinct.

Variation (male). Tibia 1 in five males (incl. holotype): 6.2-7.8 (mean 6.8). Distance between eye triads $250-270 \ \mu m$. Some males with white marks dorsally on abdomen.

Female. In general very similar to male but abdomen often wider, ocular area slightly less raised and triads closer together (PME-PME 200 μ m). Tibia 1 in



Figure 57. *Micropholcus shaat* Huber, sp. nov.; male from Oman, Dhofar, Shaat sinkhole (ZFMK Ar 24677) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views. Abbreviation: hp, dorsal hinged process. Scale bars: 0.3 mm.

five females: 5.2–5.6 (mean 5.3). Epigynum (Fig. 59A) anterior plate roundish, anterior margin weakly curved, posterior margin strongly curved, with distinctive median sclerotised band, membranous knob at anterior end of sclerotised band, directed towards posterior; lateral internal sclerites clearly visible in untreated specimens; posterior epigynal plate very short and indistinct. Internal genitalia (Figs 58C, 59B–E) with pair of large sclerotised pore plates, with



Figure 58. *Micropholcus shaat* Huber, sp. nov.; from Oman, Dhofar, Shaat sinkhole (ZFMK Ar 24677) **A**, **B** male chelicerae, frontal and lateral views **C** cleared female genitalia, dorsal view. Abbreviations: k, epigynal 'knob'; pp, pore plates. Scale bars: 0.3 mm.

roughly square-shaped sclerotised opening leading into large round membranous sac (collapsed in Fig. 59D; removed in Fig. 59B, C, E); with complex system of lateral membranous structures.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from type locality only, in Dhofar, western Oman (Fig. 13C).

Natural history. The spiders were found in niches and small caverns in the walls of the wadis leading to Shaat sinkhole (Fig. 14E). They rested in the apex of very fine and poorly visible dome-shaped webs directly on the rock surface.

Micropholcus agadir (Huber, 2011)

Figs 4D, E, 60-63

Pholcus agadir Huber, 2011: 331, figs 1530–1531, 1553–1554, 1606–1611 (♂♀). Eberle et al. 2018 (molecular data).

Micropholcus agadir – Huber et al. 2018: 83. Huber and Eberle 2021, Suppl. material 1.

New records. MOROCCO: **Souss-Massa** • 3 33, 2 9 9; Paradise Valley; 30.588°N, 9.528°W; 305 m a.s.l.; 13 Sep. 2018; B.A. Huber leg.; ZFMK Ar 24679 • 2 33, 2 9 9 (one abdomen transferred to ZFMK Ar 24679), in pure ethanol; same collection data as for preceding; ZFMK Mor76 • 2 9 9, in pure ethanol; Agadir, path to Kasbah Hill; 30.4289°N, 9.6186°W; 60 m a.s.l.; 28 Nov. 2016; S. Huber leg.; ZFMK Sieg27 • 4 9, in pure ethanol; Agadir, path to Kasbah Hill; 30.4289°N, 9.6186°W; 60 m a.s.l.; 27 MK Sieg11 • 1 3, in pure ethanol; Road Agadir-Alma; 30.4864°N, 9.5650°W; 440 m a.s.l.; 27 Nov. 2016; S. Huber leg.; S. Huber leg.; ZFMK Sieg25. **Marrakech-Safi** • 1 9; NE of Tizi n'Test; 30.897°N, 8.339°W; 2075 m a.s.l.; 12 Sep. 2018; B.A. Huber leg.; ZFMK Ar 24683.



Figure 59. *Micropholcus shaat* Huber, sp. nov.; two females from Oman, Dhofar, Shaat sinkhole (ZFMK Ar 24677) **A** epigynum, ventral view **B**, **C** cleared female genitalia, ventral and dorsal views, same specimen as in A; membranous sac removed **D**, **E** cleared female genitalia of second female, dorsal views; membranous sac is shown in D (arrows), but was removed for E. Abbreviation: k, epigynal 'knob'. Scale bars: 0.3 mm.

Diagnosis. Distinguished from similar congeners (*M. tegulifer*, *M. ghar* sp. nov.) by short and distally widened dorsal hinged process of procursus (Fig. 61C), by rounded uncus with scales (Fig. 61D, E; similar only in *M. khenifra* sp. nov.,



Figure 60. *Micropholcus agadir* (Huber, 2011); male from Morocco, Souss-Massa, Paradise Valley (ZFMK Ar 24679). Left palp in prolateral (**A**), dorsal (**B**), and retrolateral (**C**) views. Scale bar: 0.3 mm.

cf. Fig. 69E), and by flat and oval appendix with small proximal spine and prolateral ridge (Fig. 61D); from *M. tegulifer* also by presence of two pairs of processes proximally on male chelicerae (Fig. 62A, B; absent in *M. tegulifer*), by lateral marks on carapace (Fig. 4D, E; absent in *M. tegulifer*), and by oval rather than elongate pore plates in female internal genitalia. From *M. ghar* sp. nov. also distinguished by smaller triangular plate posteriorly on epigynum (compare Fig. 63B with Fig. 67A).

Description (amendments; see also Huber 2011). Tibia 1 length in seven males (incl. holotype): 6.0-7.4 (mean 6.7); in 12 females (including those in Huber 2011): 4.8-5.9 (mean 5.6). The drawings in Huber (2011) are from the male holotype and from a topotypical female. Compared to the newly collected males, the procursus of the holotype was slightly twisted towards prolateral; thus, the dorsal hinged process was described as "prolateral branch". The new material also shows that the dorsal process is connected to the main part of the procursus by slightly membranous cuticle, i.e., it is hinged, comparable with the dorsal processes of congeneric species. The female epigynal knob was originally said to be on the posterior plate (Huber 2011). Instead, it is situated on a small, slightly separate triangular part of the anterior plate. The posterior epigynal plate in *M. agadir* is indistinct (Fig. 63B); it was indicated by a row of hairs in Huber (2011: fig. 1610), but not explicitly drawn.

Distribution. Known from several localities in southern Morocco, in Souss-Massa and Marrakech-Safi regions (Fig. 13D).



Figure 61. *Micropholcus agadir* (Huber, 2011); male from Morocco, Souss-Massa, Paradise Valley (ZFMK Ar 24679) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views. Abbreviations: a, putative appendix; e, embolus; hp, dorsal hinged process; ps, proximal bulbal sclerite; u, putative uncus. Scale bars: 0.3 mm.

Natural history. In Paradise Valley, the spiders were found on overhanging rock-surfaces, often in very close proximity to *Holocnemus reini* (C. Koch, 1873). While the latter had large and distinct webs, the webs of *Micropholcus* were barely visible. Two egg sacs had diameters of 1.9 and 2.4 mm, respectively, contained 23/31 eggs with an egg diameter of 0.60–0.63 (Huber and Eberle 2021).



Figure 62. *Micropholcus agadir* (Huber, 2011); male from Morocco, Souss-Massa, Paradise Valley (ZFMK Ar 24679), female from Morocco, Souss-Massa, 7 km N Agadir (IRSB) **A, B** male chelicerae, frontal and lateral views **C** cleared female genitalia, dorsal view (from Huber 2011). Abbreviation: pp, pore plate. Scale bars: 0.3 mm.

Micropholcus ghar Huber, sp. nov.

https://zoobank.org/7D4B69A6-ED3D-4AA2-B9C4-AAAEADCDC383 Figs 4F, 5C, D, H, 6F, G, 8D-F, 9D, H, I, 10E, F, 11C, I, 12E-G, 64-67

Type material. *Holotype.* MOROCCO – **Fès-Meknès** • ♂; Kef el Ghar (=Rhar); 34.4788°N, 4.2766°W; 620 m a.s.l.; 22 Sep. 2018; B.A. Huber leg.; ZFMK Ar 24684.

Other material examined. MOROCCO – **Fès-Meknès** • 14 33, 12 99 (1 3, 1 9 used for SEM); same collection data as for holotype; ZFMK Ar 24685 to 24686 • 2 33, 2 99, 1 juv., in pure ethanol; same collection data as for holotype; ZFMK Mor100 • 1 3, 2 99; same locality as for holotype, 2 Jun. 1978; P. Strinati leg.; MHNG • 1 3, 4 99, 6 juvs; same locality as for holotype, 2 Jun. 1978; B. Hauser leg.; MHNG.

Diagnosis. Distinguished from similar congeners (*M. agadir, M. tegulifer*) by unique shape of uncus (Fig. 65D, E; with sickle-shaped process and series of pointed processes along edge), by unique shape of appendix (Fig. 65D, E; two small pointed processes proximally, larger process distally, and membranous distal area), and by large sclerotised triangular plate on epigynum with whitish median area (Fig. 67A); also by pair of distinctive membranous structures laterally in female internal genitalia (arrows in Fig. 66C); from *M. tegulifer* also by presence of two pairs of processes proximally on male chelicerae (Fig. 66A, B; absent in *M. tegulifer*), by lateral marks on carapace (Fig. 4F; absent in *M. tegulifer*), and by roundish rather than elongate pore plates (Fig. 66C).

Description. Male (holotype). *Measurements.* Total body length 3.6, carapace width 1.2. Distance PME-PME 200 μ m; diameter PME 85 μ m; distance PME-ALE 25 μ m; distance AME-AME 20 μ m; diameter AME 45 μ m. Leg 1:



Figure 63. *Micropholcus agadir* (Huber, 2011); female from Morocco, Souss-Massa, Paradise Valley (ZFMK Ar 24679) **A**, **C** abdomen, ventral and lateral views **B** epigynum, ventral view **D**, **E** cleared genitalia, ventral and dorsal views. Abbreviations: aep, anterior epigynal plate; k, epigynal 'knob'; pep, posterior epigynal plate. Scale bars: 0.5 mm (**A**, **C**); 0.3 mm (**B**, **D**, **E**).

37.6 (9.7 + 0.6 + 9.7 + 15.9 + 1.7), tibia 2: 6.8, tibia 3: 4.2, tibia 4: 5.7; tibia 1 L/d: 84; diameters of leg femora (at half length) ~ 0.13; of leg tibiae 0.11-0.12.

Colour (in ethanol). Prosoma and legs mostly pale ochre-yellow, carapace with light brown marks, ocular area and clypeus without darker pattern, sternum with brown margins and three light brown marks posteriorly; legs with slightly darkened patellae, tibia-metatarsus joints not darkened; abdomen monochromous pale grey to whitish.



Figure 64. *Micropholcus ghar* Huber, sp. nov.; male from Morocco, Fès-Meknès, Kef el Ghar (ZFMK Ar 24685). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views. Scale bar: 0.3 mm.

Body. Habitus as in Fig. 4F. Ocular area raised (distinct in frontal view; Fig. 5C). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.80/0.60), unmodified. Abdomen oval, approximately twice as long as wide. Gonopore with four epiandrous spigots (Fig. 10E). Spinnerets as in Fig. 9H, I.

Chelicerae. As in Fig. 66A, B; with pair of long distal frontal apophyses, each with two cone-shaped hairs (Fig. 6F, G); and two pairs of smaller proximal processes.

Palps. As in Fig. 64; coxa unmodified; trochanter with retrolateral-ventral apophysis provided with terminal modified hair (Fig. 9D); femur cylindrical with distinct ventral process proximally; femur-patella joints shifted toward prolateral side; tibia-tarsus joints slightly shifted toward retrolateral side. Procursus (Figs 8D, 65A–C) proximally with sclerotised prolateral ridge; at half length with strong prolateral-ventral sclerotised ridge or process; distally with dorsal hinged process; tip of procursus partly sclerotised and apparently also hinged against proximal part. Genital bulb (Figs 8E, F, 65D, E) with strong proximal sclerite; putative appendix with three pointed processes directed towards bulbous part, distally widened and membranous, with fringed membrane; putative uncus flat with series of pointed processes and one long curved process; and mostly semi-transparent embolus.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs (many hairs missing in holotype but confirmed in other males); retrolateral trichobothrium of tibia 1 at 5%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 20 pseudosegments, distally distinct.



Figure 65. *Micropholcus ghar* Huber, sp. nov.; male from Morocco, Fès-Meknès, Kef el Ghar (ZFMK Ar 24685) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views; numbers 1–3 denote pointed processes of appendix. Abbreviations: a, putative appendix; e, embolus; hp, dorsal hinged process; ps, proximal bulbal sclerite; u, putative uncus. Scale bars: 0.3 mm.

Variation (male). Tibia 1 in 18 males (incl. holotype): 6.2–10.2 (mean 8.1). While most elements of the bulbal processes (and procursus) appear to be very consistent, there is substantial variation in the row of pointed processes on the uncus. The number of larger processes ranges from two to four; the smaller processes may be absent or replaced by a single (sometimes larger) process; several males were asymmetric in this respect.

Female. In general very similar to male but abdomen often much wider. Tibia 1 in 14 females: 6.0–9.1 (mean 7.3). Epigynum (Figs 10F, 67A) anterior



Figure 66. *Micropholcus ghar* Huber, sp. nov.; from Morocco, Fès-Meknès, Kef el Ghar **A**, **B** male chelicerae, frontal and lateral views (ZFMK Ar 24685) **C** cleared female genitalia, dorsal view (ZFMK Ar 24686); arrows point at distinctive membranous structures. Scale bars: 0.3 mm.



Figure 67. *Micropholcus ghar* Huber, sp. nov.; female from Morocco, Fès-Meknès, Kef el Ghar (ZFMK Ar 24686) **A** epigynum, ventral view **B**, **C** cleared female genitalia, ventral and dorsal views. Scale bars: 0.3 mm.

plate divided into two sections, anterior section weakly sclerotised, with curved ridges and hairs; posterior section smooth, medially whitish, laterally brown to black, i.e., heavily sclerotised; small knob-shaped process (Fig. 11C) between posterior and anterior parts; posterior epigynal plate short and very indistinct. Internal genitalia (Figs 66C, 67B, C) with pair of small oval pore plates, distinctive median sclerite, and pair of large membranous structures laterally.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from two localities in Morocco, both in Fès-Meknès Region (Fig. 13D). We could not examine the single male specimen mentioned in Lecigne et al. (2023: 71), originating from Tazekka National Park, Ghar Admam, 34.0278°N, 4.1509°W. However, photographs of the male palp kindly provided by S. Lecigne leave little doubt that this is the same species.

Natural history. The spiders were very abundant within the first ~ 100 m of the cave; no specimens were seen outside the cave or in deeper sections. They built their fine and slightly domed webs close to the floor, often under small rock overhangs. They were hanging in the apex of the dome rather than sitting on the rock. At disturbance, they bounced slightly and walked towards the rock.

Micropholcus khenifra Huber, Lecigne & Lips, sp. nov.

https://zoobank.org/A1FA9AD3-2DCE-4303-B128-9966534808DE Figs 4G, 68-71

Type material. *Holotype.* MOROCCO – **Béni Mellal-Khénifra** • ♂; Imi n'Ifri; 31.724°N, 6.971°W; 1050 m a.s.l.; 26 Sep. 2018; B.A. Huber leg.; ZFMK Ar 24687.

Other material. MOROCCO – **Béni Mellal-Khénifra** • 5 \Diamond \Diamond , 5 \Diamond \Diamond ; same collection data as for holotype; ZFMK Ar 24688 to 24689 • 2 \Diamond \Diamond , 1 \Diamond , in pure ethanol; same collection data as for holotype; ZFMK Mor104 • 4 \Diamond \Diamond , 4 \Diamond \Diamond ; near Sidi Ben Daoud; 32.5347°N, 6.1285°W; 700 m a.s.l.; 25 Sep. 2018; B.A. Huber leg.; ZFMK Ar 24690 to 24691 • 1 \Diamond , 1 \Diamond , 2 juvs, in pure ethanol; same collection data as for preceding; ZFMK Mor102 • 4 ∂ \Diamond , 3 \Diamond \Diamond , 1 juv.; W of El Ksiba; 32.560°N, 6.053°W – 32.562°N, 6.050°W; 950 m a.s.l.; 25 Sep. 2018; B.A. Huber leg.; ZFMK Ar 24692 to 24693 • 1 \Diamond , 1 juv., in pure ethanol; same collection data as for preceding; ZFMK Mor103 • 1 \Diamond ; Jbel Bou-Guergour, Ghar-el-Ghazi; 32.869°N, 5.689°W (?); 970 m a.s.l.; 26 May 2001; C. Ribera leg.; ZFMK Ar 24700.

Diagnosis. Easily distinguished from known congeners by whitish dorsal process of male palpal tarsus (asterisk in Fig. 69C; absent in congeners), by large and prominent flat ventral process of procursus (arrowed in Fig. 69C; much smaller or absent in congeners), by complex tip of procursus with distinctive dorsal spine (Fig. 69A–C), by rounded uncus (Fig. 69E, F; similar only in *M. agadir*), by very small appendix (larger and more complex in Moroccan congeners), by long prominent embolus (Fig. 69E, F; in Moroccan congeners shorter and in prolateral view largely hidden behind uncus and appendix), by pair of dark internal structures visible at anterior margin of epigynum (Fig. 71A; absent in congeners), by distinctive m-shaped dorsal arc in female internal genitalia (Fig. 71C),



Figure 68. *Micropholcus khenifra* Huber, Lecigne & Lips, sp. nov.; male from Morocco, Béni Mellal-Khénifra, Imi n'Ifri (ZFMK Ar 24688). Left palp in prolateral (A), dorsal (B), and retrolateral (C) views. Scale bar: 0.5 mm.

and by very narrow (short) sclerotised band posteriorly on epigynum carrying epigynal 'knob' (Fig. 71A).

Description. Male (holotype). *Measurements.* Total body length 3.9, carapace width 1.5. Distance PME-PME 205 μ m; diameter PME 90 μ m; distance PME-ALE 30 μ m; distance AME-AME 20 μ m; diameter AME 50 μ m. Leg 1: 34.5 (8.7 + 0.6 + 9.0 + 14.4 + 1.8), tibia 2: 6.4, tibia 3: 4.0, tibia 4: 5.3; tibia 1 L/d: 75; diameters of leg femora (at half length) 0.14–0.15; of leg tibiae 0.12.

Colour (in ethanol). Prosoma and legs mostly ochre-yellow, carapace with brown median mark, ocular area and clypeus without darker pattern, sternum with brown margins; legs with slightly darkened patellae, anterior femora ventrally only very slightly darkened, tibia-metatarsus joints not darkened; abdomen monochromous pale grey.

Body. Habitus as in Fig. 4G. Ocular area raised (distinct in frontal view). Carapace without thoracic groove. Clypeus unmodified. Sternum wider than long (0.88/0.72), unmodified. Abdomen oval, approximately twice as long as wide.

Chelicerae. As in Fig. 70A, B; with pair of strong distal frontal apophyses, each with two cone-shaped hairs; and two pairs of smaller proximal processes.

Palps. As in Fig. 68; coxa unmodified; trochanter with ventral apophysis provided with terminal modified hair (Fig. 69D); femur cylindrical, proximally with small retrolateral process and larger prolateral-ventral process; femur-patella joints shifted toward prolateral side; tibia very large relative to femur; tibia-tarsus joints not shifted to one side; tarsus with cone-shaped light dorsal process carrying tarsal organ. Procursus (Fig. 69A–C) proximally with sclerotised prolateral ridge; proximal half with flat ventral process (arrowed in Fig. 69C), distally divided into dorsal and ventral parts and complex hinged structure between them (mostly on prolateral side), with pointed process



Figure 69. *Micropholcus khenifra* Huber, Lecigne & Lips, sp. nov.; male from Morocco, Béni Mellal-Khénifra, Imi n'Ifri (ZFMK Ar 24688) **A–C** left procursus in prolateral, dorsal, and retrolateral views; bold arrow in A points at pointed process that is absent in males from near Sidi Ben Daoud and from W of El Ksiba; asterisk in C marks distinctive whitish process of tarsus; bold arrow in C points at flat ventral process of procursus **D** tip of palpal trochanter apophysis **E**, **F** left genital bulb in prolateral and retrolateral views. Abbreviations: a, putative appendix; e, embolus; ps, proximal bulbal sclerite; u, putative uncus. Scale bars: 0.3 mm (**A–C**, **E**, **F**); 0.05 mm (**D**).

originating from membranous connection between dorsal part and hinged process. Genital bulb (Fig. 69E, F) with strong proximal sclerite; putative appendix small; putative uncus flat with retrolateral process; and long, partly sclerotised embolus.

Legs. Without spines, without curved hairs, without sexually dimorphic short vertical hairs; retrolateral trichobothrium of tibia 1 at 9%; prolateral trichobothrium absent on tibia 1; tarsus 1 with > 30 pseudosegments, distally distinct.







Figure 71. *Micropholcus khenifra* Huber,Lecigne & Lips, sp. nov.; female from Morocco, Béni Mellal-Khénifra, near Sidi Ben Daoud (ZFMK Ar 24689) **A** epigynum, ventral view **B**, **C** cleared female genitalia, ventral and dorsal views. Abbreviations: aep, anterior epigynal plate; k, epigynal 'knob'; pep, posterior epigynal plate. Scale bars: 0.5 mm.

Variation (male). Tibia 1 in 14 males (incl. holotype): 6.9–9.2 (mean 8.2). There was very slight variation in palpal structures among localities: in males from near Sidi Ben Daoud and from W of El Ksiba, the uncus was slightly rounder, the appendix slightly larger, and one small, pointed element of the dorsal part of the procursus (arrowed in Fig. 69A) was absent. The number of modified hairs on the frontal cheliceral apophyses was two or three, and was sometimes asymmetrical (as in Fig. 70A).

Female. In general very similar to male. Tibia 1 in 12 females: 7.1–8.4 (mean 7.7). Epigynum (Fig. 71A) anterior plate mostly light, anteriorly with pair of dark internal structures variably visible in untreated specimens, posteriorly with narrow darker transversal band and median 'knob'; posterior epigynal plate short and very indistinct. Internal genitalia (Figs 70C, 71B, C) with pair of small oval pore plates and distinctive ventral and dorsal anterior arches and sclerites.

Etymology. The species name is derived from Béni Mellal-Khénifra, the region in Morocco where all available specimens were collected; noun in apposition.

Distribution. Known from several localities in Morocco, all in Béni Mellal-Khénifra Region (Fig. 13D).

Natural history. At Imi N'ifri (Fig. 14G) and west of Ksiba, the spiders were found in small cavities of rocks, on the undersides of very large boulders, and in small caverns at ground level. Near Sidi Ben Daoud, the spiders were found in a small cave from which a brook emerged. The spiders sat flat on the rock and appeared very unwilling to leave the spot upon disturbance. At all localities, *M. khenifra* sp. nov. was found in close proximity with *Holocnemus reini*.

Micropholcus bukidnon Huber, sp. nov.

https://zoobank.org/F99122C7-C6F3-40B3-81BB-1DED94C7718E Figs 4H, 5E, F, 6H, 8G, H, 9E, J, 10G, H, 11J, 12H, 72–75

Micropholcus Phi114 – Eberle et al. 2018 (molecular data). Huber et al. 2018: fig. 11.

Micropholcus sp. n. Phi114 – Huber and Eberle 2021, Suppl. material 1.

Type material. *Holotype.* PHILIPPINES – **Mindanao** • ♂; Bukidnon Province, Central Mindanao University, Faculty Hill; 7.852°N, 125.048°E; 330 m a.s.l.; on rocks in degraded forest; 10 Feb. 2014; B.A. Huber leg.; ZFMK Ar 24694.

Other material. PHILIPPINES – **Mindanao** • 7 33, 10 99, 1 juv. (1 3, 1 9 used for SEM); same collection data as for holotype; ZFMK Ar 24695 to 24696 • 2 33, 3 99, in pure ethanol; same collection data as for holotype; ZFMK Phi 273 • 3 33, 2 99; Barangay San Jose, Blue Water Cave; 7.705°N, 125.035°E; 230 m a.s.l.; at rocks at cave entrance; 16 Feb. 2014; B.A. Huber leg.; ZFMK Ar 24697 • 2 33, 2 99, 1 juv., in pure ethanol; same collection data as for preceding; ZFMK Phi 250 • 7 99; Barangay San Jose, Kabyaw Cave; ~ 7.704°N, 125.038°E; ~ 260 m a.s.l.; at rocks at cave entrance; 16 Feb. 2014; B.A. Huber leg.; ZFMK Ar 24698.

Diagnosis. Easily distinguished from known congeners by unusually long proximal frontal apophyses on male chelicerae (Fig. 74B); by long ventral



Figure 72. *Micropholcus bukidnon* Huber, sp. nov.; male from Philippines, Mindanao, Central Mindanao University (ZFMK Ar 24695); left palp in prolateral (**A**), dorsal (**B**), and retrolateral (**C**) views; arrow in C points at distinctive ventral process on femur. Scale bar: 0.3 mm.

process of palpal femur (Fig. 72C); by long rod-shaped putative appendix (Fig. 73D, E), and by distinctive shapes of processes on procursus (Fig. 73A–C; in particular large flat dorsal process). Female genitalia very simple externally (Fig. 75A), distinguished from congeners by absence of external knob (Fig. 10H) and by distinctive internal structures (round pore plates; m-shaped dorsal arch; concentric ventral arches; distinctive median membranous structures posteriorly).

Description. Male (holotype). *Measurements.* Total body length 2.8, carapace width 1.1. Leg 1: 28.1 (6.7 + 0.5 + 7.1 + 12.4 + 1.4), tibia 2: 4.5, tibia 3: 2.9, tibia 4: 3.8; tibia 1 L/d: 77. Distance PME-PME 190 μ m, diameter PME 100 μ m, distance PME-ALE ~ 30 μ m; distance AME-AME 30 μ m, diameter AME 15 μ m.

Colour (in ethanol). Carapace pale ochre with dark median band widening posteriorly, ocular area and clypeus only slightly darkened; sternum pale ochre with narrow dark margins; legs ochre to light brown, with dark brown patellae and tibia-metatarsus joints; abdomen monochromous pale grey.

Body. Habitus as in Fig. 4H; ocular area slightly raised (Fig. 5E); carapace without median furrow; clypeus unmodified; sternum wider than long (0.65/0.55), unmodified. Gonopore of scanned male with five epiandrous spigots (Fig. 10G). Anterior lateral spinnerets with one strongly widened, one pointed, and six cylindrically shaped spigots (Fig. 9J).



Figure 73. *Micropholcus bukidnon* Huber, sp. nov.; male from Philippines, Mindanao, Central Mindanao University (ZFMK Ar 24695) **A–C** left procursus in prolateral, dorsal, and retrolateral views **D**, **E** left genital bulb in prolateral and retrolateral views. Abbreviations: a, putative appendix; e, embolus; ps, proximal bulbal sclerite. Scale bars: 0.3 mm.

Chelicerae. As in Fig. 74A, B; proximally with pair of long frontal apophyses and pair of short lateral processes directed towards proximal, distally with pair of dark apophyses near laminae, each provided with five modified (globular) hairs (Fig. 6H).

Palps. As in Fig. 72; coxa unmodified; trochanter with retrolateral apophysis, tip of apophysis without modified hair (Fig. 9E); femur with low retrolateral hump proximally and with distinctive ventral process; procursus (Figs 8G, 73A–C) very complex distally, with apparently hinged ventral structures and large dorsal flap; genital bulb (Figs 8H, 73D, E) with strong proximal sclerite, with long weakly sclerotised embolus and heavily sclerotised cylindrical putative appendix with proximal round protrusion and short subdistal branch.



Figure 74. *Micropholcus bukidnon* Huber, sp. nov.; from Philippines, Mindanao, Central Mindanao University **A**, **B** male chelicerae, frontal and lateral views (ZFMK Ar 24695) **C**, **D** cleared female genitalia, ventral and dorsal views (ZFMK Ar 24696). Scale bars: 0.3 mm.

Legs. Without spines and curved hairs; without sexually dimorphic short vertical hairs; retrolateral trichobothrium on tibia 1 at 10%; prolateral trichobothrium absent on tibia 1, present on other tibiae; tarsus 1 with ~ 15 pseudosegments, only distally distinct. Tarsus 4 comb-hairs as in Fig. 12H.

Variation (male). Tibia 1 in nine other males: 5.4–7.3 (mean 6.5); specimens from Barangay San Jose have consistently shorter legs than specimens from Faculty Hill (5.4–5.7 versus 6.6–7.3).

Female. In general similar to male; eye triads at almost same distance (PME-PME: 170 μ m; Fig. 5F). Dark band on carapace in some females posteriorly not widened. Tibia 1 in 19 females: 4.3–6.0 (mean: 5.2). As in males, specimens from Barangay San Jose have consistently shorter legs than specimens from Faculty Hill (4.3–5.2 versus 5.4–6.0). Epigynum very simple (Figs 10H, 75A–C), weakly sclerotised, without external 'knob'; internal genitalia (Figs 74C, D, 75D, E) with round pore plates, m-shaped dorsal arch, concentric ventral arches, and distinctive median membranous structures posteriorly.

Etymology. The species name is derived from the type locality; noun in apposition.

Distribution. Known from three localities (two of them very close to each other) in central Mindanao, Philippines (Fig. 13B).



Figure 75. *Micropholcus bukidnon* Huber, sp. nov.; females from Philippines, Mindanao, Central Mindanao University. (ZFMK Ar 24696) **A** abdomen, ventral view **B**, **C** epigyna of two females, ventral views **D**, **E** cleared genitalia, ventral and dorsal views. Scale bars: 0.5 mm (**A**); 0.3 mm (**B**–**E**).

Natural history. The spiders were found on rocks, either on the undersides of large rocks with sufficient space to the ground, or in small depressions of near-vertical rock-surfaces (Fig. 14H). Two egg sacs contained 11 and 25 eggs, respectively, with an egg diameter of 0.54–0.57 mm (Huber and Eberle 2021).



Figure 76. Acroceridae larvae in book lungs of Pholcidae; arrows point at larvae as seen in untreated abdomens **A** female abdomen of *Micropholcus bashayer* Huber, sp. nov., from Saudi Arabia, 'Asir, NW of Al Bashayer (ZFMK Ar 24666) **B–D** Acroceridae larva extracted from A **E** male abdomen of *Micropholcus darbat* Huber, sp. nov., from Oman, Dhofar, Wadi Darbat (ZFMK Ar 24672) **F** Acroceridae larva extracted from E **G** *Mesabolivar eberhardi* Huber, 2000, from Colombia, Magdalena, at Cascada Valencia (ZFMK Col138) **H** Acroceridae larva extracted from G. Scale bars: 0.5 mm (**A**, **E**, **G**); 0.1 mm (**B**, **F**, **H**); 0.05 mm (**C**, **D**).

Discussion

Species limits

Our data on Saudi Arabian *Micropholcus* are difficult to interpret. From a morphological perspective, there are consistent differences among specimens assigned herein to different nominal species. These differences are at approximately the same level of distinctness as between congeners in many other Pholcidae genera. In addition, the respective traits are very homogeneous within putative species. From a molecular perspective, however, our data suggest different species limits, in particular among the southern group of Saudi Arabian species: *M. alfara* sp. nov., *M. dhahran* sp. nov., and *M. harajah* sp. nov. Among these, the genetic distances of 3.4-6.7% are clearly below the problematic range of overlap between intra- and interspecific distances reported for Pholcidae (usually ~ 8-12%; Astrin et al. 2006; Huber et al. 2023b, 2024a, 2024b). The ASAP analysis suggests that this group contains only one or two species, rather than three. We here give more weight to the morphological evidence, but acknowledge that this is in need of further research. At this point, almost every locality at higher elevations with suitable habitats visited in Saudi Arabia has its own 'species' of *Micropholcus*, and we predict that further collecting in this area and in neighbouring Yemen will dramatically increase the number of possible species.

The geographic origin of Micropholcus fauroti

Synanthropic species, i.e., species ecologically associated with humans, have often attained their wide distributions long before they were studied in any detail (Baumann 2023), and their geographic origins and spreading histories are thus usually undocumented (e.g., Molero-Baltanás et al. 2024). Their ancestral areas can only be reconstructed by phylogenetic and geographic analyses of the most closely related taxa (e.g., García-Vázquez and Ribera 2016). In Pholcidae, a dozen species have attained worldwide or pan-tropical distributions, and a few more have probably extended their ranges with the aid of humans. Their spreading histories are mostly undocumented (but see Fürst and Blandenier 1993) and can only be reconstructed indirectly.

Micropholcus fauroti was first described from Djibouti (Simon 1887) but a few years later recorded from Myanmar (Thorell 1895, as Pholcus infirmus), and in 1929 from the New World, from Brazil (Mello-Leitão 1929, as Leptopholcus occidentalis) and from Puerto Rico (Petrunkevitch 1929, as Pholcus unicolor). It has long been thought to have an Old World origin, especially since the description of M. jacominae from Yemen (Deeleman-Reinhold and van Harten 2001), and the newly described species from Saudi Arabia support this idea. The very distinctive dorsal hinged process of *M. fauroti* resembles that of the Saudi Arabian species more than any other congener. However, M. fauroti lacks the distinctive membranous flap prolaterally on the procursus seen in Saudi Arabian species (cf. Figs 7A, D, 8B), and the bulbal processes appear easier to homologise with those of M. jacominae than with those of Saudi Arabian species. Our molecular analysis does not include M. jacominae, and it does not clearly associate M. fauroti with any sequenced congener (the sister group relationship with the Moroccan species in Fig. 1 is poorly supported and very probably an artifact). We thus hypothesise that further collecting in Yemen has good chances to find even closer relatives of M. fauroti.

Acroceridae larvae in Pholcidae book lungs

Flies (Diptera) are known to attack spiders in a variety of ways, as predators, egg parasitoids and predators, kleptoparasites, and endoparasitoids (Gillung and Borkent 2017). The latter category is, among the Diptera, the domain of a single family, the Acroceridae Leach, 1815. Acroceridae larvae are thought to develop exclusively in true spiders (Schlinger 1987), with an apparent

preference for cursorial and fossorial species. True web-building spiders are rarely attacked, which is probably due to the fact that the fly eggs are deposited on the substrate and the emerging larvae must actively search for their hosts (Schlinger 1987). Pholcidae are web-building spiders, and this may partly explain why Acroceridae have never been reported to parasitise representatives of this spider family. However, in some pholcids, the apex of the domed sheet component of the web is closely attached to the substrate, and this is the section of the web where the spider spends most of the day. This is also the case in most *Micropholcus*, where the spiders often appear to be sitting directly flat on the rock surface, due to the very delicate and sparse web (Fig. 15).

Acroceridae larvae are here reported from two species of *Micropholcus* from Saudi Arabia (Fig. 76A–F), and, for the sake of completeness, from a juvenile of *Mesabolivar eberhardi* from Colombia (Fig. 76G, H). The first author has seen a probable fourth case, but the larva was not extracted and the material has been returned to the California Academy of Sciences: a female of *Paramicromerys rothorum* Huber, 2003 from Madagascar (Antsiranana, Montagne d'Ambre). This suggests that Pholcidae may in fact be fairly common hosts for Acroceridae worldwide. Future collectors should try to keep spiders alive in which larvae can be seen through the book lung covers (Fig. 76A, E, G). Rearing of spiders is the most prolific source of information of spider-fly relationships (Kehlmaier et al. 2024). Rearing infected pholcids is particularly worthwhile because we cannot exclude that the pholcids are attacked accidentally, as is suspected to be the case at least in Acari (Schlinger 1987; Gillung and Borkent 2017).

Conclusions

Micropholcus in the Old World has a wide geographic range but seems to be largely restricted to semiarid regions, where the spiders lead reclusive lives in caves, in cave-like spaces under rocks, and in rock depressions. The genus is species-rich both on the Arabian Peninsula and in Morocco, suggesting that it should also be present in suitable habitats in the large but poorly sampled area in-between. The Philippine *M. bukidnon* sp. nov. extends the distribution of the genus far to the east, but this species is morphologically exceptional and its assignment to the genus rests on molecular evidence only.

Micropholcus appears to be exceptionally diverse in southwestern Saudi Arabia, which is generally considered as one of the richest biodiversity areas on the Arabian Peninsula (Abuzinada et al. 2005; Al-Namazi et al. 2021). We predict that a similar high species-richness will also be found in neighbouring Yemen. Saudi Arabian species seem to be restricted to high altitudes (above 1200 m a.s.l.), and most species are known from a single locality. Species from neighbouring localities are often morphologically distinct but genetically (CO1 distance) very close.

Acroceridae flies mainly attack cursorial and fossorial spider species (Gillung and Borkent 2017), probably due to the larval strategy to find a spider host (Schlinger 1987). Here we document the first cases of Acroceridae larvae developing in the book lungs of web-building Pholcidae. We suggest that *Micropholcus* spiders are accessible to Acroceridae larvae because they spend the day in the apex of the domed web that is closely attached to the rock surface. Rearing of infected spiders will be necessary to show if the larvae can actually develop in the spiders, or if pholcids are attacked accidentally.

Acknowledgements

We thank C. Etzbauer (LIB, Bonn) for her support with molecular lab work; C.S Borkent and M. Hauser kindly confirmed the family ID of the acrocerid larvae; S. Lecigne sent photos of the *M. ghar* sp. nov. palp from Ghar Admam. The first author thanks G.R. Feulner, S. Huber, and C. Ribera for the donation of specimens, and I.J. Smit (Naturalis, Leiden) and P. Schwendinger (Muséum d'histoire naturelle, Genève) for the loan of specimens. The first author thanks R. Victor and I. Al Zakwani (Sultan Qaboos University, Muscat) for support with field work in Oman, H. Belhadj (Marrakesh) for support with field work in Morocco, and A. Aldawood and M. Sharaf (King Saud University, Riyadh) for support with field work in Saudi Arabia. We appreciate the helpful comments by Z. Yao on 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 Alexander Koenig Stiftung (AKS, Bonn) provided financial support for field work in Oman and Saudi Arabia.

Author contributions

BAH: initiation of project, funding acquisition, collecting, taxonomy, writing. GM: curation and analysis of molecular data, writing.

Author ORCIDs

Bernhard A. Huber () https://orcid.org/0000-0002-7566-5424 Guanliang Meng () https://orcid.org/0000-0002-6488-1527

Data availability

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

References

- Abuzinada AH, Al-Wetaid YI, Al-Basyouni SZM (2005) The National Strategy for Conservation of Biodiversity in the Kingdom of Saudi Arabia. Prepared and issued by The National Commission for Wildlife Conservation and Development. Riyadh, Saudi Arabia, 94 pp. https://www.cbd.int/doc/world/sa/sa-nbsap-01-en.pdf
- Ahmad NFR, Abou-Setta MM (2017) Life history of the pholcid spider, *Micropholcus fauroti* (Simon, 1887) (Araneae: Pholcidae) in Egypt. Acarines 11: 31–35. https://doi. org/10.21608/ajesa.2017.164166
- Al-Namazi AA, Al-Khulaidi AWA, Algarni S, Al-Sagheer NA (2021) Natural plant species inventory of hotspot areas in Arabian Peninsula: Southwest Al-Baha region, Saudi Ara-

bia. Saudi Journal of Biological Sciences 28: 3309–3324. https://doi.org/10.1016/j. sjbs.2021.02.076

- Astrin JJ, Huber BA, Misof B, Kluetsch CFC (2006) Molecular taxonomy in pholcid spiders (Pholcidae, Araneae): evaluation of species identification methods using CO1 and 16S rRNA. Zoologica Scripta 35: 441–457. https://doi.org/10.1111/j.1463-6409.2006.00239.x
- Astrin JJ, Höfer H, Spelda J, Holstein J, Bayer S, Hendrich L, Huber BA, Kielhorn K-H, Krammer H-J, Lemke M, Monje JC, Morinière J, Rulik B, Petersen M, Janssen H, Muster C (2016) Towards a DNA barcode reference database for spiders and harvestmen of Germany. PLoS ONE 11(9): e0162624. https://doi.org/10.1371/journal.pone.0162624
- Baumann C (2023) The paleo-synanthropic niche: a first attempt to define animal's adaptation to a human-made micro-environment in the Late Pleistocene. Archaeological and Anthropological Sciences 15: 63. https://doi.org/10.1007/s12520-023-01764-x
- Brown BV (1993) A further chemical alternative to critical-point-drying for preparing small (or large) flies. Fly Times 11: 10.
- Deeleman-Reinhold CL, Prinsen JD (1987) Micropholcus fauroti (Simon) n. comb., a pantropical, synanthropic spider (Araneae: Pholcidae). Entomologische Berichten, Amsterdam 47(5): 73–77. https://natuurtijdschriften.nl/pub/1012616/EB1987047005003.pdf
- Deeleman-Reinhold CL, van Harten A (2001) Description of some interesting, new or little known Pholcidae (Araneae) from Yemen. In: Ishwar Prakash (Ed.) Ecology of Desert Environments. Scientific Publishers, India, Jodhpur, 193–207.
- Dimitrov D, Arnedo MA, Ribera C (2008) Colonization and diversification of the spider genus *Pholcus* Walckenaer, 1805 (Araneae, Pholcidae) in the Macaronesian archipelagos: Evidence for long-term occupancy yet rapid speciation. Molecular Phylogenetics and Evolution 48: 596–614. https://doi.org/10.1016/j.ympev.2008.04.027
- Dimitrov D, Astrin JJ, Huber BA (2013) Pholcid spider molecular systematics revisited, with new insights into the biogeography and the evolution of the group. Cladistics 29: 132–146. https://doi.org/10.1111/j.1096-0031.2012.00419.x
- Eberle J, Dimitrov D, Valdez-Mondragón A, Huber BA (2018) Microhabitat change drives diversification in pholcid spiders. BMC Evolutionary Biology 18: 141. https://doi. org/10.1186/s12862-018-1244-8
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791. https://doi.org/https://doi.org/10.2307/2408678
- Feulner GR, Roobas B (2016) Spiders of the United Arab Emirates: an introductory catalogue. Tribulus 23: 54–98.
- Fürst P-A, Blandenier G (1993) Psilochorus simoni (Berland, 1911) (Araneae, Pholcidae): Découvertes de nouvelles stations suisses et discussion de son écologie. Bulletin de la Société neuchâteloise des Sciences naturelles 116: 75–85.
- García-Vázquez D, Ribera I (2016) The origin of widespread species in a poor dispersing lineage (diving beetle genus *Deronectes*). PeerJ 4: e2514. https://doi.org/10.7717/ peerj.2514
- Gillung JP, Borkent CJ (2017) Death comes on two wings: a review of dipteran natural enemies of arachnids. Journal of Arachnology 45: 1–19. https://doi.org/10.1636/ JoA-S-16-085.1
- González-Sponga MA (2004) Arácnidos de Venezuela. Un nuevo género y nuevas especies de la familia Pholcidae (Araneae). Aula y Ambiente 8: 63–76.
- Huber BA (2000) New World pholcid spiders (Araneae: Pholcidae): a revision at generic level. Bulletin of the American Museum of Natural History 254: 1–348. https://doi. org/10.1206/0003-0090(2000)254<0001:NWPSAP>2.0.CO;2
- Huber BA (2009) Four new generic and 14 new specific synonymies in Pholcidae, and transfer of *Pholcoides* Roewer to Filistatidae (Araneae). Zootaxa 1970: 64–68. https://doi.org/10.11646/zootaxa.1970.1.3
- Huber BA (2011) Revision and cladistic analysis of *Pholcus* and closely related taxa (Araneae, Pholcidae). Bonner zoologische Monographien 58: 1–509. https://biostor. org/reference/137036
- Huber BA (2019) The pholcid spiders of Sri Lanka (Araneae: Pholcidae). Zootaxa 4550: 1–57. https://doi.org/10.11646/zootaxa.4550.1.1
- Huber BA, Chao A (2019) Inferring global species richness from megatransect data and undetected species estimates. Contributions to Zoology 88: 42–53. https://doi. org/10.1163/18759866-20191347
- Huber BA, Eberle J (2021) Mining a photo library: eggs and egg sacs in a major spider family. Invertebrate Biology 140: e1234 [1–13]. https://doi.org/10.1111/ ivb.12349
- Huber BA, Villarreal O (2020) On Venezuelan pholcid spiders (Araneae, Pholcidae). European Journal of Taxonomy 718: 1–317. https://doi.org/10.5852/ejt.2020.718.1101
- Huber BA, Wunderlich J (2006) Fossil and extant species of the genus *Leptopholcus* in the Dominican Republic, with the first case of egg-parasitism in pholcid spiders (Araneae: Pholcidae). Journal of Natural History 40: 2341–2360. https://doi.org/10.1080/00222930601051196
- Huber BA, Pérez-González A, Baptista RLC (2005) *Leptopholcus* (Araneae: Pholcidae) in continental America: rare relicts in low precipitation areas. Bonner zoologische Beiträge 53(1/2): 99–107.
- Huber BA, Carvalho LS, Benjamin SP (2014) On the New World spiders previously misplaced in *Leptopholcus*: molecular and morphological analyses and descriptions of four new species (Araneae, Pholcidae). Invertebrate Systematics 28: 432–450. https://doi.org/10.1071/IS13050
- Huber BA, Neumann J, Grabolle A, Hula V (2017) Aliens in Europe: updates on the distributions of *Modisimus culicinus* and *Micropholcus fauroti* (Araneae, Pholcidae). Arachnologische Mitteilungen 53: 12–18. https://doi.org/10.5431/aramit5303
- Huber BA, Eberle J, Dimitrov D (2018) The phylogeny of pholcid spiders: a critical evaluation of relationships suggested by molecular data (Araneae, Pholcidae). ZooKeys 789: 51–101. https://doi.org/10.3897/zookeys.789.22781
- Huber BA, Meng G, Král J, Ávila Herrera IM, Izquierdo MA (2023a) Revision of the South American Ninetinae genus *Guaranita* (Araneae, Pholcidae). European Journal of Taxonomy 900: 32–80. https://doi.org/10.5852/ejt.2023.900.2301
- Huber BA, Meng G, Dupérré N, Astrin J, Herrera M (2023b) Andean giants: *Priscula* spiders from Ecuador, with notes on species groups and egg-sac troglomorphism (Araneae: Pholcidae). European Journal of Taxonomy 909: 1–63. https://doi.org/10.5852/ ejt.2023.909.2351
- Huber BA, Meng G, Dederichs TM, Michalik P, Forman M, Král J (2024a) Castaways: the Leeward Antilles endemic spider genus *Papiamenta* (Araneae: Pholcidae). Invertebrate Systematics 38: IS23052. https://doi.org/10.1071/IS23052
- Huber BA, Meng G, Valdez-Mondragón A (2024b) Notes on *Chisosa* (Araneae: Pholcidae), with the description of a new species from Mexico. Zootaxa 5419: 217–244. https://doi.org/10.11646/zootaxa.5419.2.3
- Kehlmaier C, Schäfer M, Reimann A, Ares AM (2024) New host records of West Palaearctic spider flies (Diptera: Acroceridae). Integrative Systematics: Stuttgart Contributions to Natural History 7: 49–57. https://doi.org/10.18476/2024.479160

- Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581
- Lecigne S, Moutaouakil S, Lips J (2023) Contribution to the knowledge of the spider fauna of Morocco (Arachnida: Araneae) – First note – On new records of cave spiders. Arachnologische Mitteilungen 66: 44–71. https://doi.org/10.30963/aramit6607
- Letunic I, Bork P (2021) Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Research 49: W293–W296. https://doi.org/https://doi.org/10.1093/nar/gkab301
- Mello-Leitão C de (1929) Aranhas de Pernambuco, colhidas por D. Bento Pickel. Anais da Academia Brasileira de Ciências 1: 91–112 [pl. 1–24].
- Molero-Baltanás R, Mitchell A, Gaju-Ricart M, Robla J (2024) Worldwide revision of synanthropic silverfish (Insecta: Zygentoma: Lepismatidae) combining morphological and molecular data. Journal of Insect Science 24: 1 [ieae045]. https://doi. org/10.1093/jisesa/ieae045
- Petrunkevitch A (1929) The spiders of Porto Rico. Transactions of the Connecticut Academy of Arts and Sciences 30: 1–158.
- Puillandre N, Brouillet S, Achaz G (2021) ASAP: assemble species by automatic partitioning. Molecular Ecology Resources 21: 609–620. https://doi.org/10.1111/1755-0998.13281
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425. https://doi. org/10.1093/oxfordjournals.molbev.a040454
- Schlinger El (1987) The biology of Acroceridae (Diptera): True endoparasitoids of spiders. In: W. Nentwig (Ed.) Ecophysiology of Spiders. Springer, Berlin, 319–327. https://doi.org/10.1007/978-3-642-71552-5_24
- Simon E (1887) Arachnides recueillis à Obock en 1886 par M. le Dr L. Faurot. Bulletin de la Société Zoologique de France 12: 452–455.
- Tamura K, Stecher G, Kumar S (2021) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38: 3022–3027. https://doi. org/10.1093/molbev/msab120
- Thorell T (1895) Descriptive Catalogue of the Spiders of Burma. British Museum, London, 406 pp.
- Vishnudas EH, Sudhikumar AV (2021) First report of the small daddy long leg spider *Micropholcus fauroti* (Simon, 1887) (Araneae: Pholcidae) female from India with redescription of the male. Serket 18(1): 59–63.

Supplementary material 1

CO1 K2P distances of the sequenced specimens

Authors: Bernhard A. Huber, Guanliang Meng Data type: xlsx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1213.133178.suppl1



Research Article

Discovery of a new soft-bodied click-beetle genus from Namibia with a unique morphology leads to a modified diagnosis of Drilini (Coleoptera, Elateridae)

Robin Kundrata¹⁰, Gabriela Packova¹⁰

1 Department of Zoology, Faculty of Science, Palacky University, 17. listopadu 50, 77146 Olomouc, Czech Republic Corresponding author: Robin Kundrata (robin.kundrata@upol.cz)

Abstract

Drilini are soft-bodied predatory click beetles with incompletely metamorphosed females. Approximately 150 described species are distributed in the Afrotropical, Palaearctic and Oriental realms, with the highest diversity known from sub-Saharan Africa. In this study, we describe Namibdrilus albertalleni gen. et sp. nov. from Namibia which brings the total number of genera in Drilini to 16. The discovery of this unique taxon sheds new light on the diversity and evolution of the enigmatic paedomorphic beetle lineage and is interesting for several reasons. This new species is the only known representative of Drilini that has unidentate mandibles and lacks a hook on the dorsal part of the aedeagal median lobe, two of the few characters heretofore used for the unambiguous identification of members of this group. Furthermore, based on its morphology it belongs to a group of genera (Drilus clade) which heretofore contained only taxa from the Palaearctic Realm. We provide an updated diagnosis of the tribe Drilini, as well as an updated diagnosis and an identification key for the genera of the Drilus clade based on adult males. Further, we explain how to easily recognize adult Drilini from similar-looking soft-bodied elateroids like Elateridae: Omalisinae, Rhagophthalmidae, and Lampyridae: Ototretinae.

Key words: Afrotropical Realm, Agrypninae, click beetle, distribution, Elateroidea, identification key, male genitalia, new genus, new species, paedomorphosis, taxonomy

Introduction

The tribe Drilini (Elateridae, Agrypninae) contains soft-bodied click beetles with flight capable males, incompletely metamorphosed larviform females, and larvae which feed on land snails (Crowson 1972; Baalbergen et al. 2014; Kundrata et al. 2015a; Kundrata and Bocak 2011, 2019). Although the center of diversity of this group lies in the Afrotropical Realm, Drilini are also well represented in the Palaearctic fauna, and several species are also known from the Oriental Realm (Kundrata and Bocak 2019). This group was earlier treated as a separate family, Drilidae, and contained various taxa which are currently placed in several other families within Elateriformia (e.g., Wittmer 1944; Crowson 1972; Geisthardt 2009; Kazantsev 2010). Kundrata and Bocak (2011)



Academic editor: Vinicius S. Ferreira Received: 5 July 2024 Accepted: 13 August 2024 Published: 26 September 2024

ZooBank: https://zoobank. org/8FF3F5AB-3DF7-4DE9-AAC4-DADA05435A8E

Citation: Kundrata R, Packova G (2024) Discovery of a new softbodied click-beetle genus from Namibia with a unique morphology leads to a modified diagnosis of Drilini (Coleoptera, Elateridae). ZooKeys 1213: 183–197. https://doi. org/10.3897/zookeys.1213.131283

Copyright: © Robin Kundrata & Gabriela Packova. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). placed the group in Elateridae as tribe Drilini in Agrypninae based on a molecular phylogenetic approach. They also transferred genera *Euanoma* Reitter, 1889 and *Pseudeuanoma* Pic, 1901 to Omalisidae (currently a subfamily of Elateridae), and redefined Drilini to contain only *Drilus* Olivier, 1790, *Malacogaster* Bassi, 1834, *Selasia* Laporte, 1838, and tentatively also *Paradrilus* Kiesenwetter, 1866. The latter genus was soon after transferred to Omalisidae by Kundrata et al. (2015b) based on results of a molecular phylogenetic analysis. This concept of Drilini with only three genera lasted only until Kundrata and Bocak (2017) investigated the diversity of Drilini in western Africa and described five new genera from there. Kundrata and Bocak (2019), in the most comprehensive phylogeny of Drilini yet published, defined five main clades, and established six new genera, of which five were from tropical Africa and one from Pakistan. Kovalev et al. (2019) then described a new monotypic genus from Iran.

Here, we report the discovery of a morphologically unique and extremely interesting Drilini specimen from Namibia, which represents a new genus and species. Surprisingly, it is morphologically similar to geographically distant Palaearctic genera. The unique morphology of its mandibles and male genitalia compelled us to modify the diagnosis of the tribe Drilini.

Material and methods

The genitalia were dissected after a short treatment in hot 10% KOH. Images of habitus and main diagnostic characters were photographed using a digital camera Canon EOS M6 Mark II attached to a stereoscopic microscope Olympus SZX12. Stacks of photographs were combined with the software Helicon Focus Pro (version 7.6.4, Kharkiv, Ukraine), applying the 'depth map' or 'weighted average' rendering methods. We did not clean the surface of the holotype in order not to damage the unique specimen. The measurements were taken with a scale bar in an eyepiece. Body length was measured from the fore margin of the head to the apex of elytra (since abdomen is highly flexible in soft-bodied elateroids), body/elytra width at humeri, head width including eyes, minimum interocular distance in the frontal part of the cranium, maximum eye diameter in lateral view, pronotal length at midline, pronotal width at the widest part, scutellar shield length at midline, scutellar shield width at the widest part, aedeagus length medially from base to the apex of the median lobe, and aedeagus width at the widest part. We follow the morphological terminology and the classification of Drilini by Kundrata and Bocak (2019). Label data are cited verbatim. The holotype of the here-described new species is deposited in the National Museum, Prague, Czech Republic (NMPC). The Drilini specimens used for comparison of the here-described new species with its congeners are deposited in the following collections: The Natural History Museum, London, United Kingdom (BMNH), Koninklijk Museum voor Midden-Afrika, Tervuren, Belgium (RMCA), Museum National d'Histoire Naturelle, Paris, France (MNHN), Natural History Museum, Budapest, Hungary (HNHM), Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN), Naturkundemuseum Erfurt, Germany (NKME), Naturhistorisches Museum, Vienna, Austria (NHMW), Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH), Naturhistorisches Museum, Basel, Switzerland (NHMB), Hessisches Landesmuseum, Darmstadt, Germany (HLMD), Oxford University Museum of Natural History, Oxford, United Kingdom (**OUMNH**), Museo Civico di Storia Naturale, Genova, Italy (**MSNG**), Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin, Germany (**MFNB**), Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany (**SDEI**), Lund Museum of Zoology, Lund University, Sweden (**MZLU**), Natural History Museum, Copenhagen, Denmark (**NHMD**), and the collections of Albert Allen, Idaho, USA (**PCAA**) and Robin Kundrata, Olomouc, Czech Republic (**PCRK**). The updated diagnosis of the tribe Drilini, as well as an updated diagnosis and an identification key for the genera of the *Drilus* clade, are partly based on Kundrata and Bocak (2019).

Systematics

Genus Namibdrilus gen. nov.

https://zoobank.org/81EF4D52-F9D3-4BF0-B7FD-8BDBD216032E Figs 1-4

Type species. Namibdrilus albertalleni sp. nov.; here designated.

Etymology. The generic name is derived from the Republic of Namibia, plus *Drilus*, a genus name in Elateridae: Drilini. Gender: masculine.

Diagnosis. Namibdrilus gen. nov. can be unequivocally distinguished from its congeners by the robust unidentate mandibles (Fig. 2A-D) and the aedeagal median lobe dorsally without a subapical hook (Fig. 4B, E-G). Additionally, the following combination of characters can be used to recognize the genus: body (Fig. 1A, B) elongate; frontoclypeal region (Fig. 2A-D) strongly produced forwards, gradually narrowed toward apex, apically carinate and widely rounded; eyes (Fig. 2A–D) large, their frontal separation 1.15 times eye diameter; antenna (Fig. 2E) robust, strongly serrate; pronotum (Fig. 3A, B) roughly subrectangular, 1.30 times as wide as long, lateral carina not developed; prosternum (Fig. 3C) distinctly transverse, without well-developed prosternal process; scutellar shield (Fig. 3A) apically subtruncate and medially emarginate; mesoventrite (Fig. 3C) narrow, v-shaped; elytra (Figs 1A, 3E) elongate, with relatively rough surface and without any apparent striae or rows of punctures; abdomen (Figs 1B, 4A) with eight free visible sternites, the first of which is partly membranous medially; abdominal sternite IX and tergite X (Figs 4C, D) not apparently elongate, about 1.5 times as long as wide. Based on its morphology, Namibdrilus gen. nov. is similar to genera of the clade D (Drilus clade) defined by Kundrata and Bocak (2019). For more information and the comparison of Namibdrilus gen. nov. with presumably related genera see the identification key and discussion below.

Distribution. Namibia.

Namibdrilus albertalleni sp. nov.

https://zoobank.org/CFF2DB6F-C808-411D-81B0-323F1B77859F Figs 1-4

Type material. *Holotype* • male, "Namibia, Khomas reg. 140 km SW Windhoek; 23°14.875'S, 16°17.998'E; 1382 m; 14.2.2023; J. Halada lgt." (NMPC).

Etymology. This species is named after Albert Allen (Idaho, USA), who allowed us to study the unique specimen in his possession, and who kindly





donated it to NMPC. For recent discussions about the need for protecting stable biological nomenclatural systems, which also includes the problematics of eponyms, we refer to Jiménez-Mejías et al. (2024).

Diagnosis. As for the genus (vide supra).

Description. Male. *Body* (Fig. 1A, B) slightly convex dorsally, 3.30 times longer than width at humeri (8.30 mm long, 2.50 mm wide); yellowish brown to light brown, with head and most of abdomen slightly darker, light brown to brown, and elytra reddish brown to dark brown; body surface covered with yellow pubescence.

Head (Fig. 2A–D) including eyes about 1.15 times as wide as pronotum; surface rather flat, more or less smooth basally, rougher toward apex, punctures sparse and fine but denser and larger basally; frontoclypeal region strongly produced straight forwards between antennae, gradually narrowed toward apex, lateral margins distinctly elevated and thickened above antennal insertions, forming a narrow median furrow, apically again slightly widened, subtruncate, apical margin slightly raised, carinate, very widely rounded. Eyes large, strongly prominent, their minimal frontal separation 1.15 times maximum eye diameter.



Figure 2. Namibdrilus albertalleni gen. et sp. nov., holotype, male A head with basal antennomeres, dorsal view B head, frontolateral view C head, frontal view D head, ventral view E left antenna, dorsal view. Scale bars: 1.0 mm (A–D); 2.0 mm (E).

Labrum covered by frontoclypeal region, visible from frontal view, very short, transverse, with frontal margin slightly concave; sparsely punctate and covered with long semi-erect setae. Mandible unidentate, robust, relatively wide, distinctly curved; base with rough surface covered with long semi-erect setae, apical part shiny. Maxilla with palpus tetramerous, slender, about as long as mandible, palpomere II elongate, palpomere III only slightly longer than wide, terminal palpomere longest, fusiform, apically narrowed, with apex obliquely cut. Labium with palpus trimerous, tiny, less than half length of maxillary palpus, palpomere I short, transverse, terminal palpomere elongate, narrow, fusiform, apically gradually narrowed, obliquely cut. Antenna (Fig. 2E) approximately 4.3 mm long, with 11 antennomeres, robust, pectinate from antennomere 3, reaching humeri when oriented backwards; scapus robust, about 1.8 times as long as wide; pedicel minute, shortest, slightly longer than wide; antennomere 3 about twice as long as pedicel, elongate, with ramus about 0.3 times as long as antennomere itself, antennomere 4 elongate, with ramus about as long as antennomere itself, antennomeres 5-10 subequal in length, elongate, with rami about 1.5-1.6 times as long as respective antennomeres, terminal antennomere twice as long as preceding antennomeres, simple, elongate, about 5.5 times as long as wide, apically narrowed, outer side medially shallowly emarginate.

Pronotum (Fig. 3A, B) roughly subrectangular, 0.6 times as wide as elytra, widest at posterior angles, 1.30 times as wide as long (1.20 mm long, 1.55 mm wide).



Figure 3. Namibdrilus albertalleni gen. et sp. nov., holotype, male A head and prothorax, dorsal view B head and prothorax, lateral view C prothorax and mesothorax, ventral view D metathorax, ventral view E apical portion of elytra, dorsal view F right protarsus, dorsal view G left mesotarsus, dorsal view H left metatarsus, dorsal view. Scale bars: 1.0 mm (A, D); 0.5 mm (B, C, E, F–H).

Anterior margin somewhat widely rounded, slightly produced medially, sides concave, gradually narrowed from anterior margin toward about three-fourths of pronotum length and then distinctly widened at posterior angles, posterior margin simple, trisinuate, rather arcuately and shallowly emarginate medially. Anterior angles inconspicuous; posterior angles short but distinct, divergent, with rough surface, apically narrowly rounded. Lateral carina not developed. Surface of disc relatively rough, sparsely and finely punctate, with moderately dense, long, semi-erect pubescence. Pronotosternal suture very short. Prosternum (Fig. 3C) strongly transverse, its surface uneven, sparsely punctate and covered with semi-erect setae, mainly at frontal margin; prosternal lobe absent,



Figure 4. Namibdrilus albertalleni gen. et sp. nov., holotype, male A abdomen, ventral view B male genitalia (undissected), dorsal view C abdominal tergites IX and X, dorsal view D abdominal sternite IX, ventral view E male genitalia, dorsal view F male genitalia, lateral view G male genitalia, ventral view. Scale bars: 3.0 mm (A); 0.2 mm (B); 0.5 mm (C-G).

frontal margin almost straight; prosternal process absent. Scutellar shield (Fig. 3A) flat, tongue-like, elongate, approximately 1.70 times as long as wide, with anterior margin gradually declivitous, medially slightly produced, apex subtruncate, medially slightly emarginate. Mesoventrite (Fig. 3C) small, narrow, v-shaped, with posterior margin simply rounded. Mesocoxal cavity open to both mesepimeron and mesanepisternum. Metaventrite (Fig. 3D) large, subtrapezoidal, covered with fine punctures and semi-erect pubescence; discrimen incomplete. Elytra (Figs 1A, 3E) subparallel-sided, only slightly gradually narrowed from humeri to about apical third, both combined 2.30 times as long as wide (5.70 mm long, 2.50 mm wide), 4.75 times as long as pronotum, surface uneven, basally wrinkled, without any distinct striae or lines of punctures, only with several faint costae at basal half, irregularly finely punctate, with long, semi-erect pubescence oriented posteriorly, apices internally slightly divergent, separately rounded; epipleura wider anteriorly, abruptly narrowed near posterior part of metanepisternum, then reduced. Hind wing fully developed. Leg (Figs 1A, B, 3A-D, F-H) moderately long, slightly compressed, with surface covered with moderately long, semi-erect setae, which are thickened mainly ventrally and apically; coxa robust, elongate; trochanter elongate, slightly widened apically, attached obliquely to femur; femur gradually slightly widened towards apex; tibia slightly longer than femur; tarsus (Fig. 3F–H) shorter than tibia, metatarsus relatively longer than pro- and mesotarsus; tarsomeres I-III elongate, widened apically, progressively decreasing in length, tarsomere IV short, ventrally with

short membranous lobe, terminal tarsomere long, slender; pretarsal claws simple, slender, slightly curved, basally with long setae.

Abdomen (Figs 1B, 4A) soft, highly flexible, with eight free sternites (II-IX) connected with each other by extensive membranes; sternite II semi-membranous, with two lateral sclerites and two median sclerotizations; all ventrites with sparse, shallow punctures, covered with semi-erect pubescence, which is denser and longer posteriorly and mainly at margins; penultimate ventrite (sternite XIII) with two shallow posterolateral and one rounded median emarginations. Tergites IX and X (Fig. 4C) wider than long, weakly connected by membrane, both covered with fine punctures and relatively long setae; tergite IX basally with two sublateral processes, tergite X apically widely rounded. Sternite IX (Fig. 4D) about 1.5 times as long as wide, roughly oval, with basal portion medially deeply emarginate, apex rounded, surface finely punctate and sparsely covered with setae; sternite X about 0.45 times as long as sternite IX, slightly longer than wide, somewhat rounded. Male genitalia (Fig. 4B, E-G) about 2.6 times as long as wide, about 1.3 times as long as sternite IX; median lobe elongate, distinctly longer than paramere, basally moderately curved in lateral view, with two very short basal struts, dorsally without a subapical hook, rather subparallel-sided in dorsoventral view, slightly widened after half, then slightly but abruptly constricted before apex, apically rounded; paramere distinctly longer than phallobase, with basal half robust and wide, and with apical half distinctly narrowed, divergent, apically narrowly rounded in lateral view; phallobase short, about 1.3 times as wide as long, widely u-shaped.

Females and immature stages unknown.

Distribution. Namibia.

Updated diagnosis of Drilini based on adult males

Body soft, only weakly sclerotized; mandible bidentate (unidentate in *Namibdri-lus* gen. nov.); antenna with 11 antennomeres; antennomere II minute, always distinctly shorter than antennomere 3; tarsomere IV shortest, ventrally with short membranous lobe; pretarsal claw with stout setae on outer side of base; abdomen with seven or eight visible sternites (the most basal one can be formed by two separate lateral sclerites connected by membrane); aedeagal median lobe considerably curved laterally, dorsally with subapical hook (without hook in *Namibdrilus* gen. nov.); and phallobase without any posterolateral processes.

Updated diagnosis of clade D (Drilus clade) based on adult males

Head often flattened; frontoclypeal region usually more or less produced forwards between antennae; eyes relatively small to medium-sized in Palaearctic species (their frontal separation 1.60–3.00 times eye diameter), large in Afrotropical *Namibdrilus* gen. nov. (their frontal separation 1.15 times eye diameter); antenna serrate to pectinate; pronotum usually less transverse and without sublateral carinae; prosternum without well-developed prosternal process; scutellar shield apically widely rounded to subtruncate; mesoventrite v-shaped, posteriorly simply rounded; elytra often divergent or reduced, with surface uneven, often wrinkled; abdomen with eight visible sternites; sternite IX rounded to oval, basally narrowed; posterior margin of pronotum simple and without emargination or shallowly and arcuately emarginate; abdominal ventrites I–IV never connate.

Genera included

Drilorhinus Kovalev, Kirejtshuk & Shapovalov, 2019 (Fig. 5A); *Drilus* Olivier, 1790 (Fig. 5B); *Malacodrilus* Kundrata & Bocak, 2019 (Fig. 5C); *Malacogaster* Bassi, 1834 (Fig. 5D); and *Namibdrilus* gen. nov. (Figs 1–4). For more information on individual genera see e.g., Kundrata et al. (2015a), Sormova et al. (2018), Kovalev et al. (2019), Kundrata and Bocak (2019) and Hoffmannova and Kundrata (2022).



Figure 5. Representatives of Drilini from the clade D (*Drilus* clade), adult males in dorsal view A *Drilorhinus klimenkoi* Kovalev, Kirejtshuk & Shapovalov, 2019, Iran (PCAA) B *Drilus flavescens* (Geoffroy, 1785), Italy (PCRK) C *Malacodrilus hajeki* Kundrata & Bocak, 2019, Pakistan (PCRK) D *Malacogaster passerinii* Bassi, 1834, Tunisia (PCRK). Scale bars: 4.0 mm.

An identification key for the genera of Drilini in clade D (*Drilus* clade) based on adult males

- Frontoclypeal region if produced forwards then relatively short and wide; mandibles slenderer, gradually narrowed toward apex; antenna serrate to pectinate
 3

- Frons distinctly widened; antenna serrate; pronotum subquadrate; anterior margin of prosternum concave; first visible abdominal sternite complete.
 Malacodrilus Kundrata & Bocak, 2019

Discussion

The here-described new genus of Drilini is extremely interesting from the morphological as well as from the evolutionary point of view. Its discovery has changed our view regarding the diagnosis of the tribe Drilini as well as our understanding of the morphology and distribution of one of the main clades of Drilini. Therefore, both the diagnostic characters used for the recognition of Drilini and the unique morphology of the new genus are worthy of a more detailed discussion.

Within the family Elateridae, Drilini are easily recognizable due to their various modifications connected with the soft-bodiedness and the loss of clicking mechanism. Compared to the typical well-sclerotized and clicking Elateridae, Drilini males have e.g., much softer body, reduced prosternum usually with a strongly reduced or missing prosternal process, reduced mesoventrite without a well-developed mesoventral cavity and often with a reduced mesoventral process, and the abdomen with seven or eight visible sternites (compared to usually five in hard-bodied elaterids; Kundrata and Bocak 2019). The females are larviform, lack elytra, and do not look like any other elaterid adult female (e.g., Hoffmannova and Kundrata 2022).

However, it is not always easy for some to distinguish adult Drilini from similar-looking soft-bodied elateroids, mainly Elateridae: Omalisinae, Rhagophthalmidae, and Lampyridae: Ototretinae, many of which were historically classified in the broadly defined Drilidae (Wittmer 1944). From all three above-mentioned groups, Drilini males were always easily distinguishable based on their bidentate and more robust mandibles, setae at the bases of pretarsal claws (the latter is the character typical for Agrypninae), and a distinct hook on the dorsal part of the aedeagal median lobe. All known Omalisinae, Rhagophthalmidae, and Ototretinae have simple, usually narrow and sickle-shaped mandibles; they lack setae on the pretarsal claws and lack a hook on the aedeagal median lobe (Janisova and Bocakova 2013; Bocek et al. 2018; Kundrata et al. 2022). Furthermore, Omalisinae have antennomeres 2 and 3 short and subequal in size (only antennomere 2 is short in Drilini), and the phallobase with posterolateral processes (without those processes in Drilini) (e.g., Kundrata et al. 2015b, Packova et al. 2024), and Rhagophthalmidae have always 12 antennomeres (compared to 11 in Drilini) and numerous species have deeply emarginate eyes (always simple in Drilini) (Kawashima et al. 2010; Kundrata et al. 2022; Packova and Kundrata 2023). Regarding the females, all these groups have them paedomorphic, retaining many larval characters in their adulthood. Females of many genera and species of Drilini, Omalisinae, Rhagophthalmidae, and Ototretinae are actually unknown. Nevertheless, based on available information, we can recognize females of Drilini by having only the head and

legs completely metamorphosed and the rest of the body being larviform (e.g., Kundrata et al. 2015a; for females of Omalisinae see e.g., Geisthardt 1977 and Bocek et al. 2018, for females of Rhagophthalmidae see Kawashima et al. 2010 and Kundrata et al. 2022, and for females of Ototretinae see Kawashima 1999 and Yiu and Jeng 2018).

From three main characters which were heretofore used for the unambiguous identification of Drilini males (i.e., bidentate mandibles, setae at the bases of pretarsal claws, and a distinct hook on the dorsal part of the aedeagal median lobe), Namibdrilus gen. nov. has only the setae on the claws. Namibdrilus gen. nov. has unidentate mandibles (Fig. 2A-D) that are still more robust than the sickle-shaped mandibles of omalisines, rhagophthalmids and ototretines. However, all other Drilini have bidentate mandibles although some Drilus spp. and Malacogaster spp. have the inner tooth minute (Kundrata et al. 2015a, Hoffmannova and Kundrata 2022). All Drilini species for which male genitalia was known, representing all described genera (confirmed by several hundreds of dissections by the first author and his students), had an aedeagal median lobe with a distinct hook (see e.g., Geisthardt 2007; Kundrata and Bocak 2017, 2019; Kovalev et al. 2019). However, Namibdrilus gen. nov. is surprisingly the first representative of Drilini with the median lobe of the aedeagus simple, without any hook (Fig. 4E-G). Consequently, the diagnosis of Drilini needed to be modified accordingly.

Based on the results of molecular phylogeny supported by the morphological characters, Drilini were divided into five major informal groups, i.e., clades A, W, S, M and D (letters represent the first letters of the respective genus name which is typical for the given clade; Kundrata and Bocak 2019). Four of those clades (A, W, S, M) contain solely or predominantly Afrotropical taxa (with several species of the clade S extending to the Oriental Realm) while the clade D contained exclusively Palaearctic species. One would expect that Namibdrilus gen. nov. from southern Africa belongs to one of the African clades; however, surprisingly it falls morphologically into the clade containing Palaearctic species. All known African Drilini differ from Namibdrilus gen. nov. in having a short frontoclypeal region and an aedeagal median lobe with a dorsal hook. Furthermore, the representatives of two basal-most clades A (Austroselasia Kundrata & Bocak, 2019) and W (Habeshaselasia Kundrata & Bocak, 2019, Latoselasia Kundrata & Bocak, 2017, Mashaselasia Kundrata & Bocak, 2019, Wittmerselasia Kundrata & Bocak, 2017) differ from Namibdrilus gen. nov. in many important diagnostic characters including the pronotum with posterior margin medially rectangularly emarginate (arcuately emarginate in Namibdrilus gen. nov.), the prosternal process present, platelike (absent in Namibdrilus gen. nov.), the mesoventral process well developed (absent in Namibdrilus gen. nov.), the elytral surface relatively smooth and with apparent elytral striae and interstriae or at least their remnants (elytral surface rather rough and without any striae in Namibdrilus gen. nov.), seven abdominal ventrites with the first four connate (eight free ventrites in Namibdrilus gen. nov.), and the intercoxal process present on the first abdominal ventrite (absent in Namibdrilus gen. nov.). Members of clade S (Illubaboria Kundrata & Bocak, 2019; Selasia) usually have an apparent prosternal process, well-developed mesoventral process, seven abdominal ventrites, and at least a slightly developed intercoxal process on the first abdominal ventrite. The representatives of clade M (Flabelloselasia Kundrata & Bocak, 2017, Kupeselasia Kundrata & Bocak, 2017,

Lolosia Kundrata & Bocak, 2017, *Microselasia* Kundrata & Bocak, 2017) are usually tiny forest species from the western and central Africa which differ from *Namibdrilus* gen. nov. in having a smooth glabrous pronotal disc often with distinct sublateral carinae (pronotum with relatively rough surface and no sublateral carinae in *Namibdrilus* gen. nov.) and the prosternum with a prosternal process forming a narrow plate (absent in *Namibdrilus* gen. nov.).

On the other hand, Namibdrilus gen. nov. shares many diagnostic characters with the Palaearctic genera included in clade D (Drilorhinus, Drilus, Malacodrilus, Malacogaster), including the frontoclypeal region produced forwards between antennae, the pronotum not distinctly transverse and without sublateral carinae, the posterior margin of pronotum shallowly and arcuately emarginate, the prosternum without a well-developed prosternal process, the scutellar shield apically subtruncate, the mesoventrite v-shaped and without a well-developed mesoventral process, the elytral apices not conjointly rounded and instead being divergent at internal margins, the elytral surface rough and without distinct striae, and the abdomen with eight free visible sternites. Based on the above-listed characters, we place this genus within clade D (Drilus clade) as the only Afrotropical member in this otherwise Palaearctic group. Namibdrilus gen. nov. differs from all other genera in the group by having much larger eyes, with their frontal separation 1.15 times eye diameter (eyes in the Palaearctic genera are small to medium-sized, with their frontal separation 1.60-3.00 times eye diameter; Fig. 2A-D; e.g., Kundrata et al. 2015a, Hoffmannova and Kundrata 2022), the frontoclypeal region strongly produced forwards, carinate and anteriorly widely rounded (if the frontoclypeal region in the Palaearctic genera is strongly produced then it is anteriorly narrowed and clearly emarginate; Fig. 2A-D; Packova et al. 2021), unidentate mandibles (always bidentate in the Palaearctic genera), relatively narrower prosternum, and the aedeagal median lobe without any subapical hook (Fig. 4E-G). Based on its divergent morphology and distribution in tropical Africa, we hypothesize that Namibdrilus gen. nov. may be a sister group to all remaining genera in clade D. Of course, without a strong phylogenetic hypothesis we cannot exclude the possibility that Namibdrilus gen. nov. either represents a more developed paedomorphic phenotype in one of the previously identified African clades (less likely) or it forms a separate clade on its own. All these hypotheses should be tested using a molecular approach in future research.

One of the most striking characters of *Namibdrilus* gen. nov. is the frontoclypeal region which is strongly produced forwards and is visible even from the ventral view of the head (Fig. 2D). Although Drilini have variously shaped frontoclypeal region from e.g., a very short and wide in *Selasia* spp. to a narrow and high in *Microselasia* spp., major modifications are known in genera of the *Drilus* clade. *Malacogaster ruficollis* Dodero, 1925 from Libya is the only species of genus *Malacogaster* having the frontoclypeal region produced forwards and covering labrum; however, it is very wide, sloping downwards and broadly rounded apically (Hoffmannova and Kundrata 2022). On the other hand, the monotypic *Drilorhinus* from Iran (Fig. 5A) and several Mediterranean *Drilus* spp. have the frontoclypeal region strongly produced forwards and apically narrowed, with apex medially distinctly emarginate (Kovalev et al. 2019; Packova et al. 2021). None of these conditions is similar to the strongly produced frontoclypeal region of *Namibdrilus* gen. nov., which is oriented straight forwards and apically somewhat subtruncate and widely rounded (Fig. 2A–D). In summary, the discovery of *Namibdrilus* gen. nov. is extremely important for our understanding of the diversity and evolution of Drilini and represents one of the most interesting findings regarding Drilini in recent decades. Recent increased research on Drilini has already led to a better understanding of their systematic placement (Kundrata and Bocak 2011), their phylogenetic relationships and diversity, including descriptions of several new genera (Kundrata et al. 2015a, Kundrata and Bocak 2017, 2019), or the discovery of the first Drilini from South East Asia (Kundrata and Sormova 2018). Based on morphology, *Namibdrilus* gen. nov. represents a connection between tropical Africa, the center of diversity of Drilini, with a solely Palaearctic group of genera with the highest degree of paedomorphosis-related morphological modifications (although this remains to be further tested using a molecular approach). Furthermore, its unique aedeagal morphology stresses the importance of male genitalia for the systematics of Drilini.

Acknowledgements

We thank Albert Allen (Idaho, USA), who allowed us to study the unique specimen of the here-described species, and who kindly donated it to NMPC. We also thank all curators from the institutions listed in Material and methods and other colleagues for allowing us to study Drilini specimens in their care, and Simone Policena Rosa (Brazil) and two anonymous reviewers for their helpful comments and suggestions. Our special thanks go to Jiří Halada, Pavel Kučera, František Černý and Walter Grosser (all Czech Republic) for their effort to search for more specimens of the here-described species.

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 funded by the internal grant of the Faculty of Science, UP Olomouc (IGA_ PrF_2024_029) and the National Natural Science Foundation of China (32270483).

Author contributions

Robin Kundrata: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review and editing; Gabriela Packova: Investigation, Visualization, Writing review and editing.

Author ORCIDs

Robin Kundrata https://orcid.org/0000-0001-9397-1030 Gabriela Packova https://orcid.org/0000-0001-7949-619X

Data availability

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

References

- Baalbergen E, Helwerda R, Schelfhorst R, Castillo Cajas RF, van Moorsel CHM, Kundrata R, Welter-Schultes FW, Giokas S, Schilthuizen M (2014) Predator-prey interactions between shell-boring beetle larvae and rock-dwelling land snails. PLoS ONE 9(6): e100366. https://doi.org/10.1371/journal.pone.0100366
- Bocek M, Fancello L, Motyka M, Bocakova M, Bocak L (2018) The molecular phylogeny of Omalisidae (Coleoptera) defines the family limits and demonstrates low dispersal propensity and ancient vicariance patterns. Systematic Entomology 43: 250–261. https://doi.org/10.1111/syen.12271
- Crowson RA (1972) A review of the classification of Cantharoidea (Coleoptera), with definition of two new families, Cneoglossidae and Omethidae. Revista de la Universidad de Madrid 21: 35–77.
- Geisthardt M (1977) Redeskription der weiblichen Imago von *Homalisus fontisbellaquei* Geoffroy 1762 (Coleoptera: Homalisidae). Mitteilungen des Internationalen Entomologischen Vereins e. V. 3: 113–119.
- Geisthardt M (2007) Neue und bekannte *Selasia* Laporte, 1836 Arten aus dem südliche Afrika (Coleoptera, Drilidae). Entomologica Basiliensia et Collectionis Frey 29: 31–40.
- Geisthardt M (2009) Order Coleoptera, family Drilidae. Arthropod fauna of the UAE 2: 159–163.
- Hoffmannova J, Kundrata R (2022) Diversity of the paedomorphic snail-eating click-beetle genus *Malacogaster* Bassi, 1834 (Elateridae: Agrypninae: Drilini) in the Mediterranean. Biology 11(10): e1503. https://doi.org/10.3390/biology11101503
- Janisova K, Bocakova M (2013) Revision of the subfamily Ototretinae (Coleoptera: Lampyridae). Zoologischer Anzeiger 252(1): 1–19. https://doi.org/10.1016/j. jcz.2012.01.001
- Jiménez-Mejías P, Manzano S, Gowda V, Krell F-T, Lin M-Y, Martín-Bravo S, Martín-Torrijos L, Feliner GN, Mosyakin SL, Naczi RFC, Acedo C, Álvarez I, Crisci JV, Luceño Garcés M, Manning J, Moreno Saiz JC, Muasya AM, Riina R, Sánchez Meseguer A, Sánchez-Mata D et al. (2024) Protecting stable biological nomenclatural systems enables universal communication: A collective international appeal. BioScience 74: 467–472. https://doi.org/10.1093/biosci/biae043
- Kawashima I (1999) The lampyrid beetles of the genus *Stenocladius* (Coleoptera, Lampyridae) of the Ryukyu Islands, Southwest Japan with descriptions of two new species. Elytra, Tokyo 27: 141–158.
- Kawashima I, Lawrence JF, Branham MA (2010) Rhagophthalmidae Olivier, 1907. In: Leschen RAB, Beutel RG, Lawrence JF (Eds) Coleoptera, Beetles (Vol. 2): Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). In: Kristensen NP, Beutel RG (Eds) Handbook of Zoology, Arthropoda: Insecta. Walter de Gruyter GmbH and Co KG, Berlin/New York, 135–140. https://doi.org/10.1515/9783110911213.135
- Kazantsev SV (2010) New taxa of Omalisidae, Drilidae and Omethidae, with a note on systematic position of Thilmaninae (Coleoptera). Russian Entomological Journal 19(1): 51–60. https://doi.org/10.15298/rusentj.19.1.09
- Kovalev AA, Kirejtshuk AG, Shapovalov AM (2019) *Drilorhinus*, a new genus of the family Drilidae Lacordaire, 1857 (Coleoptera: Elateroidea) from Iran. Zootaxa 4577(1): 187–194. https://doi.org/10.11646/zootaxa.4577.1.12
- Kundrata R, Bocak L (2011) The phylogeny and limits of Elateridae (Insecta, Coleoptera): is there a common tendency of click beetles to soft-bodiedness and neoteny? Zoologica Scripta 40: 364–378. https://doi.org/10.1111/j.1463-6409.2011.00476.x

- Kundrata R, Bocak L (2017) Taxonomic review of Drilini (Elateridae: Agrypninae) in Cameroon reveals high morphological diversity, including the discovery of five new genera. Insect Systematics & Evolution 48: 441–492. https://doi. org/10.1163/1876312X-48022161
- Kundrata R, Bocak L (2019) Molecular phylogeny reveals the gradual evolutionary transition to soft-bodiedness in click-beetles and identifies sub-Saharan Africa as a cradle of diversity for Drilini (Coleoptera: Elateridae). Zoological Journal of the Linnean Society 187: 413–452. https://doi.org/10.1093/zoolinnean/zlz033
- Kundrata R, Sormova E (2018) *Selasia dembickyi* sp. nov., the first member of Drilini (Coleoptera: Elateridae) from South East Asia, with the description of *S. jenisi* sp. nov. from Nepal. Acta Entomologica Musei Nationalis Pragae 58: 513–518. https://doi. org/10.2478/aemnp-2018-0039
- Kundrata R, Baalbergen E, Bocak L, Schilthuizen M (2015a) The origin and diversity of Drilus Olivier, 1790 (Elateridae: Agrypninae: Drilini) in Crete based on mitochondrial phylogeny. Systematics and Biodiversity 13(1): 52–75. https://doi.org/10.1080/147 72000.2014.968236
- Kundrata R, Baena M, Bocak L (2015b) Classification of Omalisidae based on molecular data and morphology, with description of Paradrilinae subfam. nov. (Coleoptera: Elateroidea). Zootaxa 3915(3): 413–422. https://doi.org/10.11646/zootaxa.3915.3.6
- Kundrata R, Hoffmannova J, Hinson KR, Keller O, Packova G (2022) Rhagophthalmidae Olivier, 1907 (Coleoptera, Elateroidea): Described genera and species, current problems, and prospects for the bioluminescent and paedomorphic beetle lineage. ZooKeys 1126: 55–130. https://doi.org/10.3897/zookeys.1126.90233
- Packova G, Kundrata R (2023) A new species of *Rhagophthalmus* Motschulsky, 1854 (Coleoptera, Rhagophthalmidae) from Laos represents the smallest known member of the genus. ZooKeys 1184: 81–89. https://doi.org/10.3897/zookeys.1184.112437
- Packova G, Hoffmannova J, Kundrata R (2021) Drilini (Coleoptera: Elateridae: Agrypninae) of Iran. The Coleopterists Bulletin 75(3): 579–586. https://doi.org/10.1649/0010-065X-75.3.579
- Packova G, Brus J, Kazantsev SV, Kundrata R (2024) New species and distributional records of the paedomorphic click-beetle genus *Euanoma* Reitter, 1889 (Coleoptera, Elateridae, Omalisinae) from the Western Palaearctic. Journal of Asia-Pacific Entomology 27: 102260. https://doi.org/10.1016/j.aspen.2024.102260
- Sormova E, Kramp K, Kundrata R (2018) Diversity and phylogenetic relationships of Drilus Olivier, 1790 (Elateridae: Agrypninae: Drilini) in Cyprus. Zoologischer Anzeiger 275: 1–12. https://doi.org/10.1016/j.jcz.2018.04.001
- Wittmer W (1944) Catalogue des Drilidae E. Oliv. (Coleoptera Malacodermata). Revista de la Sociedad Entomológica Argentina 12(3): 203–221.
- Yiu V, Jeng ML (2018) Oculogryphus chenghoiyanae sp. n. (Coleoptera, Lampyridae): A new ototretine firefly from Hong Kong with descriptions of its bioluminescent behavior and ultraviolet-induced fluorescence in females. ZooKeys 739: 65–78. https://doi. org/10.3897/zookeys.739.21502



Research Article

A new species of the rare genus *Endogeophilus* from southern France, with a key to the European genera of Geophilidae s.l. (Chilopoda)

Étienne Iorio¹⁰, Lucio Bonato^{2,30}

1 El – Entomologie & Myriapodologie, 36 impasse des Acacias, F-84260 Sarrians, France

2 Dipartimento di Biologia, Università di Padova, via U. Bassi 58b, I-35131 Padova, Italy

3 National Biodiversity Future Centre, Palermo, Italy

Corresponding author: Étienne Iorio (cingulata@hotmail.fr)

Abstract

The geophilid centipede *Endogeophilus alberti* **sp. nov.** is described and illustrated based on a single specimen collected from Provence, southern France. It is very similar to *E. ichnusae* Bonato, Zapparoli, Drago & Minelli, 2016, which is known only from three specimens from south-western Sardinia, and was the only species in the genus *Endogeophilus* Bonato, Zapparoli, Drago & Minelli, 2016. Both species share a remarkably narrow body, very short setae, and an unusually high number of legs, which are relatively stout. All these traits are rare among geophilids and suggest an endogeic life style. Despite of the very few specimens available for comparison and the difficulties to distinguish inter-specific differences from intra-specific variation, the two species differ at least in the shape of the pretarsi of the second maxillae and the shape of the forcipules. A revised diagnosis of the genus *Endogeophilus* is also provided, with an identification key to all genera of Geophilidae s.l. recorded so far in Europe, based on selected characters to evaluate without anatomical dissection and illustrated with original pictures.

Key words: Disjunct distribution, endogeic, Europe, Geophilomorpha, morphology, Provence, Sardinia

Introduction

Endogeophilus Bonato, Zapparoli, Drago & Minelli, 2016 is a peculiar lineage of European geophilids, showing morphological traits that suggest strictly endogeic life (Bonato et al. 2016). However, it is one of the most infrequently found geophilids, despite the European soil biotas have been intensely sampled in the past: up to date, only three specimens have been reported, from two sites in south-western Sardinia, and they have been recognized as conspecific (*Endogeophilus ichnusae* Bonato, Zapparoli, Drago & Minelli, 2016).

Here we report on a new record of *Endogeophilus*, the first from continental Europe, precisely from Provence, southern France (Fig. 1). Only a single specimen has been found; nevertheless the critical examination of its morphological features indicates that it represents a new species, clearly related to *Endogeophilus ichnusae*.



Academic editor: Luis Pereira Received: 26 July 2024 Accepted: 13 August 2024 Published: 26 September 2024

ZooBank: https://zoobank.org/ CCDC5C64-CBF4-4F02-AAD9-979642899A68

Citation: Iorio É, Bonato L (2024) A new species of the rare genus *Endogeophilus* from southern France, with a key to the European genera of Geophilidae s.l. (Chilopoda). ZooKeys 1213: 199–224. https://doi. org/10.3897/zookeys.1213.133171

Copyright: © Étienne Iorio & Lucio Bonato. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Étienne Iorio & Lucio Bonato: A new Endogeophilus with a key to the European genera of Geophilidae



Figure 1. Distribution of all known records of Endogeophilus.

Besides describing *Endogeophilus alberti* sp. nov. and discussing differences between the two species of *Endogeophilus*, we also provide a revised diagnosis of the genus and an identification key to assist in distinguishing it from all other European geophilids.

Materials and methods

A total of 758 centipedes (Chilopoda) were collected by the first author in the Port-Cros National Park (both in the central and the peripheral area, from Ramatuelle to Hyères, islands included). They were collected mainly by hand and by soil and litter sieving, in several sessions between 2019 and 2024. All specimens have been examined by the first author, with a Motic SMZ168 T-LED stereo microscope and a Motic Elite B1-223E-SP trinocular microscope. Geophilids have been identified at the species level. For the taxonomy and nomenclature, we followed Bonato and Minelli (2014), lorio (2014), and subsequent papers (Bonato et al. 2016, 2023). For the diagnostic characters, we followed Brolemann (1930) and Bonato et al. (2014a).

Only one specimen of the new species was found, despite several further attempts aimed at collecting other specimens. The single specimen was compared to representatives of other known geophilid species, including a paratype of *Endogeophilus ichnusae* (in the collection of the University of Padova, code PD 1373; see Bonato et al. 2016).

Measurements were taken with two micrometres applied to the B1-223E-SP microscope, with precision 0.1 mm or 0.01 mm. Photos were taken with a Moticam 5 camera applied to the same microscope and stacked with Helicon Focus

8.2.2. For describing the morphology, we followed the terminology recommended by Bonato et al. (2010). Abbreviation: **LBS** = leg-bearing segment(s).

In order to facilitate the distinction of specimens of Endogeophilus from similar centipedes, and to provide a practical tool for sorting samples of European geophilids, we produced an original identification key to all genera of Geophilidae known from Europe. We considered the Geophilidae sensu lato (i.e., comprising genera previously separated in other families, like Dignathodontidae Cook, 1896 and Linotaeniidae Cook, 1899, to encompass a probably monophyletic group; see Bonato et al. 2014b) and we followed the conventional geographic boundaries adopted by Fauna Europaea (de Jong 2016) and by recent synopses on European Geophilomorpha (Bonato and Minelli 2014; Bonato et al. 2014a). The key was based on both published information on morphology (Brolemann 1930; Verhoeff 1941; Machado 1952; Minelli 1982; Lewis et al. 1988; Bonato et al. 2006, 2011, 2012a, 2012b, 2014a, 2016; Bonato and Minelli 2008; Barber 2009; Tuf and Dányi 2015; Iorio 2016; Dányi and Tuf 2017; Iorio and Quindroit 2018; Iorio et al. 2022b; Desmots and Racine 2023; Dyachkov and Bonato 2024) and original observations on specimens representative of different genera. Priority was given to characters that are effective for both adult and immature specimens, easier to evaluate and less prone to subjective interpretation or misinterpretation.

Photographs illustrating the key were taken of the following specimens:

- Acanthogeophilus spiniger (Meinert,1870): 1 ♀, Edough Massif (Algeria), 23.X.1984, leg. unknown, det. L. Bonato, PD-G 5153
- Arctogeophilus inopinatus (Ribaut, 1911): 2 ♂, 2 ♀, Mervent (Vendée, France), forest of Mervent, old oak forest, 05.V.2015, leg./det. É. Iorio
- Arenophilus peregrinus Jones, 1989: 1 ♀, Noirmoutier-en-l'Île (Vendée, France), les Cents, leg. D. Desmots, det. A. Racine. Specimen reported by Desmots and Racine (2023)
- Clinopodes vesubiensis Bonato, Iorio & Minelli, 2011: ♀, Lucéram (Alpes-Maritimes, France), Peira-Cava, La Cabanette, 1320–1450 m a.s.l., mixed forest, 09.III.2007, leg. É. Iorio, det. L. Bonato and É. Iorio
- *Dignathodon microcephalus* (Lucas, 1846): ♀, Roquebillière (Alpes-Maritimes, France), Berthemont, 950 m a.s.l., deciduous forest, 07.VII.2007, leg./det. É. Iorio
- *Eurygeophilus pinguis* (Brolemann, 1898): ♀, Loubens (Ariège, France), cave of Portel, 16.III.2014, leg. O. Courtin, det. É. Iorio
- *Galliophilus beatensis* Ribaut & Brolemann, 1927: *(*), Escouloubre (Aude, France), forest of Carcanet, 04.VI.2015, leg. H. Brustel, det. A. Racine and É. Iorio
- *Geophilus electricus* (Linnaeus, 1758): ♂♀, la Chapelle-en-Valgaudémar (Hautes-Alpes, France), 44.8179°N, 6.1816°E (WGS84), 1146 m a.s.l., forest edge, 04.V.2021, leg. F. Noël, det. É. Iorio
- G. flavus (De Geer, 1778): ♂♀, la Chapelle-en-Valgaudémar (Hautes-Alpes, France), 44.8179°N, 6.1816°E (WGS84), 1146 m a.s.l., forest edge, 04.V.2021, leg. F. Noël, det. É. Iorio
- G. fucorum Brolemann, 1909: ∂♀, Hyères (Var, France), Port-Cros island, sandy beach of Port-Man, 43.0100°N, 6.4113°E (WGS84), west side with a thick stranded *Posidonia* "banquette", 04.IV.2019, leg./det. É. Iorio

- G. gavoyi Chalande, 1910: ♂, Mandelieu (Alpes-Maritimes, France), mixed forest, 07.V.2010, leg./det. É. Iorio
- G. osquidatum Brolemann, 1909: ♂♀, Torcé-Viviers-en-Charnie (Mayenne, France), forest of the Grande Charnie, 48.0693°N, -0.2591°E (WGS84), oak forest with temporary stream, 05.IV.2016, leg./det. É. Iorio
- G. richardi Brolemann, 1904: 1 ♂, 2 ♀, Sospel (Alpes-Maritimes, France), river of the Bévéra, riparian forest, 26.III.2018, leg. J.-M. Lemaire, det. É. Iorio
- G. studeri Rothenbühler, 1899: ♀, la Chapelle-en-Valgaudémar (Hautes-Alpes, France), 44.8243°N, 6.2625°E (WGS84), 1386 m a.s.l., beech forest, 03.V.2021, leg. F. Noël, det. É. Iorio
- Gnathoribautia bonensis (Meinert,1870): ♀, Colares (Lisbon district, Portugal), 38.8302°N, -9.4686°E, on a cliff, 28.XII.2018, leg. T. Cherpitel and M. Filipe, det. A. Racine and É. Iorio
- Henia bicarinata (Meinert, 1870): ♀, La Croix-Valmer (Var, France), Cap Taillat, 43.1714°N, 6.6414°E, gravel beach with a stranded Posidonia "banquette", 24.III.2022, leg./det. É. Iorio
- *H. brevis* (Silvestri, 1896): ♀, Sospel (Alpes-Maritimes, France), 43.9042°N, 7.4481°E, 620 m a.s.l., oak forest, in a shaded valley 05.XI.2020, leg./det. É. Iorio
- *H. vesuviana* (Newport, 1844): ♂♀, Metz (Moselle, France), Fort of Queuleu, deciduous forest in old fortifications, 01.V.2002, leg./det. É. Iorio
- Pachymerium ferrugineum (C.L. Koch, 1835): 1 ♂, 2 ♀, Hyères (Var, France), Giens, beach of l'Ayguade, 43.0402°N, 6.0964°E (WGS84), under stones and stranded *Posidonia*, 26.X.2015, leg. F. Noël, det. É. Iorio
- Pleurogeophilus mediterraneus (Meinert, 1870): 3♀, Sospel (Alpes-Maritimes, France), 43.8426°N, 7.4477°E (WGS84), 729 m a.s.l., mixed forest, northern slope, 10.XI.2020, leg./det. É. Iorio
- Stenotaenia linearis (C.L. Koch, 1835): 2 ♀, Ramatuelle (Var, France), Moulin of Paillas, 43.2135°N, 6.6070°E, 250 m a.s.l., old oak forest, 13.II.2024, leg./det. É. lorio
- Strigamia carniolensis (Verhoeff, 1895): 3 ♀, Chantepérier (Isère, France), 45.0123°N, 5.9757°E, 1442 m a.s.l., small beech forest, 10.V.2021, leg. F. Noël, det. É. Iorio
- Tuoba poseidonis (Verhoeff, 1901): 7 ♂, 11 ♀, Hyères (Var, France), Port-Cros island, "Fond de la Rade", 43.0068°N, 6.3820°E, well preserved gravel beach with stranded *Posidonia*, 05.IV.2019, leg./det. É. Iorio.

All specimens are in the collection of Él, with the exception of *Acanthogeophilus spiniger*, which is in the Minelli-Bonato collection, and *Arenophilus peregrinus*, which is in the collection of Antoine Racine. All photos were taken by Él, with the exception of those of *Acanthogeophilus spiniger*, taken by LB, and of *Arenophilus peregrinus*, taken by A. Racine.

Results

Endogeophilus Bonato, Zapparoli, Drago & Minelli, 2016

Diagnosis. Geophilids with the following combination of characters: body remarkably narrow (length/width ratio \sim 70); setae relatively short (not surpassing 30 µm on the head); head slightly longer than wide; clypeus uniformly areolate;

labrum with tubercles on the intermediate part and bristles on the side-pieces, which are distinct from the clypeus; second maxillary coxosternite with a long isthmus, without inner processes and without sclerotized ridges; pretarsus of second maxillae claw-like; forcipular tergite relatively broad (posterior margin about as wide as the subsequent tergite); forcipular coxosternite without anterior denticles, with complete coxopleural sutures distinctly diverging anteriorly, and with complete chitin-lines; forcipule with a single denticle, relatively small, on the tarsungulum; metasternites of the anterior part of the trunk with carpophagus pits and with pore-fields, a sub-ovoid/sub-triangular pore-field (approximately as long as wide or slightly longer than wide) on the posterior part of each metasternite; metasternites slightly longer than wide at ~ 20% of the series of trunk segments; > 90 pairs of legs, all relatively short (length/width ratio of leg tarsi < 2.5) and with slender accessory spines; metasternite of the ultimate leg-bearing segment trapezoid, wider than long; coxal organs opening through separate pores, most of them close to the metasternite, one isolated on the ventral side of the coxopleuron and some on the dorsal side; legs of the ultimate pair distinctly longer than the penultimate legs, with a claw-like pretarsus.

Type species. *Endogeophilus ichnusae* Bonato, Zapparoli, Drago & Minelli, 2016, by original designation.

Endogeophilus alberti sp. nov.

https://zoobank.org/D21886AF-5DFE-4248-8532-8D84E673C6DA

Type specimen. *Holotype* (Figs 2, 3): • ♀, 31.III.2023, É. Iorio leg. In ethanol. Body in four pieces: head, maxillae, mandibles, and trunk. Original label: Cavalaire-sur-Mer (Var), Malatra, "MALAT3", 43.1795°N, 6.5068°E (WGS84), 31.III.2023, É. Iorio leg. Deposited in the Muséum national d'Histoire naturelle, Paris (Chilopoda collection, number M370).

Type locality. FRANCE: Var department: Cavalaire-sur-Mer: near Malatra: 43.1795°N, 6.5068°E (WGS84), 215 m a.s.l., north-eastern slope (Fig. 1).

Diagnosis. An *Endogeophilus* species with claw of the second maxillae slender and hooked at its tip; forcipular trochanteroprefemur ~ 1.1× as long as wide; forcipular tarsungulum > 2.0× as long as wide, almost as long as the trochanteroprefemur, distinctly curved, fairly slender, and gradually narrowing. See also Table 1, Figs 2–4, and Discussion.

Description of the holotype (Figs 2, 3). *General features.* Body remarkably slender, 24.5 mm long, uniformly ~ 0.3 mm wide for most part of the trunk, only very slightly narrowing anteriorly along ~ 20 most anterior leg-bearing segments and backwards along approximately the five most posterior leg-bearing segments. Legs relatively short. Colour almost uniformly pale yellow, only the forcipules and the head slightly darker, both pale orange.

Cephalic capsule and antennae. Head 0.4 mm long, sub-rectangular, $1.15 \times$ longer than wide (Fig. 3A); the anterior margin slightly angulated with a small medial notch. Transverse suture absent. Approximately 40 setae on the head, the majority being short except those of the lateral margins, longer, reaching up to ~ 22 µm. Clypeus uniformly areolate, without clypeal areas and without plagulae, with six setae, arranged in a longitudinal series of three pairs on the anterior median part of the clypeus. Pleurites uniformly areolate, with some



Figure 2. Endogeophilus alberti sp. nov., holotype A labrum B first maxillae C right part of the forcipular segment D LBS 18 E ultimate LBS without distal part of legs F left part of the ultimate LBS without distal part of leg. Views: ventral (A-E), dorsal (F). Scale bars: 20 μ m (A); 40 μ m (B); 100 μ m (C-F).

setae. Labrum composed of an intermediate part narrow and almost negligible, having one tubercle (Fig. 2A); side pieces not clearly distinct from the intermediate part of labrum, each with four bristles. Antennae with 14 articles, 1.3 mm long, $3.2 \times$ longer than the head (Fig. 3B). article I ~ $0.7 \times$ longer than



Figure 3. *Endogeophilus alberti* sp. nov., holotype **A** head and forcipular segment **B** antennae **C** distal articles of left antenna **D** right telopodite of second maxillae **E** head and forcipular segment **F** right forcipule **G** LBS 15 and anterior part of 16 **H** ultimate LBS without right leg. Views: ventral (**D**–**H**), dorsal (**A**–**C**). Abbreviations: cls club-like sensilla, cmx claw of the second maxillae, cp coxopleural pores, cs carpophagus-structure. Scale bars: 200 μm (**A**–**B**, **E**, **G**–**H**); 50 μm (**C**–**D**, **F**).

wide, articles II–VII up to 1.7× longer than wide, article VIII ~ 1.2× longer than wide, articles IX–XIII approximately as long as wide; setae gradually denser and shorter from basal to distal articles, both ventrally and dorsally. Article XIV 2.0× longer than wide, with numerous setae and with club-like sensilla grouped on the distal parts of both internal and external sides (Fig. 3C).

Table 1. Differences between the single specimen of *Endogeophilus alberti* sp. nov. and the three specimens of *E. ichnusae*. For each character, each possible explanation (intraspecific variability, errors in character evaluation or divergence between species) has been evaluated as probable (x), possible (?) or improbable (–), based on what is known from other Geophilidae. Data are from Bonato et al. (2016) and this study.

Species	E. alberti sp. nov.	E. ichnusae	Explanations				
Specimens examined	1, female, 24.5 mm	3, both sexes, 31-34 mm	Differences in body size	Differences between sexes	Variability between individuals	Errors in character evaluation	Differences between species
Head: setae: max length	22 µm	15 µm	-	_	?	х	?
Labrum: distinction between side- pieces and intermediate part	faint	distinct	х	_	?	?	_
Labrum: number of tubercles	1	2	?	_	х	-	-
Antenna: length/width	3.2	3.6	?	_	х	?	-
First maxillae: coxosternal lappet	absent	small	?	-	х	-	?
First maxillae: telopodital lappet	large	small	?	_	х	-	?
Second maxillae: pretarsus	slender, hooked at the tip	stout, not hooked	_	_	_	-	X
Forcipular metatergite: width/length	2.3	1.8	?	_	х	?	?
Forcipular trochanteroprefemur: length/width	1.1	1.3	-	-	-	-	х
Forcipular tarsungulum: length/width	2.2	1.9	-	-	-	-	х
Forcipule/coxosternite length	0.9-1.0	0.7-0.8	-	_	_	_	х
Forcipular tarsungulum: shape	narrowing gradually	narrowing abruptly	-	-	-	-	x
Anterior part of trunk: metasternite: carpophagus pit	distinct, fairly deep	faint, shallow	?	-	х	-	?
Legs in female: number	99	107	-	-	х	-	?
Coxopleuron: pores: number	5 or 6	9-15	х	-	-	-	?
Ultimate/penultimate telopodite length	1.7	2.0	х	-	-	?	?

Mandibles and maxillae. A single pectinate lamella on each mandible. Coxosternite of the first maxillae entire, without mid-longitudinal sulcus. Coxal projection sub-triangular, longer than wide, bearing one or two basal setae and some more distal spine-like sensilla. Telopodite comprises two articles, the basal one without setae, the distal one with three setae and five or six spinelike sensilla. No coxosternal lappets; telopodital lappets present and pointed (Fig. 2B). Coxosternite of the second maxillae entire, the intermediate part uniformly sclerotised as the remaining parts, its anterior margin concave; no sclerotised ridges. Telopodite of the second maxillae composed of three articles: article 1 with two or three very short external setae, article 2 with one or two very short external setae, article 3 with five or six long distal setae; a simple long apical claw, subconical but hook-shaped at its extremity, with a dorsal bulge at its mid length (Fig. 3D).

Forcipular segment. Tergite trapezoid, the lateral margins distinctly converging anteriorly, $\sim 2.3 \times$ wider than long, posteriorly almost as wide as the subsequent metatergite (Fig. 3A). Exposed part of the coxosternite $\sim 1.1 \times$

wider than long; anterior margin with a medial shallow concavity, without denticles. Coxopleural sutures complete, entirely ventral or almost, only very slightly sinuous, and strongly converging posteriorly. Chitin-lines well distinct, reaching the condyles, moderately curved and converging posteriorly (Figs 2C, 3E). Trochanteroprefemur ~ 1.1× as wide as long, the external side ~ 2.2× longer than the internal side, without denticles and with some setae (Figs 2C, 3F). Forcipular intermediate articles distinct, without denticles, each with some long setae. Tarsungulum curved, gradually narrowing, ~ 2.2× as long as wide and with a basal sub-conic tubercle; slightly crenulated in its concavity, with 3-6 shallow projections, less pronounced on the left than on the right (Figs 2C, 3F).

Leg-bearing segments. 99 LBS. No paratergites. Metatergite 1 wider than the subsequent one, lateral margins converging posteriorly, without pretergite. Metatergites with two paramedian sulci. Metasternites slightly longer than wide (length/width ratio ~ 1.1 at ~ 20% of the LBS), uniformly areolate; setae very sparse. Metasternites of LBS 10–20 with a carpophagus pit on the anterior margin, fairly deep on LBS 14–19, ~ 0.6× as wide as the margin of metasternite (Figs 2D, 3G). Glandular pores on the metasternites from the 2nd to the penultimate, up to 30–35 pores and loosely arranged in a sub-ovoid/sub-triangular pore-field on the posterior half of each metasternite in the anterior part of the trunk (Fig. 2D); the pore-fields being much less visible in the posterior half of the trunk, loosely arranged in a transverse band of pores in the ~ 5–10 penultimate LBS. Length/width ratio of leg tarsus ~ 2.3 at ~ 20% of the LBS. Leg claws simple.

Ultimate leg-bearing segment. Setae uniformly sparse. Pleuropretergite entire, lacking sutures or sulci. Metatergite sub-trapezoid, ~ 1.5× wider than long, lateral margins very slightly convex and distinctly converging posteriorly, posterior margin convex. Presternite not medially constricted. Metasternite trapezoid, ~ 1.3× wider than long, anteriorly 1.4× wider than posteriorly (Fig. 2E); lateral margins slightly convex, converging backwards; posterior margin straight. Coxal organs of each coxopleuron opening through five or six independent pores: two dorsal pores, one covered by the pleuropretergite and one exposed on the dorso-lateral side (Fig. 2F); three or four ventral pores including two or three more or less aligned under the edge of the corresponding metasternite and a single pore isolated on the postero-central part of the ventral side (Figs 2E, 3H). Telopodite 0.43 mm long, 7.8× as long as wide, ~ 1.7× as long as the penultimate telopodite; six articles, all covered dorsally with sparse long setae, ventrally with dense shorter setae; a fairly well-developed apical claw (Fig. 3H).

Postpedal segments. Gonopods in the shape of a short, slightly bilobate lamina. A pair of anal pores.

Distribution and ecology. The new species is only known from the type locality (see above; Fig. 1). The single specimen was collected in a shaded maquis of old *Arbutus unedo* L., 1753 with also some large *Quercus suber* L., 1753 (Fig. 5). It was found after sieving with a Winkler apparatus, at a depth of 10–20 cm in the soil.

Etymology. This species is dedicated to Prince Albert II de Monaco, because the Foundation Prince Albert II de Monaco has supported the field work of this study. The epithet *alberti* is intended as a noun in the genitive case.



Figure 4. *Endogeophilus ichnusae*, paratype PD 1373, ventral **A** head and forcipular segment **B** right forcipule and apical claw of the second maxillae. Abbreviation: cmx claw of the second maxillae. Scale bars: 100 µm.



Figure 5. Habitat of *Endogeophilus alberti* sp. nov., near Malatra, Cavalaire-sur-Mer, France (Fig. 1).

Identification key to the European genera of Geophilidae s.l.

Within Europe, the Geophilidae s.l. can be distinguished from all other Chilopoda by means of the combination of the following two characters: > 25 pairs of legs and second maxillary pretarsi in shape of either subconical, non-spatulate, pointed claw or a tubercle with only one or two tiny spines. A total of 20 genera of Geophilidae s.l. are recorded in Europe (Table 2).

The key should be applied by examining any specimen through a microscope. A magnification of 50× is recommended, even 100× for examining labrum and pore-fields. For characters defined on the leg-bearing segments, examination between the 5th and the 20th LBS is recommended.

In addition to the dichotomic characters included in the key, additional information is given for each genus (number of species recorded in Europe, geographical distribution within Europe, and additional morphological characters), between square brackets, to assist in the identification.

1	Legs of the ultimate pair with an apical tubercle having minute spines
	(Fig. 10B: t)Arenophilus Chamberlin, 1912
	[In Europe: a single species, A. peregrinus Jones, 1989; recorded in very
	few localities on and near the coasts of south-western England, western
	France, and western Iberian peninsula. Always with 45 LBS.]
_	Legs of the ultimate pair with an apical claw or missing the pretarsus at all
	(Figs 3H, 10A, C-I) 2
2	Forcipular tergite sub-rectangular, more or less headband-shaped (Fig. 6D,
	E: ft)3
	The genera included here have a large basal denticle on the tarsungulum
	(Fig. 6F) or a deep median diastema on the forcipular coxosternite (Fig. 6G, H).]
_	Forcipular tergite trapezoid, posteriorly wider than anteriorly (Fig. 6A-C:
	ft)5
	[With the exception of Gnathoribautia (Fig. 6I), the basal denticle of the
	tarsungulum, when existing, is smaller (Fig. 7C, G). No deep median dias-
	tema on the forcipular coxosternite (Figs 6I, 7C, G).]
3	A large basal denticle on the tarsungulum (Fig. 6F: d). No deep median
	diastema and no chitin-lines on the forcipular coxosternite (Fig. 6F)
	Strigamia Gray, 1843
	[In Europe: many species, across most continental lands.]
_	No large basal denticle on the tarsungulum (Fig. 6G, H). A deep median
	diastema and distinct chitin-lines on the forcipular coxosternite (Fig. 6G,
	H: cl)
4	2 denticles on the mesial side of the tarsungulum (Fig. 6G: dc). Pore-fields
	absentDignathodon Meinert, 1870
	[In Europe: only two species, D. microcephalus (Lucas, 1846) (southern
	Europe; 65–89 LBS) and D. gracilis (Attems, 1952) (only recorded in Anda-
	lusia; 59–71 LBS.]
_	No denticles on the mesial side of the tarsungulum (Fig. 6H). Pore-fields pres-
	ent, sub-circular to longitudinally much elongate
	[In Europe: many species, across most continental lands and major
	islands.]

5	Coxal pores grouped in 1, 2, 3, or 4 ventral pits, fossae, or distinct clus-
	ters on each coxopleuron, all close to the metasternite or covered by it
	(Fig. 9G–I), but sometimes accompanied by 1 or 2 lateral or posterior sep-
	In France and in porth western Europe, with the execution of Nothergoph
	ilus only recorded in England the labrum of the species of the two other
	genera present in this area (Clinopodes and Stenotaenia) generally has
	numerous bristles and no tubercles (Fig. 7A).]
_	All coxal pores separate, not in pits, fossae, or distinct clusters (Figs 2E, F,
	3H, 9A-F, 10E) 12
	[In France and in north-western Europe, the genera included here have one
	to several tubercles on the intermediate part and bristles on the side piec-
	es of the labrum (Figs 2A, 7B).]
6	Forcipular coxosternite with a pair of distinctly sclerotised denticles
	(Fig. 7E) 7
_	Forcipular coxosternite without distinctly sclerotised denticles (e.g., Fig. 7G)
7	Carpophagus structures absent (e.g., Fig. 8G, H). Coxal pores mostly or
	entirely in 1 ventral pit on each coxopleuron (Fig. 9I)
	Diphyonyx Bonato, Zapparoli & Minelli, 2008
	[In Europe: a few species, from the Balkan peninsula to Caucasus. Claws
	of many anterior legs bearing an enlarged accessory spine.]
-	Carpophagus structures present (Fig. 8A, B: cs). Coxal pores mostly or entire-
	ly in 2–4 ventral pits or clusters (e.g., Fig. 9G) <i>Clinopodes</i> C.L. Koch, 1847
0	[In Europe: many species, across most of central and eastern Europe.]
0	Repekium Verhoeff 1941
	In Europe: a single species <i>B</i> mirabile Verhoeff 1941: recorded only in
	the eastern part of the Balkan peninsula; 39–41 LBS.]
_	Pore-fields present (Fig. 8D, E, G, H: pf). Labrum with bristles, sometimes
	also with tubercles9
9	All coxal pores in 2 or 3 ventral pits on each coxopleuron (Fig. 9G, H). Pore-
	fields oval or sub-trapezoid (Fig. 8G: pf) Stenotaenia C.L. Koch, 1847
	[In Europe: many species, across most of central and eastern Europe.]
-	All coxal pores in 1 ventral pit on each coxopleuron (Fig. 9I). Pore-fields
	sub-triangular (e.g., Fig. 8D, E: pt) or reniform or in a transverse band
10	(FIQ. OT. PI)
10	(e.g. Fig. 8D F ⁻ pf) 57–71 BS Algerophilus Brolemann, 1925
	[In Europe: a single species, Algerophilus hispanicus (Meinert, 1870); only
	from southern Iberian peninsula.]
-	Carpophagus structures absent (Fig. 8H). Pore-fields reniform or in a
	transverse band (Fig. 8H: pf); 37–55 LBS11
11	Pore-fields more or less reniform, $\leq 1/2$ of the width of the metasternite;
	37–39 LBS Nothogeophilus Lewis, Jones & Keay, 1988
	[In Europe: a single species, <i>N. turki</i> Lewis, Jones & Keay, 1988; only recorded
_	From the isles of Scilly and Wight (Southern England). Body length < 15 mm.] Dero fields not replication > $1/2$ of the width of the metastamits (Fig. 91)):
_	Pole-lieus not reniform, $> 1/2$ of the Width of the metasternite (Fig. 8H); 47-551 RS

[In Europe: only two species, *T. poseidonis* (Verhoeff, 1901) (with 49–55 LBS; strictly halophilic, only present on the Mediterranean seashores) and *T. zograffi* (Brolemann, 1900) (47 LBS; not halophilic; only recorded in the Canary islands).]

- Legs of the ultimate pair with an apical claw, usually well-developed² (Fig. 10C, D: c, E, F: c, I). Pore-fields usually present³, sub-diamond or in a tranverse band (Fig. 8D, E: pf)......17
- No pore-fields (Fig. 8B). Legs of the ultimate pair usually < 2× as long as the penultimate legs⁴
 15

4 *Gnathoribautia bonensis* has a fairly elongated ultimate pair of legs, but generally less so than *Pleurogeophilus mediterraneus*. Also, *G. bonensis* has a much longer head than *P. mediterraneus* (Fig. 6B, C) and distinct forcipules (Fig. 6I).

¹ Apart from *Endogeophilus*, only one species of European geophilids has > 90 LBS: *Stenotaenia sturanyi* (Attems, 1903) (105–115 LBS), recorded between Macedonia and the Chalcidic peninsula, possibly also in Crete (Bonato and Minelli 2008).

² European species of *Geophilus* with poorly developed claws on the ultimate legs of both sexes include *G. fucorum*, *G. joyeuxi* Léger & Duboscq, 1903 (variable), and *G. seurati* Brolemann, 1924. Other species may have poorly developed claws on the ultimate legs of males only, e.g., *G. flavus* (variable) and *G. carpophagus* Leach, 1815.

³ A few European species of *Geophilus* have no pore-fields: *G. guanophilus* Verhoeff, 1939, *G. ribauti* Brolemann, 1908, *G. richardi* Brolemann, 1904, *G. truncorum* Bergsøe & Meinert, 1866, and other species of uncertain identity (*G. madeirae* Latzel, 1895, *G. ibericus* (Attems, 1952), *G. strictus* Latzel, 1880).

- 16 39–41 LBS. Forcipular coxosternite slightly wider than long. All forcipular articles with a denticle (Fig. 7C)......Arctogeophilus Attems, 1909 [In Europe: three species, A. inopinatus (Ribaut, 1911) (western and central France), A. attemsi Folkmanová, 1956, and A. macrocephalus Folkmanova & Dobroruka, 1960 (both only recorded in Ukraine).]

Macaronesia, Iberian peninsula, and Sicily) and *G. syriaca* (Attems, 1903) (~ 87 LBS; body length ≤ 11 cm; Aegean islands]

- Forcipules without denticles. Legs of the ultimate leg-bearing segment with elongate projections (Fig. 10I) *Acanthogeophilus* Minelli, 1982 [In Europe: only 1 species, *A. dentifer* Minelli, 1982, only recorded in the Italian peninsula; ~ 67 LBS.]

[In Europe: only 2 species, *E. pinguis* (Brolemann, 1898) (< 50 LBS) and *E. multistiliger* (Verhoeff, 1899) (> 50 LBS). Pore-fields in a very stretched transverse band in both species (Fig. 8C: pf).]

Discussion

Taxonomic remarks

After the discovery of a specimen of *Endogeophilus* among the centipedes collected in the Port-Cros National Park, further sessions of field research were conducted with the aim to find other specimens, either in the collection locality or other sites. However, efforts have remained ineffective up to now. Worth notice is that the only three specimens of *Endogeophilus* previously



Figure 6. Head and forcipular segment. Species A Pachymerium ferrugineum B Gnathoribautia bonensis C Pleurogeophilus mediterraneus D Strigamia carniolensis E Henia vesuviana F Strigamia carniolensis G Dignathodon microcephalus H Henia bicarinata I Gnathoribautia bonensis. Views: dorsal (A–E), ventral (F–I). Abbreviations: cl chitin-lines, d forcipular denticle, dc denticles of the mesal side of the forcipular tarsungulum, ft forcipular tergite. Arrow: median diastema of the forcipular coxosternite. Scale bars: 400 µm (A–F, H–I); 300 µm (G).

reported were found through intense field research and among a huge sample of collected centipedes (Bonato et al. 2016), and no other specimens have been found subsequently.



Figure 7. A, B labrum **C, D, F, G** forcipular segment **E** distal part of the forcipular coxosternite. Species **A** *Clinopodes vesubiensis* **B** *Geophilus studeri* **C** *Arctogeophilus inopinatus* **D** *Eurygeophilus pinguis* **E** *Clinopodes vesubiensis* **F** *Galliophilus beatensis* **G** *Geophilus gavoyi*. Views: all ventral. Abbreviations: cl chitin-lines, d forcipular denticle, tp trochanteroprefemur. Arrow: denticles on the forcipular coxosternite. Scale bars: 50 µm (**A, B**); 300 µm (**C–G**).



Figure 8. Metasternite of an anterior LBS. Species **A** *Clinopodes vesubiensis* LBS 16 **B** *Galliophilus beatensis* LBS 19 **C** *Eurygeophilus pinguis* LBS 10 **D** *Geophilus osquidatum* LBS 11 **E** *Geophilus electricus* LBS 11 **F** *Pleurogeophilus mediterraneus* LBS 15 **G** *Stenotaenia linearis* LBS 20 **H** *Tuoba poseidonis* LBS 12. All ventral views. Abbreviations: cs carpophagus-structure, pf pore-field. Scale bars: 200 µm.



Figure 9. Ultimate LBS without distal part of legs. Species A Arctogeophilus inopinatus female B Galliophilus beatensis male C Geophilus flavus female D Pachymerium ferrugineum male E Pleurogeophilus mediterraneus female F Pleurogeophilus mediterraneus male G, H Stenotaenia linearis female I Tuoba poseidonis female. Views: ventral (A–G), lateral (H, I). Abbreviations: p pit or fossa (H wide fossa from dorsal to ventral side, indicated by dotted lines), um metasternite of the ultimate LBS (medial length and maximum width are indicated by dotted lines). Scale bars: 300 µm.


Figure 10. Legs of the ultimate pair **A**, **C** right leg **B**, **D** distal articles of the right leg **E**–**G** both legs **H** left leg. Species **A** Arctogeophilus inopinatus male **B** Arenophilus peregrinus female **C** Eurygeophilus pinguis female **D** Geophilus fucorum female **E** Geophilus richardi female **F** Pachymerium ferrugineum female **G** Pleurogeophilus mediterraneus male **H** Pleurogeophilus mediterraneus female **I** Acanthogeophilus spiniger female. Views: ventral (**A**-**E**, **I**), lateral (**F**) and dorsal (**G**-**H**). Abbreviations: c claw, t tubercle with spines. Scale bars: 300 µm (**A**, **C**, **F**); 50 µm (**B**); 100 µm (**D**, **E**, **I**); 1 mm (**G**-**H**).

We observed several morphological differences between the single individual of Endogeophilus collected in Provence and the three individuals previously collected in Sardinia (Table 1). However, only some differences may be attributed confidently to evolutionary divergence (thus suggesting that they represent different species), while other differences may be explained by variability between individuals (including variation associated to body size or sex). Moreover, the effect of "errors" in measurement and character evaluation may not be negligible in such a small sample. The very few specimens available for comparison did not allow testing these alternative explanations empirically or statistically. However, we tentatively tabulate what is known for other better-studied geophilids (Table 1). Following a cautionary approach, at least a few differences are very difficult to explain by intraspecific variation or measurement errors alone. Thus, these differences indicate an evolutionary morphological divergence between the population of Endogeophilus discovered in Provence (represented by the single specimen here described) and the populations inhabiting south-western Sardinia (the three specimens described as E. ichnusae). These differences are in the shape of the pretarsi of the second maxillae and the forcipules.

The maxillary pretarsi are more slender and distinctly hooked in the specimen from Provence, while they are stouter and not hooked in all three Sardinian specimens (Fig. 3D vs Fig. 4B). There is no asymmetry between right and left pretarsi. The observed difference is larger than the interindividual variation observed within well-studied species of geophilids (unpublished data), and the effect of body size may be ruled out considering the close lengths of the specimens (Table 1).

The forcipules of the specimen from Provence differ from those of all three Sardinian specimens because they are slightly more elongate in comparison with the coxosternite, with stouter trochanteroprefemora and more slender tarsungula, which are also more gradually narrowing (Fig. 3E, F vs Fig. 4A, B). There is no asymmetry between right and left forcipules, and no differences between sexes among the Sardinian specimens. The observed differences are larger than usually observed in other geophilids (unpublished data), even in comparison to changes observed or expected with growth (Table 1).

Other observed differences may turn out to be variable characters between the two species, but further specimens are necessary to rule out alternative explanations (Table 1). These putative characters include the length of the setae, the relative size of the first maxillary lappets, the elongation of the forcipular metatergite, the depth of the carpophagus pits, the range of variation of the number of legs, the number of coxal pores in relation to the body size, and the relative length of the legs of the ultimate pair.

Identification key to genera of Geophilidae

The redefinition of the diagnosis of *Endogeophilus* prompted us to build a key to all geophilid genera recorded from Europe thus far, according to the current taxonomy and nomenclature in use, and leveraging all recently published information on the morphology of European geophilids. Presently, the Geophilidae s.l. living in Europe (~ 120 species; Bonato et al. 2014a) are recognised in 20 genera (Table 2).

Table 2. Genera of Geophilidae s.l. and approximate number of species recognised in Europe. Only genera recorded within the geographic boundaries adopted by Fauna Europaea are considered (see Materials and methods). Occurrences are also indicated for the biogeographical subregions of SW Europe and the two countries where *Endogeophilus* occurs.

Conuc	Approximate number of	Occurrence			
Genus	species in Europe	South-western Europe	France	Italy	
Acanthogeophilus Minelli, 1982	1	x	_	х	
Algerophilus Brolemann, 1925	1	X	-	-	
Arctogeophilus Attems, 1909	3	_	х	-	
Arenophilus Chamberlin, 1912	1	X	х	-	
Bebekium Verhoeff, 1941	1	_	_	-	
Clinopodes C.L. Koch, 1847	9	X	х	х	
Dignathodon Meinert, 1870	2	х	х	х	
Diphyonyx Bonato, Zapparoli & Minelli, 2008	2	-	-	-	
Endogeophilus Bonato, Zapparoli, Drago & Minelli, 2016	2	X	х	х	
Eurygeophilus Verhoeff, 1899	2	X	х	х	
Galliophilus Ribaut & Brolemann, 1927	1	X	х	-	
Geophilus Leach, 1814	49	X	х	х	
Gnathoribautia Brolemann, 1909	2	X	-	х	
Henia C.L. Koch, 1847	17	X	х	х	
Nothogeophilus Lewis, Jones & Keay, 1988	1	-	-	-	
Pachymerium C.L. Koch, 1847	6	х	х	х	
Pleurogeophilus Verhoeff, 1901	3	X	х	х	
Stenotaenia C.L. Koch, 1847	9	X	х	х	
Strigamia Gray, 1843	7	X X		х	
Tuoba Chamberlin, 1920	2	x x		х	
Total	121 species	16 genera	14 genera	13 genera	

For a long time, the identification of geophilids collected in Europe have relied on consulting a few specialists with personal expertise, browsing descriptions that are sparse in the taxonomic literature (often heterogeneous and only partially comparable) or by using a few available keys. After the keys published by Attems (1929) within his global monograph on Geophilomorpha (now largely outdated), more recent keys (still suitable for European geophilids) have been published to cover single countries or regions (e.g., Koren 1986; Andersson et al. 2005; Barber 2009; Dányi 2010; Iorio and Labroche 2015; Iorio et al. 2022b). Indeed, an interactive digital key to all European species of Geophilomorpha has been recently delivered on-line (Bonato et al. 2014a); however, it includes many characters often requiring anatomical dissection, and most of the characters are not accompanied by illustrations. Given the current state of uncertainty of the species-level taxonomy of many European genera of geophilids (e.g., Del Latte et al. 2015; Bonato et al. 2023), a simplified tool assisting in distinguishing genera may turn out to be useful and desirable for many faunistic and ecological investigations. Moreover, we have given priority to characters that are easier to evaluate (not requiring high magnification, dissection, or clarification of the integument) and of broader applicability (effective for adult and large immature individuals, and without knowing the sex of the specimen).

Biogeographic and conservation remarks

The new species of *Endogeophilus* has been discovered in southern Provence, ca 2 km from the coast, while the known range of *E. ichnusae* is limited to south-western Sardinia (Fig. 1). The two areas are > 450 km far apart, and are separated by a broad branch of sea. Similar distributional disjunctions of strictly related species between Sardinia and Provence are apparently rare among soil arthropods, even though single species have been reported with separate populations in Sardinia and narrow parts of continental Europe (e.g., Soldati and Soldati 2014; Ponel et al. 2023). Among centipedes, for instance, the himantariid *Stigmatogaster sardoa* (Verhoeff, 1901) has been recently recorded in Provence while it was previously recorded only in Sardinia (Él, pers. obs.).

Provence is one of the most intensely surveyed areas in Europe for centipedes: in June 2024, ~ 6,100 records and 12,300 identified specimens had been collected for the entire Provence-Alpes-Côte d'Azur region (Myria-France 2024; lorio and Racine, unpublished data). However, even within Provence, several sectors and/or habitats remain poorly studied, like the south of the Var department and generally the strictly endogeic centipedes. In particular, *E. alberti* has been found in the peripheric area ("zone d'adhésion") of the Port-Cros National Park, which – like the massif of the Maures – was poorly studied before 2022, except for coastal habitats (Iorio 2014, 2022; Iorio et al. 2020, 2023; Myria-France 2024).

Because the Alpes-Maritimes department, as well as secondarily the northern part of the Var department, have been relatively well studied, it seems possible that *E. alberti* sp. nov. has a narrow distribution in Provence, possibly limited to the massif of the Maures and its surroundings. However, as only one specimen was found after > 50 hours dedicated to its search and the single specimen corresponds to only 0.6% of the total number of collected geophilomorphs, this species shows a very low probability of detection in comparison with most other centipedes.

While Provence belongs to a well-known biodiversity hotspot within Europe (Médail and Quézel 1997), its biodiversity is highly threatened by the demographic pressure and various human impacts (e.g., urbanisation, artificialisation of natural environments, fires) (Daligaux 2003; Médail and Diadema 2006; Vimal et al. 2012). In particular, in the last decades, the forest habitats of the south of the Maures have been disturbed by urbanisation and artificialisation. Also, the fires caused a strong impact in this area, as in 2021 with 7,000 hectares burned (Ballouard et al. 2023). These impacts also concern several species of centipedes (Chilopoda), which are already considered as threatened (lorio et al. 2015, 2020, 2023; Geoffroy and lorio 2019; lorio 2022; lorio et al. 2022a).

Further studies would be necessary to precisely define the real distribution and abundance of *E. alberti*, its ecology, and its conservation status.

Acknowledgments

We are grateful to the Fondation Prince Albert II de Monaco and the Port-Cros National Park for the funding of the field work in the Park (Stoechas project; Él is supported by the partnership contract 2022-598). Guillelme Astruc (Port-Cros National Park, Stoechas project) and Élodie Debize (Port-Cros National Park, terrestrial fauna expert) are thanked for their valuable help with the organisation of the mission as is Antoine Racine (Groupe d'Etude des Invertébrés Armoricains) for information on *Galliophilus beatensis* and the photograph of *Arenophilus peregrinus*. Hervé Brustel, Thomas Cherpitel, Jean-Michel Lemaire, Franck Noël, and Olivier Courtin are gratefully acknowledged for sending specimens. We are also grateful to L.A. Pereira and A. Minelli, who reviewed the manuscript and helped us to improve it, as well as to Nathalie Yonow. LB was supported by the Italian Ministry of University and Research (project funded by the European Union - Next Generation EU: PNRR Missione 4 Componente 2, "Dalla ricerca all'impresa", Investimento 1.4, Progetto CN00000033).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The authors acknowledge the support of: Fondation Prince Albert II de Monaco and Port-Cros National Park (Stoechas project); Italian Ministry of University and Research, funded by the European Union - Next Generation EU: PNRR Missione 4 Componente 2, "Dalla ricerca all'impresa", Investimento 1.4, Progetto CN00000033.

Author contributions

Both authors have contributed equally.

Author ORCIDs

Étienne Iorio I https://orcid.org/0009-0003-2550-3480 Lucio Bonato I https://orcid.org/0000-0002-8312-7570

Data availability

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

References

- Andersson G, Meidell B, Scheller U, Djursvoll P, Budd G, Gärdenfors U (2005) Mångfotingar. Myriapoda. Nationalnyckeln till Sveriges Flora och Fauna, Uppsala, 351 pp.
- Attems C (1929) Myriapoda. 1. Geophilomorpha. Das Tierreich 52. De Gruyter, Berlin, 388 pp. https://doi.org/10.1515/9783111430638
- Ballouard JM, Afériat M, Gillet G, Daneluzzi A, Yvin M, Garnier E, Moreaux L, Delorme O, Caron S, Laboure L, Rodriguez C, Catard A, Bonnet X, Vercoutter F, Levasseur R, Guicheteau D (2023) Impact d'un incendie en fonction de l'habitat sur la population de tortues d'Hermann *Testudo hermanni hermanni* de la Réserve Naturelle Nationale de la Plaine des Maures. Bulletin de la Société herpétologique de France 183: 1–17. https://doi.org/10.48716/bullshf.183-2
- Barber AD (2009) Centipedes. Synopses of the British Fauna 58. Linnean Society of London and the Estuarine and Brackish-Water Sciences Association/Field Studies Council, Shrewsbury, 228 pp.

- Bonato L, Minelli A (2008) *Stenotaenia* Koch, 1847: a hitherto unrecognized lineage of western Palaeartic centipedes with unusual diversity in body size and segment number (Chilopoda: Geophilidae). Zoological Journal of the Linnean Society 153: 253–286. https://doi.org/10.1111/j.1096-3642.2008.00394.x
- Bonato L, Minelli A (2014) Chilopoda Geophilomorpha of Europe: a revised list of species, with taxonomic and nomenclatorial notes. Zootaxa 3770: 1–136. https://doi. org/10.11646/zootaxa.3770.1.1
- Bonato L, Barber A, Minelli A (2006) The European centipedes hitherto referred to *Eury-geophilus*, *Mesogeophilus*, and *Chalandea* (Chilopoda, Geophilomorpha): taxonomy, distribution, and geographical variation in segment number. Journal of Natural History 40: 415–438. https://doi.org/10.1080/00222930600661839
- Bonato L, Edgecombe GD, Lewis JGE, Minelli A, Pereira LA, Shelley RM, Zapparoli M (2010) A common terminology for the external anatomy of centipedes (Chilopoda). ZooKeys 69: 17–51. https://doi.org/10.3897/zookeys.69.737
- Bonato L, Iorio É, Minelli A (2011) The centipede genus Clinopodes C. L. Koch, 1847 (Chilopoda, Geophilomorpha, Geophilidae): reassessment of species diversity and distribution, with a new species from the Maritime Alps (France). Zoosystema 33: 175–205. https://doi.org/10.5252/z2011n2a3
- Bonato L, Dányi L, Socci AA, Minelli A (2012a) Species diversity of *Strigamia* Gray, 1843 (Chilopoda: Linotaeniidae): a preliminary synthesis. Zootaxa 3593: 1–39. https://doi.org/10.11646/zootaxa.3593.1.1
- Bonato L, Voigtländer K, Minelli A (2012b) *Algerophilus*, a neglected lineage of Western Mediterranean centipedes (Chilopoda: Geophilidae). Zootaxa 3235: 23–34. https://doi.org/10.11646/zootaxa.3235.1.2
- Bonato L, Minelli A, Lopresti M, Cerretti P (2014a) ChiloKey, an interactive identification tool for the geophilomorph centipedes of Europe (Chilopoda, Geophilomorpha) Zoo-Keys 443: 1–9. https://doi.org/10.3897/zookeys.443.7530
- Bonato L, Drago L, Murienne J (2014b) Phylogeny of Geophilomorpha (Chilopoda) inferred from new morphological and molecular evidence. Cladistics 30: 485–507. https://doi.org/10.1111/cla.12060
- Bonato L, Zapparoli M, Drago L, Minelli A (2016) An unusually elongate endogeic centipede from Sardinia (Chilopoda: Geophilidae). European Journal of Taxonomy 231: 1–19. https://doi.org/10.5852/ejt.2016.231
- Bonato L, Bortolin F, De Zen G, Decker P, Lindner EN, Orlando M, Spelda J, Voigtländer K, Wesener T (2023) Towards elucidating species diversity of European inland Strigamia (Chilopoda: Geophilomorpha): a first reassessment integrating multiple lines of evidence. Zoological Journal of the Linnean Society 199: 945–966. https://doi. org/10.1093/zoolinnean/zlad070
- Brolemann HW (1930) Eléments d'une faune des myriapodes de France. Chilopodes. Faune de France 25. Imprimerie Toulousaine, Toulouse/P. Lechevalier, Paris, 405 pp.
- Daligaux J (2003) Urbanisation et environnement sur les littoraux: une analyse spatiale. Rives méditerranéennes 15: 1–7. https://doi.org/10.4000/rives.165
- Dányi L (2010) Magyarország százlábúi (Chilopoda) II. Határozókulcs. Állattani Közlemények 95: 3–24.
- Dányi L, Tuf IH (2017) On the identity of *Photophilus* Folkmanová, 1928: A new generic synonymy (Chilopoda: Geophilomorpha: Geophilidae). Zootaxa 4347: 361–370. https://doi.org/10.11646/zootaxa.4347.2.10
- de Jong Y (2016) Fauna Europaea. Fauna Europaea Consortium. https://doi. org/10.15468/ymk1bx [accessed 10.VII.2024]

- Del Latte L, Bortolin F, Rota-Stabelli O, Fusco G, Bonato L (2015) Molecular-based estimate of species number, phylogenetic relationships and divergence times for the genus *Stenotaenia* (Chilopoda, Geophilomorpha) in the Italian region. ZooKeys 510: 31–47. https://doi.org/10.3897/zookeys.510.8808
- Desmots D, Racine A (2023) *Arenophilus peregrinus* Jones, 1989 sur l'île de Noirmoutier (Vendée), une nouvelle espèce de chilopode pour la faune de France (Geophilomorpha: Geophilidae). Invertébrés Armoricains 25: 7–11.
- Dyachkov Y, Bonato L (2024) An updated synthesis of the Geophilomorpha (Chilopoda) of Asian Russia. ZooKeys 1198: 17–54. https://doi.org/10.3897/zookeys.1198.119781
- Geoffroy JJ, Iorio É (2019) Myriapodes. In: Johanet A, Kabouche B (Eds) La faune des Bouches-du-Rhône. Parthénope, Mèze, 325–335.
- lorio É (2014) Catalogue biogéographique et taxonomique des chilopodes (Chilopoda) de France métropolitaine. Mémoires de la Société linnéenne de Bordeaux 15:1–372.
- Iorio É (2016) Première observation en France de Lithobius (Lithobius) derouetae Demange, 1958 et autres données nouvelles sur les Chilopodes du Sud-Ouest (Chilopoda). Bulletin de la Société linnéenne de Bordeaux (NS) 44: 71–96.
- Iorio É (2022) Étude des chilopodes du Cap Lardier et de la Croix-Valmer (Parc national de Port-Cros, Var) avec focus sur les espèces des plages et prise en compte des araignées halophiles. Rapport d'El - Entomologie & Myriapodologie pour le Parc national de Port-Cros.
- Iorio É, Labroche A (2015) Les chilopodes (Chilopoda) de la moitié nord de la France: toutes les bases pour débuter l'étude de ce groupe et identifier facilement les espèces. Invertébrés Armoricains 13: 1–108.
- Iorio É, Quindroit C (2018) New records of Henia (Chaetechelyne) duboscqui (Verhoeff, 1943) and of other centipedes from Corsica (Chilopoda) together with some notes on the French species of Henia. Bulletin of the British Myriapod & Isopod Group 30: 71–83.
- Iorio É, Zapparoli M, Ponel P, Geoffroy JJ (2015) Les myriapodes chilopodes (Chilopoda) du Parc national du Mercantour, du département des Alpes-Maritimes et de leurs environs: description d'une nouvelle espèce du genre *Lithobius* Leach, 1814 s. s., synthèse des connaissances et espèces menacées. Zoosystema 37: 211–238. https://doi.org/10.5252/z2015n1a11
- Iorio É, Geoffroy D, Pétillon J (2020) Distribution and indicator value of intertidal centipedes from Mediterranean beaches within and around Port-Cros National Park (Southern France), with proposal of a simplified monitoring (Chilopoda). Bulletin de la Société entomologique de France 125: 41–62. https://doi.org/10.32475/bsef_2107
- Iorio É, Dusoulier F, Soldati F, Noël F, Guilloton JA, Doucet G, Ponel P, Dupont P, Krieg-Jacquier R, Chemin S, Tillier P, Touroult J (2022a) Les Arthropodes terrestres dans les études d'impact: limites actuelles et propositions pour une meilleure prise en compte des enjeux de conservation. Naturae 2022 (4): 43–99. https://doi.org/10.5852/naturae2022a4
- Iorio É, Labroche A, Jacquemin G (2022b) Les chilopodes (Chilopoda) de la moitié nord de la France: toutes les bases pour débuter l'étude de ce groupe et identifier facilement les espèces. Version 2. Document basé sur la première version parue dans la revue "Invertébrés Armoricains" n°13 (épuisée), 90 pp.
- Iorio É, Carnet M, Cherpitel T, Desmots D, Geoffroy JJ, Jacquemin G, Quindroit C, Racine A (2023) Les Chilopodes de France métropolitaine (Myriapoda, Chilopoda): liste commentée des espèces avec état des connaissances et proposition de noms français. Naturae 2023(1): 1–20. https://doi.org/10.5852/naturae2023a1

- Koren A (1986) Die Chilopoden-Fauna von Kärnten und Osttirol. 1. Geophilomorpha, Scolopendromorpha. Carinthia II 43: 1–87.
- Lewis JGE, Jones RE, Keay AN (1988) On a new genus and species of centipede (Chilopoda Geophilomorpha Chilenophilidae) from the British Isles. Journal of Natural History 22: 1657–1663. https://doi.org/10.1080/00222938800771031
- Machado A (1952) Miriápodes de Portugal. Primeira parte: Quilópodes. Publicações do Instituto de Zoologia "Dr. Augusto Nobre" 43: 65–169.
- Médail F, Diadema K (2006) Biodiversité végétale méditerranéenne. Annales de Géographie 115: 618–640. https://doi.org/10.3406/geo.2006.21290
- Médail F, Quézel P (1997) Hot-spots analysis for conservation of plant biodiversity in the Mediterranean basin. Annals of the Missouri Botanical Garden 84: 112–127. https://doi.org/10.2307/2399957
- Minelli A (1982) Un nuovo chilopodo italiano *Acanthogeophilus dentifer*, n.g. n.sp. (Geophilomorpha Geophilidae). Bollettino del Museo Civico di Storia Naturale di Verona 8: 235–240.
- Myria-France (2024) La base entomo. Myriapodes. https://bd.cettia.fr/myriapodes [Accessed 15.I.2024]
- Ponel P, Perez C, Médail F (2023) Prospections entomologiques hivernales sur l'île de Porquerolles et ses îlots périphériques (Parc national de Port-Cros, Provence, France). *Amaurorhinus sardous sardous* Folwaczny, 1972 et *Teplinus matthewsi* (Reitter, 1885), coléoptères nouveaux pour la faune de la France continentale. Scientific Reports of Port-Cros National Park 37: 419–437.
- Soldati F, Soldati L (2014) Tenebrionidae. In: Tronquet M (Ed.) Catalogue des Coléoptères de France. Revue de l'Association Roussillonnaise d'Entomologie, 23 (suppl.): 535–549.
- Tuf IH, Dányi L (2015) True identity of *Folkmanovius paralellus* Dobroruka (Chilopoda: Geophilomorpha: Geophilidae). Zootaxa 4058: 444–450. https://doi.org/10.11646/ zootaxa.4058.3.11
- Verhoeff KW (1941) Untersuchungen über die Fauna des Hologaeum: Chilopoden, Diplopoden und Land-Isopoden. Zoologischer Anzeiger 135: 35–41.
- Vimal R, Geniaux G, Pluvinet P, Napoleone C, Lepart J (2012) Detecting threatened biodiversity by urbanization at regional and local scales using an urban sprawl simulation approach: Application on the French Mediterranean region. Landscape and Urban Planning 104: 343–355. https://doi.org/10.1016/j.landurbplan.2011.11.003



Research Article

A taxonomic review of the genus *Fignya* Solovyev & Witt, 2009 (Lepidoptera, Limacodidae) with descriptions of three new species and one new combination

Jun Wu^{1,2}, Huilin Han^{1,2,3}

2 Northeast Asia Biodiversity Research Center, Northeast Forestry University, Harbin 150040, China

3 Ministry of Education, Key Laboratory of Sustainable Forest Ecosystem Management, Northeast Forestry University, Harbin 150040, China

Corresponding author: Huilin Han (hanhuilin@aliyun.com)

Abstract

Three new species of the genus *Fignya* Solovyev & Witt, 2009, *F. qiana* **sp. nov.**, *F. trigo-num* **sp. nov.**, and *F. samkosa* **sp. nov.**, are described from China and Cambodia. Additionally, a new combination, *F. brachygnatha* (Wu & Fang, 2008), **comb. nov.**, is proposed. The new species are illustrated with images of the adults and male genitalia, and compared with similar species. A key to all known species in the genus, along with their geographical distributions, is provided.

Key words: China, identification key, morphology, new combination, slug caterpillar moths, taxonomy, Zygaenoidea

Introduction

The genus *Fignya* is a recently established small genus within the family Limacodidae. Currently, only two species are included in this genus: *F. melkaya* Solovyev & Witt, 2009 and *F. ravalba* Wu, Solovyev & Han, 2022. The type species, *F. melkaya*, was first discovered in northern Vietnam on Mt. Fan-si-pan (West) and later also found in Sichuan, China; *F. ravalba* was discovered in Medog County, Xizang Autonomous Region, China, and is so far only known from its type locality (Ji 2018; Wu et al. 2022).

The diagnostic characteristics of this genus have been thoroughly described in previous studies: small in size, filiform antennae in both sexes; slightly upcurved labial palpi; and a large white spot on the forewing in the Cu area with a brown border. The forewing also features a sinusoidal vein R_1 , with veins R_3+R_4 branched from R_5 . The tibial spurs are in a 0-2-4 formula. In the male genitalia, the gnathos is fishtail-shaped with a comb-like apex; and the vesica bears large, strongly sclerotized cornuti (Solovyev and Witt 2009; Wu et al. 2022).

In this study, we propose a minor expansion of the definition of this genus, focusing mainly on the male genitalia: (1) the gnathos is fishtail-shaped with a comb-like apex, or the gnathos is spoon-shaped, with a weakly sclerotized, nearly membranous apex densely covered with small scobinations; (2) the



Academic editor: Reza Zahiri Received: 17 July 2024 Accepted: 21 August 2024 Published: 26 September 2024

ZooBank: https://zoobank. org/3E178C26-C21C-4BD9-B6AD-5DBCDD3D88E1

Citation: Wu J, Han H (2024) A taxonomic review of the genus *Fignya* Solovyev & Witt, 2009 (Lepidoptera, Limacodidae) with descriptions of three new species and one new combination. ZooKeys 1213: 225–235. https://doi.org/10.3897/ zookeys.1213.132318

Copyright: © Jun Wu & Huilin Han. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

¹ School of Forestry, Northeast Forestry University, Harbin 150040, China

base of the valva usually has an obvious, large, hairy process which bears large lateral bristles, though the process may be reduced or absent in some taxa; and (3) the vesica bears large, strongly sclerotized cornuti, or the phallus is bifurcated with a pointed spine terminally.

During the examination of moths from southwestern China and Cambodia, we identified several specimens of *Fignya*. While these specimens morphologically resemble the two known congeners, dissection revealed significant differences in the structure of the male genitalia. Consequently, we formally describe them as new species, increasing the number of species within *Fignya* to six. A diagnosis, illustrations, and a key to species with distributions are also provided.

Materials and methods

The type series was collected with a 220V/450W mercury vapor light and a DC black light in China and Cambodia. Standard methods for dissection and preparation of the genitalia slides were used (Kononenko and Han 2007). The specimens were photographed using a Canon M6 II camera, whereas the genitalia slides were photographed with an Olympus photo microscope aided by Helicon Focus 7 software and further processed in Adobe Photoshop CS6. Terminology of genitalia follows Epstein (1996) and Kristensen (2003).

All type materials of the new species are deposited in the collection of the Northeast Forestry University (NEFU), Harbin, China.

Abbreviations used:

IZCAS	Institute of Zoology, Chinese Academy of Sciences, Beijing, China
MWM/ZSM	Museum Witt München/Zoologische Staatssammlung München,
	Munich, Germany
NEFU	Northeast Forestry University, Harbin, China
нт	Holotype
РТ	Paratype

Taxonomic account

Genus Fignya Solovyev & Witt, 2009 Common name. 微刺蛾属

Fignya Solovyev & Witt, 2009: 197. Type species (by original designation): *Fignya melkaya* Solovyev & Witt, 2009. Type locality: Vietnam, Mt. Fan-si-pan (West).

Fignya qiana sp. nov.

https://zoobank.org/42F53B62-6D7A-4E42-B089-95704BC8A17D Figs 1, 7, 8, 18, 19 Common name. 黔微刺蛾

Туре material. *Holotype*: Сніма • ♂, Guizhou Province, Xingren City, Linggangshang, 1470 m, 29.vi.2022, J Wu, B Gao & RT Xu leg., genit. prep. WuJ-1129-1, in NEFU.

Paratypes • 3 ♂, same data as for holotype, genit. prep. WuJ-806-1, 1130-1, all in NEFU.



Figures 1–6. Adults of Fignya spp. Depositories of the specimens 1–2, 4–6 in NEFU 3 in MWM/ZSM. Scale bars: 5 mm.

Diagnosis. The new species is not noticeably different in appearance from *F. melkaya* (Fig. 3), *F. ravalba* (Fig. 4), and the next new species, *F. trigonum* sp. nov. (Fig. 2), but it seems that the overall color of the present new species is lighter compared to the three species mentioned above. It can be clearly distinguished from others by the characteristics of male genitalia.

In the male genitalia, *F. qiana* sp. nov. (Figs 7, 8) is most similar to *F. trigo-num* sp. nov. (Figs 9, 10), as both have almost flat or beveled cucullus and a large, bristled process at the basal part of the valva. However, *F. qiana* sp. nov. has a pair of stronger and asymmetrical apical spines on the juxta, with the left (when viewed ventrally) spine usually slightly longer than the right; a more elongated and finger-like saccus; a smaller, claw-like, curved cornutus at the

base of the vesica; and lacks the triangular sclerite near the base of the vesica. In contrast, *F. trigonum* sp. nov. has a pair of thinner, almost equal-length, finger-like spines on the juxta apically; a shorter, wider, triangular saccus; a thicker and slightly curved cornutus at the base of the vesica; and a triangular sclerite near the base of the vesica. Additionally, *F. qiana* sp. nov. differs from *F. melkaya* (Fig. 11) and *F. ravalba* (Fig. 12) by the latter two having a rounded cucullus on the valva, a small bristled process at the base of the valva, and a shorter, stouter phallus.

Description. Male. *Adult.* Forewing length 7.5-8.0 mm, wingspan 16-17 mm (N = 4). Antennae filiform, brown. Labial palpi upcurved, pale brown. Head and thorax grayish-white with a tinge of brown; abdomen grayish-white to pare brown. Forewings grayish-white with a series of serrated reddish-brown transverse lines, among which the antemedial line distinct, brown, zigzag; medial and postmedial areas of forewing, and tornus relatively darker, with two large, white, oval spots with black scales around the edges and a series of indistinct small circular spots near tornus, and a small, white, round dot near distal part of cell; postmedian line distinct, forming a broad, indistinct reddish-brown band extending from c. 3/5 of the costal margin from the wing base to tornus. Fringe grayish-white with a tinge of light brown. Hindwings reddish-brown, with dark brown scales mixed in the costal and inner margin areas; fringe light brown.

Male genitalia. Uncus short, pointed apically. Gnathos flat, fish-tail-shaped, comb-like distally. Valva wider at the base; inner side of the cucullus obviously protruding, causing the end of the cucullus to look flat or beveled; basal part of valva with a near-elliptical sclerotized plate, covered with dense, long bristles laterally; sacculus slightly swollen at base. Juxta flat, with a pair of asymmetrical, slender, sclerotized apical spines apically, the left one always slightly longer than the right. Saccus long, broad at the base, gradually narrowing to the middle, then finger-like at distal half part. Phallus slender, tubular, slightly curved, sclerotized at terminal part. Vesica bearing 4–5 cornuti in total, basal one large, hook-shaped; second one small, near the basal cornutus; subapical cornuti 1 or 2 in numbers, apical and subapical cornuti same in size.

Female genitalia. Unknown.

Phenology and habitat. The type specimens were collected in June at an altitude of c. 1470 m. The collection site is close to a mixed coniferous and broad-leaved forest, with bamboo forest, bushes, and farmland surrounding it (Fig. 16). The immature stages are still unknown.

Distribution. China (Guizhou).

Etymology. The new species name is derived from the abbreviation of Guizhou Province in China, "Qian", which is the type locality of *F. qiana* sp. nov.

Fignya trigonum sp. nov.

https://zoobank.org/0F6D83BD-C06D-4303-A109-4E3DB4B037D8 Figs 2, 9, 10, 20 Common name. 三角微刺蛾

Type material. *Holotype*: CHINA • ♂, Yunnan Province, Zhaotong City, Xiaocaoba Town, Yutang Village, 1864 m, 16.vii.2023, RT Xu & MX Han leg., genit. prep. WuJ-1136-1, in NEFU.

Paratypes: CHINA • 2♂, same data as for holotype, genit. prep. WuJ-1111-1, 1112-1, all in NEFU.

Diagnosis. *F. trigonum* sp. nov. (Fig. 2) is closest to *F. qiana* sp. nov. (Fig. 1) both in appearance and male genitalia. The differences between the two have been listed in the diagnosis section of *F. qiana* sp. nov. The most significant



Figures 7–15. Male genitalia of *Fignya* spp. Depositories of the slides **7–10**, **12–15** in NEFU **11** in MWM/ZSM. Scale bars: 1 mm, except unscaled Fig. 11.

difference in male genitalia from the other two species, *F. melkaya* (Fig. 11) and *F. ravalba* (Fig. 12), are that the new species has an obviously protruding inner side of the cucullus, a well developed thick and long horn-like cornutus at the base of the vesica, and a triangular sclerite. In contrast, the inner side of the cucullus in *F. melkaya* and *F. ravalba* is smooth, and the developed horn-shaped basal cornutus and triangular sclerite are absent.

Description. Male. *Adult.* Forewing length 7.5–8.0 mm, wingspan 16.6–18.0 mm (N = 3). Antennae filiform, brown. Labial palpi upcurved, light brown. Head and thorax grayish-white with a tinge of brown; abdomen grayish-white to light brown, terminal with grayish-white to light brown scale tuft. Forewing grayish-brown with a series of serrated reddish-brown to dark brown, serrated transverse lines, among which the antemedial line distinct, dark brown, zigzag; medial and postmedial areas of forewing, and tornus brown to dark brown, with two large, white, oval spots with black scales around the edges and a series of indistinct small circular spots near tornus, and a small, indistinct, white dot located at the end of discal cell; postmedian line grayish-white, extending from c. 3/5 of the costal margin from the wing base to tornus; terminal area black, mixed with some brown and white scales. Fringe grayish-brown with a tinge of dark brown. Hindwings reddish-brown, with dark brown scales mixed in the costal and inner margin areas; fringe pale yellow.

Male genitalia. Uncus short, pointed apically. Gnathos flat, fish-tail-shaped, comb-like distally. Valva narrow and long, swollen at the base, parallel-sided in the middle; inner side of the cucullus slightly protruding; basal part of valva with a near-elliptical, sclerotized plate, which is densely covered with long bristles laterally; sacculus slightly swollen. Juxta flat, with a pair of slender, sclerotized lateral processes. Saccus long, broad at the base, gradually narrowing to the end, rounded terminally. Phallus tube-shaped, sclerotized at terminal part. Vesica with 3 diverticula, bearing 3 cornuti; basal diverticulum with a strongly sclerotized, slightly curved, long, large cornutus, and with a sclerotized triangular sclerite next to it; medial and apical diverticula each with one strongly sclerotized, nail-shaped cornutus.

Female genitalia. Unknown.

Phenology and habitat. The type specimens were collected in July at an altitude of c. 1864 m. The collection site is located in a high mountainous area, surrounded by various types of broad-leaf trees. The ground cover consists of herbs and small shrubs (Fig. 17). The immature stages are still unknown.

Distribution. China (Yunnan).

Etymology. The new species name is derived from the Latin "trigonum", meaning triangle, referring to a unique sclerotized triangular sclerite at the base of vesica.

Fignya brachygnatha (Wu & Fang, 2008), comb. nov.

Figs 6, 15 Common name. 短颚微刺蛾

Kitanola brachygnatha Wu & Fang, 2008, *Acta Entomol. Sinica* 51 (8): 866. Type locality: China, Yunnan, Xishuangbanna. Holotype: male, in IZCAS.

Material examined. CHINA • ♂, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Jinghong City, Mengyang Town, Yexianggu, 4–5.viii.2018, HL Han & MR Li leg., genit. prep. WuJ-243-1, in NEFU. **Diagnosis.** The external characters of *F. brachygnatha* (Wu & Fang, 2008) comb. nov. (Fig. 6) are characterized by: the forewing with a grayish-brown ground color, scattered with ochre scales; the antemedial line is ochre-yellow, zigzag; the postmedial line is reddish-brown; at the end of the discal cell, there is a round white spot, below which is a larger white spot surrounded by dark brown scales; and the terminal area is triangular, grayish-white. Hindwings grayish-white, with a slightly darker coloration at the apex.

In the male genitalia (Fig. 15), the uncus is triangular with a small apical spine; the gnathos is short and broad, with a rounded tip; the valva is narrow and elongated, with a broad and rounded cucullus; the juxta is shield-shaped, with a pair of weakly sclerotized finger-like lateral process apically. The phallus is slender and bifurcate at the apex.

Distribution. China (Yunnan).

Remarks. Wu and Fang (2008) placed this species in the genus *Kitanola* Matsumura, 1925, based on its similarity to *K. speciosa* Inoue, 1956 [currently *Mediocampa speciosa* (Inoue, 1956), see Solovyev 2008: 23]. However, both the forewing pattern and the male genitalia of *F. brachygnatha* comb. nov. show significant differences from those of *Kitanola* species and are more closely aligned with the genus *Fignya*.

F. brachygnatha comb. nov. can be distinguished from the type species of the genus *Kitanola*, *K. sachalinensis* Matsumura, 1925 [currently a synonym of *K. uncula* (Staudinger, 1887)], by several morphological features. The new-ly combined species displays a grayish-brown forewing coloration, characterized by two distinct white circular spots near the end of the discal cell, which are surrounded by dark brown scales. The male genitalia are marked by a spoon-shaped gnathos, an absence of any processes in the valva, and a well-developed saccus. The aedeagus is stout and long, apical half forked, with a pointed apex.

In contrast, *K. uncula* exhibits highly variable forewing coloration, typically presenting 1–2 irregular discal spots. The male genitalia of *K. uncula* are distinguished by a large, hook-shaped gnathos, a valva with a fairly large fold on its inner surface, an inconspicuous saccus, and a curved aedeagus with a large, hook-shaped apical process.

Despite some discrepancies in the male genitalia characteristics compared to typical *Fignya* species, such as a rounded gnathos apex instead of comb-like, and the absence of a basal hairy process on the valva, *F. brachygnatha* shares several key characters with *Fignya*: (1) similar forewing patterns; (2) short uncus with a small apical spine; (3) juxta with a pair of finger-like lateral process at the apex; and (4) the saccus is long. Therefore, we formally transfer this species to the genus *Fignya*.

The newly combined species, as well as *F. samkosa* sp. nov., exhibit some differences in appearance and male genitalia from the rest of the genus as follows (the corresponding characters of the rest of the genus are in brackets): (1) the antemedial line and fringe of the forewing are golden (mostly grayish-brown to reddish-brown); (2) the gnathos is spoon-shaped, with the apical part nearly membranous and rough in surface (the gnathos is fishtail-shaped, with a comblike tip); (3) the bristled process at the base of the valva is reduced to a small triangular flap or absent (the basal process of valva is well developed and bears laterally large bristles); and (4) the phallus is slender and bifurcated apically

(the phallus is relatively short and thick, containing cornuti of varying sizes and numbers in the vesica).

We hypothesize that *F. brachygnatha* comb. nov. and *F. samkosa* sp. nov. may represent a lineage within the genus *Fignya* and might form a sister-group relationship with other congeners in this genus. However, this hypothesis requires further confirmation through molecular analysis.

Fignya samkosa sp. nov.

https://zoobank.org/90E4D02D-3315-4D83-BA1A-F62A75D65064 Figs 5, 13, 14 Common name. 東微刺蛾

Type material. *Holotype*: CAMBODIA • ♂, Samkos, 7–8.ii.2015, YS Bae leg., genit. prep. WuJ-1132-1, in NEFU.

Paratypes • 2♂, same data as for holotype, genit. prep. WuJ-1131-1, all in NEFU. **Diagnosis.** The new species (Fig. 5) is most similar to the newly combined species, *F. brachygnatha* comb. nov. (Fig. 6), described in this paper. There is no obvious difference in appearance between these two species, but the male genitalia can be clearly distinguished: *F. samkosa* sp. nov. (Figs 13, 14) has a relatively straight valva, a small triangular hairy flap at the base of the valva, and a slender phallus; whereas the valva of *F. brachygnatha* comb. nov. (Fig. 15) is slightly curved inward, lacks the hairy flap at the base of the valva, and has a thicker phallus. The differences between *F. samkosa* sp. nov. and the other four *Fignya* species are provided in the remarks section of *F. brachygnatha* comb. nov.



Figures 16–20. Biotope and living habitus 16, 18–19 *F. qiana* sp. nov., the collecting site is Xingren City, Guizhou Province, China; 17, 20 *F. trigonum* sp. nov., the collecting site is Zhaotong City, Yunnan Province, China.

Description. Male. *Adult.* Forewing length 6.0-6.5 mm, wingspan 14–15 mm (N = 3). Antennae filiform, brown. Labial palpi upcurved, brown. Head and thorax grayish-white with a tinge of pale brown; abdomen grayish-white to pale brown. Forewings grayish-brown with a series of serrated, golden and red-dish-brown transverse lines, among which the antemedial line distinct, golden, zigzag; medial and postmedial lines fuzzy, reddish-brown; discal cell and tornus bear a total of three obvious white spots with black edges. Apex area white, brown on outer edge. Fringe golden. Hindwing reddish-brown; fringe long, pale yellow.

Male genitalia. Uncus narrow and long, pointed apically. Gnathos spoonshaped, slightly sclerotized and nearly membranous at the end, with densely packed small granular scobination on the surface. Tegument wide. Valva straight, lateral margins nearly parallel; valva with a hairy, small, triangular flap near the base; cucullus rounded. Juxta flat, shield-shaped, upper margin slightly concave in the middle. Saccus long, wide at base, then finger-like. Phallus slender, slightly curved, bifurcated from near the middle, each with a claw-like spine apically.

Female genitalia. Unknown.

Phenology and habitat. The type specimens were collected in February in the Phnom Samkos Wildlife Sanctuary of western Cambodia. The region has a typical tropical monsoon climate, characterized by distinct wet and dry seasons. The sanctuary's vegetation includes a mix of evergreen forests, montane forests, and bamboo groves. The immature stages are still unknown.

Distribution. Cambodia (Samkos).

Etymology. The new species is named after its type locality, the Phnom Samkos Wildlife Sanctuary in western Cambodia.

Key to the species of *Fignya* based on appearance and male genitalia, with distributions

- 2 Juxta with a pair of developed, spin-like lateral processes apically; phallus without long spines on the surface......**3**

- 5 Valve straight, with a small, triangular, hairy flap near the base; the phallus is thin......**F. samkosa sp. nov.** (Cambodia: Samkos)

Acknowledgements

We are profoundly grateful to the reviewers for their invaluable suggestions, comments, and precise linguistic refinements of this paper. Our sincere thanks go to Dr Yang-Seop Bae (Division of Life Sciences, Incheon National University, Incheon, Korea) for collecting and generously providing the specimen of *F. samkosa* sp. nov. We are also deeply indebted to Dr Alexey V. Solovyev (Department of Biology and Chemistry, Ulyanovsk State Pedagogical University, Russia) for providing the holotype images of *F. melkaya* Solovyev & Witt, 2009.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study is supported by the project of National Nature Science Foundation of China (No. 31572294), the financial assistance under Heilongjiang Postdoctoral Fund (No. 415486), Full-time Postdoctoral Support Program (No. 415895), and Northeast Asia Biodiversity Research Center (NABRI202303; 2572022DS09).

Author contributions

Investigation: JW. Writing - original draft: JW. Writing - Review and Editing: HH.

Author ORCIDs

Jun Wu lo https://orcid.org/0000-0002-8032-2522 Huilin Han lo https://orcid.org/0000-0002-2045-6182

Data availability

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

References

Epstein ME (1996) Revision and phylogeny of the Limacodid-group families, with evolutionary studies on slug caterpillars (Lepidoptera: Zygaenoidea). Smithsonian Contributions to Zoology 582: 1–102. https://doi.org/10.5479/si.00810282.582 Inoue H (1956) A new species of Heterogeneidae. Kontyu 24: 159-160. [In Japanese]

- Ji SQ (2018) Taxonomic study on the Limacodidae from South-west China (Lepidoptera: Limacodidae). MSc Thesis, South China Agricultural University, Guangzhou, 97 pp. [In Chinese]
- Kononenko VS, Han HL (2007) Atlas Genitalia of Noctuidae in Korea (Lepidoptera). In: Park KT (Ed.) Insects of Korea. Series 11. Junhaeng-Sa, Seoul, 464 pp.
- Kristensen NP (2003) 4. Skeleton and muscles: adults. In: Kristensen NP (Ed.) Vol.
 4. Arthropoda: Insecta, teilband/Part 36. Lepidoptera, moths and butterflies. Vol.
 2. Morphology, physiology, and development. Handbook of Zoology/Handbuch der Zoologie. Part IV. Walter de Gruyter, Berlin and Boston, 39–131. https://doi. org/10.1515/9783110893724.39
- Matsumura S (1925) An enumeration of the butterflies and moths from Saghalien, with description of new species and subspecies. Journal of the College of Agriculture, Hokkaido Imperial University 15: 83–196.
- Solovyev AV (2008) The limacodid moths (Lepidoptera: Limacodidae) of Russia. Eversmannia 15(4): 17–43. [In Russian]
- Solovyev AV, Witt TJ (2009) The Limacodidae of Vietnam. Entomofauna, Supplement 16: 33–229.
- Staudinger O (1887) Neue Arten und Varietäten von Lepidopteren aus dem Amur-Gebiete. In: Romanoff NM (Ed.) Mémoires sur les Lépidoptères 3. Imprimerie de M.M. Stassuléwitch, St. Petersburg, 126–232 [pls 6–12 + 16–17]. [In German]
- Wu CS, Fang CL (2008) Discovery of the genus *Kitanola* Matsumura from China, with descriptions of seven new species (Lepidoptera, Limacodidae). Acta Entomologica Sinica 51(8): 861–867.
- Wu J, Solovyev AV, Han HL (2022) Four new species and two newly recorded species of Limacodidae (Lepidoptera, Zygaenoidea) from China. ZooKeys 1123: 205–219. https://doi.org/10.3897/zookeys.1123.77217



Research Article

A new gorgonian *Pseudopterogorgia nanjiensis* sp. nov. (Cnidaria, Octocorallia, Gorgoniidae) from the Nanji Islands, China

Tingzai Sun^{1,2}, Yu Xu¹⁰, Kuidong Xu^{1,2,3,4}, Shun Chen⁵, Shangwei Xie⁶, Zifeng Zhan^{1,40}

- 1 Laboratory of Marine Organism Taxonomy and Phylogeny, Qingdao Key Laboratory of Marine Biodiversity and Conservation, Shandong Province Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
- 2 University of Chinese Academy of Sciences, Beijing 100049, China
- 3 Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519082, China
- 4 Laboratory for Marine Biology and Biotechnology, Qingdao Marine Science and Technology Center, Qingdao 266237, China
- 5 National Engineering Research Center of Marine Facilities Aquaculture, Zhejiang Ocean University, Zhoushan 316022, China
- 6 Nanji Islands National Marine Nature Reserve Administration, Wenzhou 325400, China

Corresponding author: Zifeng Zhan (zzhan@qdio.ac.cn)



Academic editor: James Reimer Received: 13 May 2024 Accepted: 19 August 2024 Published: 27 September 2024

ZooBank: https://zoobank. org/9582F9A4-9DF0-4706-AFB4-D288A3A0B8F8

Citation: Sun T, Xu Y, Xu K, Chen S, Xie S, Zhan Z (2024) A new gorgonian *Pseudopterogorgia nanjiensis* sp. nov. (Cnidaria, Octocorallia, Gorgoniidae) from the Nanji Islands, China. ZooKeys 1213: 237–249. https://doi. org/10.3897/zookeys.1213.126841

Copyright: [©] Tingzai Sun 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).

Abstract

Members of the genus *Pseudopterogorgia* Kükenthal, 1919 are widely distributed in shallow water of the Indo-West Pacific. During an investigation of benthic biodiversity in the subtidal zone surrounding the Nanji Islands in the East China Sea, two specimens of *Pseudopterogorgia* were collected and described as a new species based on an integrated morphological-molecular approach. *Pseudopterogorgia nanjiensis* **sp. nov.** is most similar to *P. fredericki* Williams & Vennam, 2001 in the irregular branching form and indistinct scaphoids, but differs by the coenenchymal sclerite content of distinct capstans and a few warty spindles and radiates (vs. mostly warty spindles and a few capstans), and a purplish colony (vs. white, pink to deep rose). Molecular phylogenetic analyses, based on the mtMutS-COI gene sequences, delineated a monophyletic clade encompassing all assessed *Pseudopterogorgia* species. Within this clade, *P. nanjiensis* **sp. nov.** showed a close phylogenetic affinity with both *P. fredericki* and *P. australiensis* Ridley, 1884.

Key words: Anthozoa, COI, Malacalcyonacea, morphology, mtMutS, new species, phylogeny, *Pseudopterogorgia nanjiensis*, taxonomy, 28S rDNA

Introduction

Octocorals fulfill a pivotal ecological function, with their three-dimensional configurations enhancing the structural intricacy and variety of reefs (Poliseno 2016; Lau et al. 2020). Gorgonian corals in octocoral animal forests offer nourishment and habitat for a variety of interrelated organisms (Sánchez 2016). Among these, the genus *Pseudopterogorgia* Kükenthal, 1919 represents a rare group of shallow-water octocorals found in the Indo-West Pacific (Williams and Chen 2012). The members of this genus are characterized by pinnate or irregular branching structures, absence of anastomoses, and C-shaped or scaphoid-like sclerites present in the coenenchyme (Kükenthal 1919; Bayer 1961; Grasshoff and Alderslade 1997). To date, this genus has seven valid species, all of which are distributed in the Indo-West Pacific (Williams and Chen 2012; WoRMS Editorial Board 2024).

During the investigation of benthic biodiversity in the subtidal zone of the Nanji Islands on the Chinese coast of the East China Sea, two specimens of *Pseudopterogorgia* were collected. They are described as a new species *Pseudopterogorgia nanjiensis* sp. nov. based on morphological and phylogenetic analyses. The phylogenetic placement of *P. nanjiensis* sp. nov. within the genus is further explored.

Material and methods

Specimen collection and morphological examination

Two specimens were obtained by scuba diving from the subtidal zone of the Nanji Islands (27°28.53'N, 121°08.13'E) on the Chinese coast of the East China Sea during investigation of the benthic biodiversity in 2022 and 2023 (Fig. 1). The specimens were photographed in situ using a Nikon D850 before being sampled and on board using a Canon EOS 5D Mark IV, and then stored in 75% ethanol after collection.

A stereo dissecting microscope (Zeiss SteREO Discovery. V20) was used to examine the general morphology and anatomy of the specimens. Sclerites of the polyps and branches were isolated by digesting the tissues in sodium hypochlorite and subsequently rinsed thoroughly with deionized water. To investigate the ultrastructure of polyps and sclerites, they were air-dried, mounted on carbon double adhesive tape, and coated with Pd/Au for scanning electron microscope (SEM) observation. SEM scans were obtained by using a TM-3030Plus SEM at 15 kV, and the optimum magnification was selected for each type of sclerite. The terminology used in this study follows Bayer et al. (1983).

The type specimens of the new species have been deposited in the Marine Biological Museum of Chinese Academy of Sciences (MBMCAS) at Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China.



Figure 1. Sampling site (red dot) off the coast of the Nanji Islands in the East China Sea.

DNA extraction and sequencing

Genomic DNA was extracted from the polyps of each specimen using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the instructions. Specific regions of the mitochondrial *mutS* homolog (mtMutS) and cytochrome *c* oxidase subunit I (COI), as well as an approximately 800-nt fragment of the 28S nuclear ribosomal gene (28S rDNA), were selected for phylogenetic analysis. For the amplification of mtMutS and COI, the primer pairs AnthoCorMSH (5'-AGGAGAATTATTCTAAGTATGG-3'; Herrera et al. 2010) and Mut-3458R (5'-TSGAGCAAAAGCCACTCC-3'; Sánchez et al. 2003); COI8414-F (5'-CCAGGTAGTATGTTAGGRGA-3') and HCO2198 (5'-TAAACTTCAGGGTGAC-CAAAAAATCA-3') were utilized, respectively. Sequencing of 28S rDNA was performed using the primers 28S-Far (5'-CACGAGACCGATAGCGAACAAGTA-3') and 28S-Rar (5'-TCATTTCGACCCTAAGACCTC-3') (McFadden and van Ofwegen 2012). Amplifications and sequencing of the markers followed Xu et al. (2021).

Genetic distance and phylogenetic analyses

The sequences of Gorgoniidae species and two species of Eunicellidae as an out-group were downloaded from GenBank (Table 1). The sequences were aligned using MAFFT v.7 (Katoh and Standley 2013) with the L-INS-I algorithm. Genetic distances of mtMutS, COI and 28S between species/populations were calculated with MEGA 6.0 using Kimura 2-parameter model (Tamura et al. 2013).

Species	Veucher Number	Gen	nBank Accession Numbers		
Species	voucher Number	mtMutS	COI	28S rDNA	
Pseudopterogorgia nanjiensis sp. nov.	MBM287892	PP558212	PP556906	PP572455	
Pseudopterogorgia nanjiensis sp. nov.	MBM287893	PP558213	PP556907	PP572456	
Pseudopterogorgia australiensis	-	– AY268442 –		-	
Pseudopterogorgia fredericki	CAS:118507	JX152766	-	-	
Pseudopterogorgia rubrotincta	CAS:155043	JX152768	-	-	
Leptogorgia alba	-/HMG71/MECN Ant0018	AY268452	HG917083	KX721241	
Leptogorgia cuspidata	-/HMG97/MCZ:7002	AY268450	HG917088	KX767433	
Leptogorgia mariarosae	MECN Ant0012	KX721193	KX721174	KX721231	
Leptogorgia obscura	Ant 0066/Ant 0066/BEIM 0080	KX767321	KX767383	KX721248	
Leptogorgia cf. palma	USNM:1516865/-	ON109715	MW401657	-	
Eugorgia daniana	MECN Ant0033	KX721208	KX721189	KX721246	
Pacifigorgia irene	Ant 0044/Ant 0044/MNCN 2.04/1174	KX767346 KX767406		KX767449	
Gorgonia flabellum	-/-/RMNH Coel.40827	AY126427	GQ342418	JX203708	
Phyllogorgia dilatata	-	AY126428	-	-	
Antillogorgia bipinnata	-/ABBAC034/RMNH Coel.40828	GQ342499	MK153463	JX203712	
Adelogorgia osculabunda	MZUCR:2496_OCT0088	MF579541	-	-	
Psammogorgia cf. arbuscula	HMG38/HMG15/HMG38	HG917043	HG917055	LT221092	
Chromoplexaura marki	_	KX904972	KX904954	-	
Callistephanus sp.	USNM:1606569	ON109735 –		-	
Eunicella tricoronata	RMNH Coel.40814	JX203795	JX203853	JX203707	
Complexum monodi	CSM-SEN3/CSM-SEN4/CSM-SEN4	KP036906	KP036909	KP036912	

Table 1. The sequences used in this study. Newly sequenced species are in bold.

Phylogenetic analyses were conducted on the 28S rDNA and mtMutS-COI datasets. The phylogenetic frameworks were established using PhyloSuite v.1.2.3 (Zhang et al. 2020; accessible at http://phylosuite.jushengwu.com), a comprehensive platform for phylogenetic analysis. ModelFinder v.2.2.0 (Kalyaanamoorthy et al. 2017) was employed to identify the optimal evolutionary models based on the Bayesian Information Criterion (BIC). HKY+F+I and TIM3e+I were selected as the best-fit substitution models for the mt-MutS-COI and 28S rDNA alignments, respectively. Maximum likelihood (ML) trees were inferred using IQ-TREE v.2.2.0 (Nguyen et al. 2015) with 1000 standard bootstraps. Following Hillis and Bull (1993), ML bootstrap values were categorized into low (<70%), moderate (70-90%), and high (\geq 90%) confidence levels. Bayesian inference (BI) phylogenies were reconstructed using MrBayes v.3.2.7a (Ronquist et al. 2012) with dual parallel runs, each consisting of 10,000,000 generations and a burn-in phase that discarded the initial 25% of sampled data. Following Alfaro et al. (2003), the Bayesian posterior probabilities < 0.95 and ≥ 0.95 were considered as low and high, respectively.

Results

Phylum Cnidaria Hatschek, 1888 Subphylum Anthozoa Ehrenberg, 1834 Class Octocorallia Haeckel, 1866 Order Malacalcyonacea McFadden, van Ofwegen & Quattrini, 2022 Family Gorgoniidae Lamouroux, 1812 Genus *Pseudopterogorgia* Kükenthal, 1919

Pseudopterogorgia nanjiensis Sun, Xu & Zhan, sp. nov. https://zoobank.org/37902653-255A-46C8-9867-7C8DDC045BA3 Figs 2, 3, Table 2

Material examined. *Holotype*: MBM287893, CHINA-Zhejiang Prov. • Nanji Islands; 27°28.53'N, 121°08.13'E; 11 May 2023. *Paratype*: MBM287892, CHINA-Zhejiang Prov. • Nanji Islands; 27°28.53'N, 121°08.13'E; 14 m; 11 May 2022.

Diagnosis. Colony non-pinnate and purplish. Branches divided dichotomously and irregularly, nearly in one plane. Polyp retracted and forming a small and oval-shaped protrusion. Sclerites in polyps small flat rodlets, colorless and rare to absent in numbers. Sclerites in coenenchyme red, mostly capstans, a few radiates and warty spindles, and rare scaphoids. Capstans have two whorls of tubercles and blunt ends with irregular arrangements of complex tubercles. Radiates and immature sclerites with two whorls of projections, spindles with 4–8 whorls of tubercles, and scaphoids with similar ornamentation of tubercles on both convex and concave sides.

Description. Holotype upright and nearly planar but not pinnate, about 70 mm in length (Fig. 2B). Holdfast nearly round, about 8 mm long and 7 mm wide at maximum. Trunk cylindrical, about 3 mm in length before first branch and 2 mm in diameter at base, divided into two primary stems, one with three secondary branches and the other with five secondary branches. Branches divided dichotomously and irregularly, nearly in one plane (Fig. 2D). Distances between



Figure 2. The external morphology and polyps of *Pseudopterogorgia nanjiensis* sp. nov. A holotype in situ B holotype after collection C paratype after fixation D part of branch under a light microscope E single polyp aperture under a light microscope F sclerites under a light microscope G coenenchyme under SEM H single dissolved polyp aperture under a light microscope. Scale bars: 2 cm(B); 3 cm(C); 2 mm(D); 0.2 mm(E); 0.1 mm(F, G, H).

adjacent branches up to 20 mm. Branches usually cylindrical in the proximal part of the colony, and became flattened in the distal part of the colony. Terminal branchlets slender and a little curved, typically measuring 20–60 mm in length and 1–2 mm in width.

Table 2. (Comparison of morpholog	gical characters of	Pseudopterogorgia	species (based o	on Williams and	Vennam 2001).
"-" means	s unavailable data.					

Characters/ Species	P. nanjiensis sp. nov.	P. fredericki	P. formosa	P. australiensis	P. luzonica	P. oppositipinna	P. rubrotincta	P. torresia
Branching	irregular	irregular	irregular	pinnate/ plumose	lateral	pinnate to irregular	pinnate	pinnate/ sparse
Sclerite forms	capstans, radiates, warty spindles, scaphoids, flat rodlets	elongated spindles, some shorter and more robust spindles, scaphoids	small or double or girdled spindles, a few double heads, scaphoids	straight or slightly curved spindles, eight radiates, scaphoids	girdled spindles, scaphoids	elongated spindles, scaphoids	rough spindles, scaphoids	warty spindles, quadriradiate, scaphoids
Scaphoids	indistinct	indistinct	some distinct	some distinct	mostly distinct	mostly distinct	some distinct	some distinct
Colony color	purplish	white, pink to deep rose	dark bright pink to carmine	yellow, orange or deep crimson	bright red	brown to amber-yellow.	orange yellow with red line	pale yellow
Sclerites color	red or colorless	reddish or colorless	red, carmine	yellow or red	red	purple-red or crimson red	red	_
Maximum sclerite length	0.18 mm	0.23 mm	0.17 mm	0.17 mm	0.16 mm	0.21 mm	0.15 mm	0.16 mm
Type locality	Nanji Islands on East China Sea	western India	Lombok	Torres Strait	Luzon	Mergui Archipelago	Sri Lanka	Torres Strait
Distribution	Western Pacific	Indian Ocean	Western Pacific	Western Pacific	Western Pacific	Indian Ocean	Indian Ocean	Western Pacific
Depth (m)	14	6-8	22	12-37	-	_	-	-
References	present study	Williams and Vennam 2001; Williams and Chen 2012; Ramvilas 2023	Nutting 1910; Grasshoff and Alderslade 1997	Ridley 1884; Thomson and Henderson 1905; Kükenthal 1919, 1924; Williams and Vennam 2001	Kükenthal 1919; Grasshoff and Alderslade 1997	Ridley 1888; Kükenthal 1924	Thomson and Henderson 1905; Williams and Vennam 2001; Williams and Chen 2012; Ramvilas 2023	Wright and Studer 1889; Williams and Vennam 2001

Polyp retracted, forming small and oval-shaped protrusions with a recessed orifice (Fig. 2E), measuring on average 1.00 mm in length and 0.65 mm in width. Each polyp's orifice appears as a tiny and pale yellowish slit aligned lengthwise. Polyps usually closely and irregularly spaced on the branches and sparse at the basal part of the colony, some of them arranged into two opposing longitudinal rows on the terminal branchlets. Distance between polyps no more than 4 mm.

Coenenchyme covered with dense sclerites, while the polyps sclerites rare to absent (Fig. 2G, H). Sclerites in coenenchyme red with various shapes, arranged in two inconspicuous layers without a clear boundary, including abundant capstans, rare indistinct scaphoids, and occasional tuberculate spheroids and crosses in outer layers, a few radiates and warty spindles in inner layers. These sclerites range in length from 0.05 mm to 0.18 mm, but mostly less than 0.12 mm long (Figs 2F, 3A–E). Among them, capstans have two whorls of tubercles, up to 0.13 mm long, and blunt ends with irregular arrangements of complex tubercles (Fig. 3A). The radiates and immature sclerites with two whorls of projections and often blunt ends, some of them covered with shallow longitudinal grooves on surface, measuring 0.05–0.09 mm (Fig. 3B). The warty spindles with 4 to 8 transversely-aligned whorls of tubercles, up to 0.18 mm in length (Fig. 3C). The scaphoids, not distinctly developed and resembling curved spindles, with 4–8 noticeable whorls of tubercles, up to 0.14 mm long



Figure 3. The sclerites of *Pseudopterogorgia nanjiensis* sp. nov. A capstans B radiates and immature sclerites C warty spindles D scaphoids E tuberculate spheroids and cross F flat rodlets. Scale bars: 0.1 mm (all in the same scale).

(Fig. 3D). The convex and concave sides have a similar degree of fine ornamentation on the whorls of tubercles, and there seems to be minimal variation in the tubercles between the two sides with those on the convex sides potentially being slightly shorter. Tuberculate spheroids and crosses occasionally present in coenenchyme, are up to 0.08 mm and 0.06 mm long, respectively (Fig. 3E). Small rodlets in polyps flat and colorless, rare in numbers and only found in a few polyps, covered with sparse warts on the edges, up to 0.09 mm (Fig. 3F).

Holotype purplish in situ and after collection, and became deep purplish red after fixation.

Variation of paratypes. The two specimens exhibited a high degree of morphological concordance, with discrepancies primarily noted in colony size and branching density. Paratype irregularly branched, about 140 mm in length and 90 mm in width (Fig. 2C). The main trunk before first branch about 5 mm in length and 2 mm in diameter at base. The terminal branchlets 30–100 mm long and 1–2 mm wide. The oval-shaped protrusions formed by retracted polyps about 1.00 mm long and 0.75 mm wide. The warty spindles in coenenchyme up to 0.16 mm long.

Type locality. The subtidal zone of Nanji Islands with water depth of 14 m. **Etymology.** Named after the type locality Nanji Islands.

Distribution and habitat. Known only from the subtidal zone of the Nanji Islands on the Chinese coast of the East China Sea with a water depth of 14 m. Colony attached to a rocky substrate. The water temperature was 18 °C and the pH was 8.13.

Remarks. Given the non-uniform nature of these variations, they are treated as manifestations of intraspecific variability. *Pseudopterogorgia nanjiensis* sp. nov. is most similar to *P. fredericki* Williams & Vennam, 2001 in the irregular branching form and indistinct scaphoids, but differs by the sclerite forms and sizes (almost capstans, a few warty spindles and radiates, and rare indistinct scaphoids and small rodlets, mostly less than 0.12 mm vs. almost warty spindles and a few scaphoids, mostly more than 0.12 mm) and the purplish colony (vs. white, pink to deep rose) (Williams and Vennam 2001; Figs 2, 3, Table 2). *Pseudopterogorgia nanjiensis* sp. nov. is also analogous to *P. formosa* Nutting, 1910 in the irregular branching form, but distinct from *P. formosa* by the sclerite forms (capstans, spindles, radiates, and scaphoids vs. spindles, double heads, and scaphoids) (Nutting 1910; Table 2). In addition, the new species can be readily distinguished from the remaining congeners by its irregular branching form (vs. pinnate or lateral; Table 2).

Genetic distance and phylogenetic analyses

The new sequences were deposited in GenBank (Table 1). The alignments comprised 720, 575 and 717 nucleotide positions for the mtMutS, COI, and 28S rDNA regions, respectively. Based on the aligned region of mtMutS, the interspecific distance of *P. australiensis* Ridley, 1884, *P. fredericki*, *P. nanjiensis* sp. nov. and *P. rubrotincta* Thomson & Henderson, 1905 ranged from zero to 0.44%, while the intraspecific distance was zero, which was calculated from only two specimens of *P. nanjiensis* sp. nov. (Suppl. material 1: table S1). The mtMutS genetic distances between *P. nanjiensis* sp. nov. and congeners are in the range of 0–0.28%. For the 28S rDNA and COI regions, only sequences of *P. nanjiensis* sp. nov. are currently available within the genus *Pseudopterogorgia*, and no genetic variation was detected between the two specimens analyzed (Suppl. material 1: tables S2, S3). The genetic distances between *P. seudopterogorgia* and other genera of Gorgoniidae are greater than 5.48% for mtMutS, 5.74% for 28S, and 2.04% for COI (Suppl. material 1: tables S1–S3).

The Bayesian inference (BI) tree is nearly identical to the maximum likelihood (ML) tree in topology for both the mtMutS-COI and 28S rDNA regions, and thus only the BI tree annotated with support values from both inference methods is presented (Figs 4, 5). In the mtMutS-COI trees, all the *Pseudopterogorgia* species formed a monophyletic clade with full node support. The new species *P. nanjiensis* sp. nov. clustered with the clade comprising *P. fredericki* and *P. australiensis* (Fig. 4). In the 28S rDNA trees, *P. nanjiensis* sp. nov. emerged as a distinct clade, with a basal branching position within the family Gorgoniidae (Fig. 5).



Figure 4. Bayesian inference (BI) tree constructed by mtMutS and COI showing phylogenetic relationships among *Pseu-dopterogorgia* and related genera and species. The maximum likelihood (ML) tree is identical to the BI tree in topology. Node support is as follows: ML bootstrap/BI posterior probability. The ML bootstrap < 70% or BI posterior probability < 0.95 is not shown. Newly sequenced species are in bold. The GenBank accession numbers of the mtMuts sequences are listed next to the species names.



Figure 5. Bayesian inference (BI) tree constructed by 28S rDNA showing phylogenetic relationships among *Pseudopterogorgia* and related genera and species. The maximum likelihood (ML) tree is identical to the BI tree in topology. Node support is as follows: ML bootstrap/BI posterior probability. The ML bootstrap < 70% or BI posterior probability < 0.95 is not shown. Newly sequenced species are in bold. The GenBank accession numbers of the 28S rDNA sequences are listed next to the species names.

Discussion

The genus *Pseudopterogorgia* is distinguished by its pinnate or irregular branching structures, absence of anastomoses, and C-shaped or scaphoid-like sclerites present in the coenenchyme (Kükenthal 1919; Bayer 1961; Grasshoff and Alderslade 1997). Based on the branching structure and sclerite form, the present species is undoubtedly assigned to this genus. In the mtMutS-COI trees, *Pseudopterogorgia nanjiensis* sp. nov. showed a phylogenetic affinity with *P. fredericki* and *P. australiensis*, congruent with morphological assessments (Fig. 4). All three species are characterized by the absence of anastomoses and the presence of scaphoid-like sclerites within the coenenchyme. However, *P. nanjiensis* sp. nov. can be differentiated from *P. fredericki* based on sclerite morphology (see above remarks), and it is easily separated from *P. australiensis* by the branching form (irregular vs. pinnate/plumose; Table 2; Ridley 1884; Williams and Vennam 2001). With the addition of the new species *P. nanjiensis* sp. nov. discovered on the Nanji Islands, there are currently a total of eight valid species in *Pseudopterogorgia* (Williams and Vennam 2001; WoRMS Editorial Board 2024).

In the present study, the precise phylogenetic placement of the genus *Pseu-dopterogorgia* remains vague, primarily attributed to the low support value assigned to the corresponding node in the mtMutS-COI trees (Fig. 4). Although the 28S rDNA trees exhibit high support values for the majority of internal branches, the systematic position of *Pseudopterogorgia* remains unresolved (Fig. 5). This uncertainty arises from the inclusion of sequences representing only seven out of a total of thirteen genera currently recognized within the family Gorgoniidae, and is further compounded by the acknowledged reality that many of these genera exhibit polyphyletic or paraphyletic relationships (Vargas et al. 2014; Poliseno et al. 2017). Therefore, to achieve a clearer resolution, further genomic data are essential. These data should encompass a more exhaustive collection of 28S rDNA sequences and potentially include ultra-conserved elements plus exon (UCEs+exon).

Acknowledgements

We thank Dr Xinming Liu for help in sample collection.

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 (No. 42176128 and No. 41930533), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB42000000), the Senior User Project of RV KEXUE (KEX-UE2020GZ02), and Science and Technology Program of Nanji Islands National Marine Nature Reserve Administration (JJZB-PYCG-2021112901).

Author contributions

All authors have contributed equally.

Author ORCIDs

Yu Xu [©] https://orcid.org/0000-0002-4937-122X Zifeng Zhan [©] https://orcid.org/0000-0003-4386-0687

Data availability

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

References

- Alfaro ME, Zoller S, Lutzoni F (2003) Bayes or Bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Molecular Biology and Evolution 20(2): 255–266. https://doi.org/10.1093/molbev/msg028
- Bayer FM (1961) The shallow-water Octocorallia of the West Indian region. Studies on the Fauna of Curaçao and other Caribbean Islands 12(1): 1–373.
- Bayer FM, Grasshoff M, Verseveldt J (1983) Illustrated Trilingual Glossary of Morphological and Anatomical Terms Applied to Octocorallia. E. J. Brill/Dr. W. Backhuys, Leiden, 75 pp. https://doi.org/10.1163/9789004631915

Grasshoff M, Alderslade P (1997) Gorgoniidae of Indo-Pacific reefs with description of two new genera (Coelenterata: Octocorallia). Senckenbergiana biologica 77(1): 23–35.

- Herrera S, Baco A, Sánchez JA (2010) Molecular systematics of the bubblegum coral genera (Paragorgiidae, Octocorallia) and description of a new deep-sea species. Molecular Phylogenetics and Evolution 55(1): 123–135. https://doi.org/10.1016/j. ympev.2009.12.007
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42(2): 182–192. https://doi. org/10.1093/sysbio/42.2.182
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software version
 7: improvements in performance and usability, Molecular Biology and Evolution 30:
 772–780. https://doi.org/10.1093/molbev/mst010
- Kükenthal W (1919) Gorgonaria. Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899 13(2): 1–946.
- Kükenthal W (1924) Lfg. 47 Coelenterata: Gorgonaria. Berlin, Boston: De Gruyter. https://doi.org/10.1515/9783111569642
- Lau YW, Poliseno A, Kushida Y, Quéré G, Reimer JD (2020) The Classification, Diversity and Ecology of Shallow Water Octocorals. In: Goldstein MI, DellaSala DA (Eds) Encyclopedia of the World's Biomes. Elsevier, Oxford, 597–611. https://doi.org/10.1016/ B978-0-12-409548-9.12109-8
- McFadden CS, van Ofwegen LP (2012) A second, cryptic species of the soft coral genus *Incrustatus* (Anthozoa: Octocorallia: Clavulariidae) from Tierra del Fuego, Argentina, revealed by DNA barcoding. Helgoland Marine Research 67: 137–147. https://doi. org/10.1007/s10152-012-0310-7

- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ–TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- Nutting CC (1910) The Gorgonacea of the Siboga Expedition. VII. The Gorgoniidae. Siboga Expedition Monograph 13b⁴: 1–11.
- Poliseno A (2016) Speciation, evolution and phylogeny of some shallow-water octocorals (Cnidaria: Anthozoa). Dissertation, LMU München: Faculty of Geosciences https://doi.org/10.5282/edoc.19888
- Poliseno A, Feregrino C, Sartoretto S, Aurelle D, Wörheide G, McFadden CS, Vargas S (2017) Comparative mitogenomics, phylogeny and evolutionary history of *Leptogor-gia* (Gorgoniidae). Molecular Phylogenetics and Evolution 115: 181–189. https://doi. org/10.1016/j.ympev.2017.08.001
- Ridley SO (1884) Alcyonaria. In: Report on the Zoological Collections made in the Indo– Pacific Ocean during the Voyage of H.M.S. 'Alert' 1881-2. British Museum, London, 327–365.
- Ridley SO (1888) Report on the Alcyoniid and Gorgoniid Alcyonaria of the Mergui Archipelago, collected for the Trustees of the Indian Museum, Calcutta, by Dr. John Anderson, F.R.S. Zoological Journal of the Linnean Society 21(131): 223–247. https://doi. org/10.1111/j.1096-3642.1888.tb00976.x
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Sánchez JA (2016) Diversity and Evolution of Octocoral Animal Forests at Both Sides of Tropical America. In: Rossi S, Bramanti L, Gori A, Orejas Saco del Valle C (Eds) Marine Animal Forests. Springer, Cham, 1–33. https://doi.org/10.1007/978-3-319-17001-5_39-1
- Sánchez JA, Lasker HR, Taylor DJ (2003) Phylogenetic analyses among octocorals (Cnidaria): mitochondrial and nuclear DNA sequences (Isu-rRNA, 16S and ssu-rRNA, 18S) support two convergent clades of branching gorgonians. Molecular Phylogenetics and Evolution 29(1): 31–42. https://doi.org/10.1016/S1055-7903(03)00090-3
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197
- Thomson JA, Henderson WD (1905) Report on the Alcyonaria collected by Professor Herdman at Ceylon in 1902. In: Herdman WA (Eds) Report of the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar. Part 3. Supplementary report. The Royal Society, London, 269–238.
- Vargas S, Guzman HM, Breedy O, Wörheide G (2014) Molecular phylogeny and DNA barcoding of tropical eastern Pacific shallow-water gorgonian octocorals. Marine Biology 161: 1027–1038. https://doi.org/10.1007/s00227-014-2396-8
- Williams GC, Chen JY (2012) Resurrection of the octocorallian genus Antillogorgia for Caribbean species previously assigned to Pseudopterogorgia, and a taxonomic assessment of the relationship of these genera with Leptogorgia (Cnidaria, Anthozoa, Gorgoniidae). Zootaxa 3505(1): 39–52. https://doi.org/10.11646/zootaxa.3505.1.3.
- Williams GC, Vennam JS (2001) A revision of the Indo-West Pacific taxa of the gorgonian genus *Pseudopterogorgia* (Octocorallia: Gorgoniidae), with the description of a new species from western India. Bulletin of the Biological Society of Washington 10: 71–95.

- WoRMS Editorial Board (2024) World Register of Marine Species. https://www.marinespecies.org [at VLIZ] [Accessed 2024-05-04] https://doi.org/10.14284/170
- Wright EP, Studer T (1889) Report on the Alcyonaria collected by H.M.S. Challenger during the years 1873–76. Report on the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873–76. Zoology 31(64): 1–314.
- Xu Y, Zhan ZF, Xu KD (2021) Morphology and phylogeny of *Chrysogorgia pinniformis* sp. nov. and *C. varians* sp. nov., two golden corals from the Caroline seamounts in the tropical Western Pacific Ocean. Journal of Oceanology and Limnology 39: 1767–1789. https://doi.org/10.1007/s00343-021-0386-5
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Supplementary material 1

Genetic distance analyses

Authors: Tingzai Sun, Yu Xu, Kuidong Xu, Shun Chen, Shangwei Xie, Zifeng Zhan Data type: docx

- Explanation note: **table S1.** Interspecific and intraspecific uncorrected pairwise distances at mtMutS of *Pseudopterogorgia* and species of other genera within Gorgoniidae. **table S2.** Interspecific and intraspecific distances at 28S of *Pseudopterogorgia* and species of other genera within Gorgoniidae. **table S3.** Interspecific and intraspecific distances at COI of *Pseudopterogorgia* and species of other genera within Gorgoniidae.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1213.126841.suppl1



Research Article

Four new species of *Laelius* Ashmead (Hymenoptera, Bethylidae) from Korea with an updated key to species in the Eastern Palaearctic region

Jongok Lim^{1,2}, Seunghwan Lee^{3,4}

- 1 Department of Life and Environmental Sciences, College of Agriculture and Food Sciences, Wonkwang University, Iksan, Jeonbuk Province, Republic of Korea
- 2 Institute of Life Science and Natural Resources, Wonkwang University, Iksan, Jeonbuk Province, Republic of Korea
- 3 Laboratory of Insect Biosystematics, Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea
- 4 Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

Corresponding author: Jongok Lim (jjongok79@gmail.com)

Abstract

The genus *Laelius* Ashmead, 1893 (Hymenoptera, Bethylidae) is a cosmopolitan bethylid genus with 68 valid species distributed across most zoogeographic regions worldwide. This taxonomic study on Korean species of *Laelius* has led to the description of four new species, namely *L. afores* **sp. nov.**, *L. atratus* **sp. nov.**, *L. sulcatus* **sp. nov.**, and *L. tricuspis* **sp. nov.** Additionally, illustrations of the diagnostic characteristics of each species are provided, along with an updated key for 11 *Laelius* species from the Eastern Palaearctic region.

Key words: Asia, Epyrinae, flat wasps, identification key, new species, taxonomy

Introduction

The genus *Laelius* Ashmead, 1893 (Hymenoptera, Bethylidae) is a cosmopolitan genus belonging to the subfamily Epyrinae. Currently, it comprises 68 valid species distributed across all zoogeographic regions, except for the Oceanian region as suggested by Holt et al. (2013). Three extinct fossil species have been recorded from Baltic and Rovno amber (Azevedo et al. 2018; Colombo et al. 2021). Approximately 33% (22 species) of the species were described from the Palaearctic region, with seven species reported from Eastern Asia.

Taxonomic information on *Laelius* species in the European Palaearctic region, mainly from Finland and Sweden, was provided by Vikberg and Koponen (2005). *Laelius sinicus* Xu, He & Terayama in the Eastern Palaearctic region was described by Xu et al. (2003), and Terayama (2006) published a book on Japanese Bethylidae, which included four *Laelius* species from Japan. *Laelius yamatonis* Terayama, 2006 was later recorded from South Korea by Lim et al. (2010a), and Lim et al. (2010b) provided a key to the Eastern Palaearctic



Academic editor: Wesley Dondoni Colombo Received: 25 February 2024 Accepted: 19 April 2024 Published: 27 September 2024

ZooBank: https://zoobank. org/03EC1A44-E47F-4FF3-9F57-68568C76AEDD

Citation: Lim J, Lee S (2024) Four new species of *Laelius* Ashmead (Hymenoptera, Bethylidae) from Korea with an updated key to species in the Eastern Palaearctic region. ZooKeys 1213: 251–265. https://doi. org/10.3897/zookeys.1213.121630

Copyright: © Jongok Lim & Seunghwan Lee. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0) species with a description of *L. jilinensis* Lim & Lee, 2010 from China. Currently, only one species, *L. yamatonis* Terayama, 2006, has been recorded in South Korea (Lim et al. 2010a).

Laelius species can be distinguished by the presence of thick black setae on the body and wings, a projected median clypeal lobe, and complete occipital carinae (Azevedo et al. 2018). The presence and relative length of the carinae on the metapostnotum and the metapectal-propodeal disc, as well as the relative length of the 2r-rs&Rs vein of the forewings, are commonly used in taxonomic and systematic studies on *Laelius* (Marques Jr. et al. 2023).

Laelius species parasitize larvae of various families, including Dermestidae and Scolytinae (Coleoptera), and Glossinidae (Diptera) (Vance 1932; Mertins 1980; Azevedo et al. 2018). Furthermore, *Laelius* species have been utilized for biological control purposes, as documented in many reports (Barbosa and Azevedo 2011).

This manuscript describes four new species of *Laelius* from South Korea, providing illustrations of their diagnostic characteristics and an updated taxonomic key for 11 *Laelius* species from the Eastern Palaearctic region.

Materials and methods

All materials examined were collected using Malaise traps from the northern and southern regions of South Korea. The abbreviations for collection localities in Korea are as follows: **GW**, Gangwon-do; **JN**, Jeollanam-do; **JJ**, Jeju-do. Detailed information on provincial localities in South Korea can be found in Lim et al. (2011).

The abbreviations for biometric measurements used in the text are as follows: **AOL**, the minimum distance between the anterior ocellus and the posterior ocellus; **DAO**, the diameter of the anterior ocellus; **HE**, the height (maximum length) of the eye in lateral view; **LFW**, the maximum length of the forewing; **LH**, the length of the head, from the apical margin of the clypeus to the posterior margin of the head in full dorsal view; **POL**, the distance between the posterior ocelli in full dorsal view; **VOL**, the vertex-ocular line, which is the distance between the top of the eye and the vertex line; **WE**, the minimum width of the eye in lateral view; **WF**, the width of the frons, the minimum distance between the eyes in full dorsal view; **WH**, the maximum width of the head in full dorsal view; **WOT**, the width of the ocellar triangle, including the width of the posterior ocelli.

Terms describing integument sculptures followed Eady (1968) and Harris (1979). Additionally, general morphological terms followed Azevedo et al. (2018), Lanes et al. (2020), and terms regarding mesoplueral structures followed Brito et al. (2021).

The specimens were examined under a Leica M205 C stereomicroscope (Leica Microsystems, Solms, Germany), and images were captured with a Dhyana 400D camera (TUCSEN CMOS, Fujian, China) attached to a Leica M205C. Multi-stacked images were produced using the Delta Multifocus ver. 24 program (Delta, South Korea) and Helicon Focus ver. 8.2.2 software (HeliconSoft, Ukraine). The final images were edited using Adobe Photoshop 2021 (Adobe Systems, Inc., San Jose, CA, USA).

The examined specimens were deposited at the Laboratory of Insect Taxonomy and Ecology at Wonkwang University (W-LITE), Iksan, Republic of Korea.
Systematic accounts

Family Bethylidae Haliday, 1839 Subfamily Epyrinae Kieffer, 1914

Genus Laelius Ashmead, 1893

- Laelius Ashmead, 1893. Bull. U.S. Nat. Mus., 45: 50. Type-species: Laelius trogodermatis Ashmead, 1893.
- Paralaelius Kieffer, 1905. Ann. Soc. Sci. Bruxelles, 29: 129. Type-species: Laelius pedatus (Say, 1836).
- *Allepyris* Kiffer, 1905. Ann. Soc. Sci. Burxelles, 29: 106. Type-species: *Allepyris microneura* (Kieffer, 1905).

Prolaelius Kieffer, 1905. Type-species: Paralaelius firmipennis (Cameron, 1905).

Diagnosis. The genus *Laelius* can be distinguished from other genera by having the mesoscuto-scutellar suture with an evident sulcus and thick black setae on the body (Colombo et al. 2022).

Laelius afores Lim, sp. nov.

https://zoobank.org/6742C3C5-B66C-4E1C-8EDF-1F9A874FF85A Fig. 1A-E

Description. Holotype (female). Body length 2.61 mm; LFW 1.48 mm.

Color. Head black; mandible dark castaneous except apical half-light castaneous; antenna dark castaneous except scape and pedicel light castaneous in dorsal view. Mesosoma black; legs light castaneous except basal half of procoxae and metafemora castaneous; wings hyaline, tegula light castaneous, veins pale castaneous. Metasoma black.

Morphology. *Head* (Fig. 1A, B). 1.1× as long as wide with broadly outcurved vertex crest in dorsal view in dorsal view (Fig. 1B). Ventral and lateral surface with suberect long setae, some setae longer than LE. Mandible with four apical teeth; two uppermost teeth small, 3rd tooth from top most thick and one ventralmost sharpened. Clypeus short, broadly rounded with one median small blunt tooth, median area longitudinally elevated (Fig. 1B). First five antennomeres in ratio of 2.5: 1.7: 2.5: 1.0: 1.0 in length; scape, pedicel and flagellomere III–V and XI 2.7, 2.4, 3.3, 1.2, 1.3 and 1.3× as long as wide, respectively. Frons coriaceous with sparse punctures; frontal line absent. Vertex slightly outcurved and round marginally. WF 1.9× LE, WF 0.7× WH. Compound eye 0.19 mm long without setae. Medioccipito-genal suture present. Occipital carina complete. LE 1.0× OOL, WF 2.3× WOT. Anterior angle of ocellar triangle obtuse, POL 1.3× AOL, OOL 1.3× WOT (Fig. 1B).

Mesosoma (Fig. 1A, C–E). Dorsal pronotal area coriaceous as head, $0.5 \times$ as long as wide, trapezoidal, humeral angle obtuse; punctures very shallow and sparse (Fig. 1D); posterior pronotal sulcus absent. Mesoscutum coriaceous as head, pronotum with short and thin setae; notaulus absent; parapsidal signum thin, reaching posterior margin of mesoscutum (Fig. 1D). Mesoscutellar disc coriaceous; mesoscuto-scutellar ridge slightly posterad at each side (Fig. 1D).



Figure 1. *Laelius afores* Lim, sp. nov., holotype (female) **A** habitus in lateral view **B** head in dorsal view **C** mesosoma in lateral view **D** ditto, in dorsal view **E** forewing in dorsal view. Scale bars: 0.50 mm (**A**); 0.20 mm (**B**–**E**).

Metapectal-propodeal disc 1.1× as long as wide; metapostnotal median carina and first metapostnotal lateral carinae complete, strong, completely extending transverse posterior carina of metapectal-propodeal complex; second metapostnotal lateral carina extending one-fourth of disc; paraspiracular carina distinct, presented on distal half of disc, reaching transverse posterior carina of metapectal-propodeal complex; transverse posterior carina of metapectal-propodeal complex; transverse posterior carina of metapectal-propodeal complex complete and concave in dorsal view. Propodeal declivity reticulate without median carina (Fig. 1D). Mesopleuron with mesepimeral sulcus; subalar fovea oval and closed; lower mesopleural fovea opened (Fig. 1C). Femora and tibia with long setae on outer surface, getting short to tarsomeres. Tegula without some erect setae. Subcostal vein (Sc_2v), median vein (M_2v) and anal vein (A_2v) with long setae; second radial cross vein and radial sector vein of fore wing (2r-rs&Rs₂v) 0.9 mm long, 0.8× as long as Rs+M₂v. First median cell of fore wing and second median cell of fore wing with few short hairs (Fig. 1E). Hind wing with three distal hamuli.

Metasoma (Fig. 1A). Tergum I and II largely smooth, polished without distinct long erect hairs; remaining terga with microreticulation on anterior half; terga III–VI with transverse sparse setae line on dorsal surface.

Material examined. *Holotype*. Female. Seoul National University, Sinlim, Gwanak, Seoul, South Korea. 6.iv.2020. Deok-Young Park leg. (W-LITE).

Distribution. South Korea (Seoul).

Etymology. The specific epithet afores refers to the absence of notaulus on the mesoscutum and median carina on the propodeum declivity.

Remarks. The species is similar to *L. jilinensis* Lim & Lee, 2010 from China by 'overall color of body and appendages; head as long as wide with broadly outcurved vertex in dorsal view; mandible with four teeth; clypeus short with one median small blunt tooth on anterior margin; compound eye without setae; metapectal-propodeal disc as long as wide with metapostnotal median carina and first- and second metapostnotal lateral carina'. However, *L. afores* Lim, sp. nov. is distinguished from *L. jilinensis* by 'WF 1.9× LE (WF 1.4× LE in *L. jilinensis*); LE 1.0× OOL (LE 1.2× LE *in L. jilinensis*); dorsal pronotal area 0.5× as long wide (0.8× as long as in *L. jilinensis*); mesoscutum without notaulus (notaulus absent in *L. jilinensis*); propodeal declivity without median longitudinal carina (median longitudinal carina present in *L. jilinensis*)'.

Laelius atratus Lim, sp. nov.

https://zoobank.org/C90ABCF0-CAD1-4B48-98F4-0029C44130E3 Fig. 2A-E

Description. Holotype (female). Body length 3.05 mm; LFW 1.67 mm.

Color. Head black; mandible light castaneous; antenna dark castaneous except apical half of scape, pedicel and basal flagellomere I light castaneous in dorsal view. Mesosoma black; legs light castaneous except coxa and femora dark black; wings hyaline, tegula light castaneous, veins pale castaneous. Metasoma black.

Morphology. *Head* (Fig. 2A, B). 1.1× as long as wide with slightly outcurved vertex crest in dorsal view (Fig. 2B). Ventral and lateral surface with erect or suberect setae. Mandible with four apical teeth; two uppermost teeth small, 3rd tooth from top most thick and one ventralmost sharpened. Clypeus short, anterior margin straight with one median very minute triangle tooth; median area longitudinally elevated (Fig. 2B). First five antennomeres in ratio of 3.1: 1.3: 1.0: 1.1: 1.1 in length; scape, pedicel and flagellomere III–V and XI 2.5, 1.2, 1.4, 1.2, 1.0 and 1.5× as long as wide, respectively. Frons coriaceous with sparse punctures, polished; frontal line absent. Vertex slightly outcurved and round marginally. WF 1.4× LE. WF 0.7× WH. Compound eye 0.29 mm long without setae in lateral view. Medioccipito-genal suture present. Occipital carina complete. LE 1.4× OOL, WF 2.2× WOT. Anterior angle of ocellar triangle slightly obtuse, POL 1.2× AOL, OOL 1.1× WOT (Fig. 2B).



Figure 2. *Laelius atratus* sp. nov., holotype (female) **A** habitus in lateral view **B** head in dorsal view **C** mesosoma in lateral view **D** ditto, in dorsal view **E** forewing in dorsal view. Scale bars: 0.50 mm (**A**); 0.20 mm (**B**–**E**).

Mesosoma (Fig. 2A, C–E). Dorsal pronotal area coriaceous as head, 0.6× as long as wide, trapezoidal, humeral angle obtuse; punctures shallow and sparse as head (Fig. 2D); posterior pronotal sulcus absent. Mesoscutum coriaceous as head, pronotum with short and thin setae; notaulus distinct, short on distal half of mesoscutum; parapsidal signum deep and reaching posterior margin of mesoscutum (Fig. 2D). Mesoscutellar disc coriaceous; mesoscuto-scutellar ridge posterad at each side (Fig. 2D). Metapectal-propodeal disc 0.9× as long as wide;

metapostnotal median carina and second metapostnotal lateral carinae strong, completely extending transverse posterior carina of metapectal-propodeal complex; first metapostnotal lateral carinae weak, completely extending transverse posterior carina of metapectal-propodeal complex; third metapostnotal lateral carinae extending one-third of disc; paraspiracular carinae distinct, completely reaching transverse posterior carina of metapectal-propodeal complex; transverse posterior carina of metapectal-propodeal complex; transverse posterior carina of metapectal-propodeal complex complete and concave in dorsal view (Fig. 2D). Propodeal declivity reticulate with median carina (Fig. 2D). Mesopleuron with mesepimeral sulcus; subalar fovea elongated and closed; lower mesopleural fovea opened (Fig. 2C). Femora and tibia with long setae on outer surface, getting short to tarsomeres. Tegula with some erect setae. Subcostal vein (Sc_2v), median vein (M_2v) and anal vein (A_2v) with long setae; second radial cross vein and radial sector vein of fore wing (2r-rs&Rs₂v) 0.1 mm long, 0.8× as long as Rs+M₂v. First median cell of fore wing and second median cell of fore wing with few short hairs (Fig. 2E). Hind wing with three distal hamuli.

Metasoma (Fig. 2A). Tergum I and II largely smooth, polished without distinct long erect hairs; remaining terga with microreticulation on anterior half; terga III–VI with transverse sparse setae line on dorsal surface.

Material examined. *Holotype*: Female. Aguala Hotel Dorm., 1388. Hyeopjae. Hallim, JJ, South Korea. 33°23'58.8"N, 126°14'57.0"E, Malaise trap, 17.vii–20. viii.2017, Sanghyeok Nam leg. (W-LITE); *paratype*: Female. same collection data as holotype (W-LITE).

Distribution. South Korea (JJ).

Etymology. The specific epithet atratus refers to the submedian carinae parallel to the median discal carinae.

Remarks. The species is similar to *L. nigrofemoratus* Terayama, 2006 from Japan by 'color of head, mesosoma and metasoma; head as long as wide with convex posterior margin in dorsal view; metapectal-propodeal complex with one metapostnotal median carina and three pairs of metapostnotal lateral carinae'. However, *L. atratus* Lim, sp. nov. is distinguished from *L. nigrofemoratus* Terayama by 'mandible with four teeth (mandible with five teeth in *L. nigrofemoratus*); legs reddish brown except coxa and femora black (legs reddish brown including coxa in *L. nigrofemoratus*); first metapostnotal lateral carinae parallel to metapostnotal median carina (first metapostnotal lateral carinae extending to metapostnotal median carina near transverse posterior carina of metapectal-propodeal complex in *L. nigrofemoratus*)'.

Laelius sulcatus Lim, sp. nov.

https://zoobank.org/DC81C584-C302-468B-BA97-5315024018C8 Fig. 3A-E

Description. Holotype (female). Body length 4.35 mm; LFW 2.46 mm.

Color. Head black; mandible castaneous; antenna dark castaneous except scape, pedicel, flagellomere I castaneous in dorsal view. Mesosoma black; legs castaneous except coxa dark castaneous; wings hyaline, tegula light castaneous, veins pale castaneous. Metasoma black.

Morphology. *Head* (Fig. 3A, B). 1.1× as long as wide with broadly outcurved vertex crest in dorsal view in dorsal view (Fig. 3B). Ventral and lateral surface with

suberect long setae, each seta shorter than LE. Mandible with five apical teeth; three uppermost teeth small, 4th tooth from top most thick and one ventralmost long and sharpened. Clypeus short, broadly rounded with one median small blunt tooth, median area longitudinally elevated (Fig. 3B). First five antennomeres in ratio of 3.0: 1.4: 1.2: 1.0: 1.0 in length; scape, pedicel and flagellomere III–V and XI 2.5, 1.7, 1.3, 1.0, 1.0 and 1.8× as long as wide, respectively. Frons coriaceous with sparse big punctures; frontal line absent. Vertex slightly outcurved and round marginally. WF 1.3× LE, WF 0.7× WH. Compound eye 0.40 mm long without setae. Medioccipito-genal suture present. Occipital carina complete. LE 1.7× OOL, WF 2.1× WOT. Anterior angle of ocellar triangle obtuse, POL 1.4× AOL, OOL 0.9× WOT (Fig. 3B).

Mesosoma (Fig. 3A, 3C-E). Dorsal pronotal area coriaceous as head, 0.5× as long as wide, trapezoidal, humeral angle obtuse; punctures shallower and smaller than punctures on head (Fig. 3D); posterior pronotal sulcus absent. Mesoscutum coriaceous as head, pronotum with short and thin setae; notaulus long, distinct; parapsidal signum thick, present distal half, reaching posterior margin of mesoscutum (Fig. 3D). Mesoscutellar disc coriaceous; mesoscuto-scutellar ridge wide and deep, posterad at each side (Fig. 3D). Metapectal-propodeal disc 0.7× as long as wide; metapostnotal median carina and second metapostnotal lateral carinae distinct, strong, extending transverse posterior carina of metapectal-propodeal complex; second metapostnotal lateral carinae extending distal one fifth of disc; paraspiracular carinae distinct, reaching transverse posterior carina of metapectal-propodeal complex; transverse posterior carina of metapectal-propodeal complex complete and concave in dorsal view (Fig. 3D). Propodeal declivity reticulate with distinct median carina (Fig. 3D). Mesopleuron with mesepimeral sulcus; subalar fovea oval and closed; lower mesopleural fovea opened (Fig. 3C). Femora and tibia with long setae on outer surface, getting short to tarsomeres. Tegula with some erect setae. Subcostal vein (Sc_2v) , median vein (M_2v) and anal vein (A_2v) with long setae; second radial cross vein and radial sector vein of fore wing (2r-rs&Rs₂v) 0.3 mm long, 1.0× as long as Rs+M₂v. First median cell of fore wing and second median cell of fore wing with few short hairs (Fig. 3E). Hind wing with three distal hamuli.

Metasoma (Fig. 3A). Tergum I and II largely smooth, polished without distinct long erect hairs; remaining terga with microreticulation on anterior half; terga III–VI with transverse sparse setae on dorsal surface.

Material examined. *Holotype*: Female. 854 Hangye-ri, Buk-myeon, Inje-gun, GW, South Korea. 38°08'46.5"N, 128°15'47.5"E, Malaise trap, 11–29.vi.2017, Sanghyeok Nam leg. (W-LITE); *paratypes*: 2 Females, Forahn House, 703 Ong-po-ri, Hanlim-eub, JJ, South Korea. 33°12'51.1"N, 126°15'04.0"E, Malaise trap, 16.v.2018, Sanghyeok Nam leg. (W-LITE); Female, Aguala Hotel Dorm., 1388. Hyeopjae. Hallim, Jeju, South Korea. 33°23'58.8"N, 126°14'57.0"E, Malaise trap, 17.vii–20.viii.2017, Sanghyeok Nam leg. (W-LITE).

Distribution. South Korea (GW, JJ).

Etymology. The specific epithet sulcatus refers to the distinctly developed notaulus on the mesoscutum.

Remarks. The species is similar to *L. yamatonis* Terayama, 2006 from Korea and Japan by 'head slight longer than wide with convex posterior margin in dorsal view; mandible with five teeth; clypeus broadly rounded; pedicel about 1.7–1.8 times as long as wide; dorsal pronotal area 0.5× as long as wide; metapectal-propodeal complex with one metapostnotal median carina and two



Figure 3. *Laelius sulcatus* sp. nov., holotype (female) **A** habitus in lateral view **B** head in dorsal view **C** mesosoma in lateral view **D** ditto, in dorsal view **E** forewing in dorsal view. Scale bars: 0.50 mm (**A**); 0.20 mm (**B**–**E**).

pairs of metapostnotal lateral carinae '. However, *L. sulcatus* Lim, sp. nov. is distinguished from *L. yamatonis* Terayama by ' LE 1.7× OOL (LE 1.3× OOL in L. *yamatonis*; mesoscutum with distinct notalulus (mesoscutum without notaulus in *L. yamatonis*); second metapostnotal lateral carinae reaching basal three fourth of metapostnotal-propodeal disc (second metapostnotal lateral carinae reaching basal one-fourth of metapostnotal-propodeal disc in *L. yamatonis*).

Laelius tricuspis Lim, sp. nov.

https://zoobank.org/7D6FE7CC-CB2A-47A3-8D3E-E6723B42DA01 Fig. 4A-E

Description. Holotype (female). Body length 2.57 mm; LFW 1.61 mm.

Color. Head black; basal half of mandible dark castaneous and apical half-light castaneous; antenna castaneous except basal two thirds dark castaneous in dorsal view. Mesosoma black; legs castaneous except coxa and femora dark castaneous; wings hyaline, tegula light castaneous, veins pale castaneous. Metasoma black.

Morphology. *Head* (Fig. 4A, B). 1.0× as long as wide with slightly convex posterior margin in dorsal view (Fig. 4B). Ventral and lateral surface with suberect long setae, some setae as long as LE. Mandible with four apical teeth; two uppermost teeth small, 3rd tooth from top most thick and one ventralmost sharpened. Clypeus short, anterior margin straight with one median small blunt tooth, median area weakly elevated (Fig. 4B). First five antennomeres in ratio of 3.5: 1.6: 1.1: 1.1: 1.0 in length; scape, pedicel and flagellomere III–V and XI 3.3, 2.0, 1.2, 1.0, 1.0 and 1.5× as long as wide, respectively. Frons coriaceous with sparse punctures; frontal line absent. Vertex slightly outcurved and round marginally. WF 1.6× LE, WF 0.7× WH. Compound eye 0.24 mm long without setae. Medioccipito-genal suture present. Occipital carina complete. LE 1.3× OOL, WF 2.4× WOT. Anterior angle of ocellar triangle obtuse, POL 1.4× AOL, OOL 1.1× WOT (Fig. 4B).

Mesosoma (Fig. 4A, C–E). Dorsal pronotal area coriaceous as head, 0.5× as long as wide, trapezoidal, humeral angle obtuse; punctures very shallow and sparse (Fig. 4D); posterior pronotal sulcus present. Mesoscutum coriaceous as head, pronotum with short and thin setae; notaulus weak, present on distal half of mesoscutum; parapsidal signum short, present on distal one third of mesoscutum (Fig. 4D). Mesoscutellar disc coriaceous; mesoscuto-scutellar ridge slightly posterad at each side (Fig. 4D). Metapectal-propodeal complex 1.0× as long as wide; metapostnotal median carina and first metapostnotal lateral carinae distinct, completely extending transverse posterior carina of metapectal-propodeal complex; median area rugulose; submedian area strigate; paraspiracular carinae distinct, reaching transverse posterior carina of metapectal-propodeal complex; transverse posterior carina of metapectal-propodeal complex complete and concave in dorsal view (Fig. 4D). Propodeal declivity reticulate with median carina (Fig. 4D). Mesopleuron with mesepimeral sulcus; subalar fovea oval and closed; lower mesopleural fovea opened (Fig. 4C). Femora and tibia with long setae on outer surface, getting short to tarsomeres. Tegula without some erect setae. Subcostal vein (Sc₂v), median vein (M₂v) and anal vein (A₂v) with long setae; second radial cross vein and radial sector vein of fore wing (2r-rs&Rs,v) 0.2 mm long, 1.0× as long as Rs+M,v. First median cell of fore wing and second median cell of fore wing with few short hairs (Fig. 4E). Hind wing with three distal hamuli.

Metasoma (Fig. 4A). Tergum I and II largely smooth, polished without distinct long erect hairs; remaining terga with microreticulation on anterior half; terga III–VI with transverse sparse setae line on dorsal surface.

Material examined. *Holotype*. Female. Is. Geumho, Sani-myeon, Haenam-gun, JN, South Korea. 34°41'19"N, 126°21'19"E, Malaise trap, 1–27.vi.2023, Jongok Lim leg. (W-LITE).

Distribution. South Korea (JN).



Figure 4. *Laelius tricuspis* sp. nov., holotype (female) **A** habitus in lateral view **B** head in dorsal view **C** mesosoma in lateral view **D** ditto, in dorsal view **E** forewing in dorsal view. Scale bars: 0.50 mm (**A**); 0.20 mm (**B**–**E**).

Etymology. The specific epithet tricuspis refers to the long and distinct three metapectal-propodeal carinae (one metapostnotal median carina and one pair of metapostnotal lateral carinae).

Remarks. The species is similar to *L. jilinensis* Lim & Lee, 2010 from China by 'overall color of body and appendages; head as long as wide with convex posterior margin in dorsal view; mandible with four teeth; clypeus short with one median

small tooth medially on straight anterior margin; compound eye without setae'. However, *L. tricuspis* Lim, sp. nov. is distinguished from *L. jilinensis* by 'scape 3.5× as long as flagellomere III (scape 2.8× as long as flagellomere III in *L. jilinensis*); WF 2.4× WOT (WF 2.2× WOT in *L. jilinensis*); pronotal disc 0.5× as long as wide (0.8× as long as wide in *L. jilinensis*); metapectal-propodeal complex with three metapostnotal lateral carinae (five metapostnotal lateral carinae present in *L. jilinensis*).

Key to the Laelius species from the Eastern Palaearctic region

1 _	POL wider than OOLL. antropovi Gorbatovsky, 1995 (Far Eastern Russia) POL narrower than OOL2
2	Metapectal-propodeal disc with one metapostnotal median carina and one pair of first metapostnotal lateral carinae <i>Laelius tricuspis</i> sp. nov. (Korea)
_	Metapectal-propodeal disc with more than five metapostnotal carinae3
3	Metapectal-propodeal disc with five metapostnotal carinae4
-	Metapectal-propodeal disc with seven metapostnotal carinae
4	Mandible with four teeth; metapectal-propodeal disc longer than wide 5
-	Mandible with five teeth; metapectal-propodeal disc wider than long7
5	First metapostnotal lateral carinae on metapectal-propodeal disc not
	reaching transverse posterior carina of metapectal-propodeal complex Laelius sinicus Xu et al., 2003 (China)
_	First metapostnotal lateral carinae on metapectal-propodeal disc reach-
	ing transverse posterior carina of metapectal-propodeal complex6
6	WF less than 1.5× LE; mesoscutum with notaulus; propodeal declivity with
	median carina
-	WF more than 1.5× LE; mesoscutum without notaulus; propodeal declivity
	without median carina Laelius afores sp. nov. (Korea)
7	Mesoscutum without notaulus; second metapostnotal lateral carinae
	reaching basal one fourth of metapectal-propodeal disc
	Laelius yamatonis Terayama, 2006 (Japan, Korea)
-	Mesoscutum with distinct notaulus; second metapostnotal lateral carinae
	reaching basal three fourth of metapectal-propodeal disc
	Laelius sulcatus sp. nov. (Korea)
8	Head distinctly wider than long; pedicel 2.0× as long as wide
	Laelius naniwaensis Terayama, 2006 (Japan)
_	Head as long as wide; pedicel less than 1.5× as long as wide9
9	Metapectal-propodeal disc with one metapostnotal median carina and
	three pairs of metapostnotal lateral carinae10
-	Metapectal-propodeal disc with one metaposthotal median carina and
	four pairs of metapostnotal lateral carinae
10	Laellus yokonamensis Terayama, 2006 (Japan)
10	Legs readish brown including coxa; first metaposthotal lateral carinae on
	metapectal-propodeal disc connected to metapositional median carina
	near transverse posterior carina of metapectal-propodeal complex
_	Laga raddish brown except across and famore black first materiations
_	Legs requisit brown except coxa and remota black, first metapositional
	Lachus analus sp. 1104. (Roled)

Discussion

Since Ashmead established the genus *Laelius* in 1893, a total of 68 species have been described. Notably, 50% of the valid species (33 species) have been reported since 2000, reflecting recent discoveries facilitated by the exploration of diverse diagnostic characteristics (Vikberg and Koponen 2005; Barbosa and Azevedo 2009, 2011, 2014; Lim et al. 2010b; Margues Jr. et al. 2023).

The presence and relative ratio or length of the 2r-rs&Rs vein of the forewing stand out as the most useful characteristics in the taxonomy of *Laelius* (Marques Jr. et al. 2023). However, in the case of the four new species in the present study, they commonly exhibit relatively similar lengths of the 2r-rs and Rs veins.

Barbosa and Azevedo (2011) conducted cladistic analyses based on 108 female structural characters, revealing that the genus is supported by six autapomorphies: a body with thick setae present; a straight profile of the median clypeal carina; an incomplete anterior extension of the median clypeal carina; an angled anterior corner of the pronotal disc; three distal hamuli on the hindwing; and tergum II longer than the others. The four new species from the present paper, namely *L. afores* sp. nov., *L. atratus* sp. nov., *L. tricuspis* sp. nov., and *L. sulcatus* sp. nov., commonly exhibit these autapomorphies suggested by Barbosa and Azevedo (2011). Additionally, the ratios of POL, OOL, WF, LE, antennal segments, number of median discal carinae, number of mandibular teeth, and the presence of a median carina on the propodeal declivity are useful characteristics for the delimitation of new species.

In Colombo et al. (2022), a more recent study of the phylogenetic relationships of Epyrinae, a clade of six genera, including *Laelius*, was found to be monophyletic. Furthermore, a clade of four genera, namely *Anisepyris*, *Austrepyris*, *Chlorepyris*, and *Laelius*, which commonly share the characteristic of the mesoscuto-scutellar sulcus being well impressed and incurved medially, is sister to the remaining Epyrinae.

Laelius species exhibit weak sexual dimorphisms and share many common characteristics except for genitalia structures (Azevedo et al. 2018). Since most species of *Laelius* worldwide were described based on females and do not include enough species for cladistic studies on male genitalia, it is necessary to obtain many male samples for studies on the genus. Additionally, Colombo et al. (2022) analyzed nine species of *Laelius*, mostly from the Nearctic and Neotropical regions, except for one species from the Palaearctic region (Israel), for constructing Epyrinae phylogeny. Indeed, a clade including *Laelius* was supported as the sister group against the other genera of Epyrinae, necessitating further exploration of the phylogenetic relationships among the genera in the clade containing *Laelius* with more diverse species from various zoogeographic regions, including Eastern Asia.

In the present paper, four additional *Laelius* species from the Korean Peninsula, part of Far Eastern Asia, were described. Consequently, five *Laelius* species have been recorded in the nation, which represents higher species diversity compared to neighboring countries such as Japan (4), China (1), and Far Eastern Russia (1). This leads us to speculate that there are more unknown species in the Eastern Palaearctic region.

Acknowledgements

The authors would like to express their gratitude to Mr. Deok-Young Park (Seoul National University) for his assistance with sample collection, management, and for providing valuable bethylid samples.

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 supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (RS-2023-00238017, NRF2020R1I1A2069484), and the Honam National Institute of Biological Resources (HNIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (Grant number: HNIBR202101101), and the Korea Forest Service.

Author contributions

Writing - original draft: JL. Writing - review and editing: SL.

Author ORCIDs

Jongok Lim https://orcid.org/0000-0001-5700-3024 Seunghwan Lee https://orcid.org/0000-0003-3026-5328

Data availability

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

References

- Azevedo CO, Alencar IDCC, Ramos MS, Barbosa DN, Colombo WD, Vargas R, JM, Lim J (2018) Global guide of the flat wasps (Hymenoptera, Bethylidae). Zootaxa 4489(1): 1–294. https://doi.org/10.11646/zootaxa.4489.1
- Barbosa DN, Azevedo CO (2009) *Laelius* Ashmead (Hymenoptera, Bethylidae) from Madagascar, with description of two new species. Zootaxa 2170: 1–14. https://doi. org/10.11646/zootaxa.2170.1.1
- Barbosa DN, Azevedo CO (2011) Phylogeny and reclassification of *Laelius* (Hymenoptera: Bethylidae) and description of four new species. Insect Systematics & Evolution 42: 237–275. https://doi.org/10.1163/187631211x559777
- Barbosa DN, Azevedo CO (2014) Revision of the Neotropical *Laelius* (Hymenoptera: Bethylidae) with notes on some Nearctic species. Zoologia 31(3): 285–311. https://doi. org/10.1590/S1984-46702014000300012
- Brito CD, Lanes GO, Azevedo CO (2021) Anatomic glossary of mesopleural structures in Bethylidae (Hymenoptera: Chrysidoidea). Papéis Avulsos de Zoologia 61: e20216152. https://doi.org/10.11606/1807-0205/2021.61.52

- Colombo WD, Perkovsky EE, Waichert C, Azevedo CO (2021) Synopsis of the fossil wasps Epyrinae (Hymenoptera, Bethylidae), with description of three new genera and 10 new species. Journal of Systematic Palaeontology. https://doi.org/10.1080/1477 2019.2021.1882593
- Colombo WD, Tribull CM, Waichert C, Azevedo CO (2022) Integrative taxonomy solves taxonomic impasses: A case study from Epyrinae (Hymenoptera, Bethylidae). Systematic Entomology 2022: 1–26. https://doi.org/10.1111/syen.12544
- Eady RD (1968) Some illustrations of microsculpture in the Hymenoptera. Proceedings of the Royal Entomological Society of London. Series A, General Entomology 43(4–6): 66–72. https://doi.org/10.1111/j.1365-3032.1968.tb01029.x
- Harris RA (1979) A glossary of surface sculpturing. Occasional Papers in Entomology 28: 1–31.
- Holt BG, Lessard JP, Fritz SA, Arujo MB, Dimitrov D, Fabre PH, Graham CH, Graves GR, Jonsson KA, Nogues-Bravo D, Wang Z, Whittaker RJ, Fjeldsa J, Rahbek C (2013) An Update of Wallace's Zoogeographic Regions of the World. Science 339(6115): 74–78. https://doi.org/10.1126/science.1228282
- Lanes GO, Kawada R, Azevedo CO, Brothers DJ (2020) Revisited morphology and applied for systematics of flat wasp (Hymenoptera, Bethylidae). Zootaxa 4752(1): 1–127. https://doi.org/10.11646/ZOOTAXA.4752.1.1
- Lim J, Kwon H, Lee J, Koh S, Lee S (2010a) Three new records of Epyrinae (Hymenoptera: Bethylidae) from Korea, with a description of male of the genus *Allobethylus*. Journal of Asia-Pacific Entomology 13: 351–360. https://doi.org/10.1016/j.aspen.2010.06.012
- Lim J, Kwon H, Lee J, Lee S (2010b) A new *Laelius* species (Hymenoptera: Bethylidae) from China with a key to the species in Eastern Palaearctic region. Journal of Asia-Pacific Entomology 13: 365–368. https://doi.org/10.1016/j.aspen.2010.07.001
- Lim J, Lee J, Koh S, Lee B, Azevedo CO, Lee S (2011) Taxonomy of *Epyris* Westwood (Hymenoptera: Bethylidae) from Korea, with the descriptions of ten new species. Zootaxa 2866: 1–38. https://doi.org/10.11646/zootaxa.2866.1.1
- Marques Jr WJ, Colombo WD, Azevedo CO (2023) Insights into the systematics of Afrotropical *Laelius* (Hymenoptera, Bethylidae): Combining molecular and morphological data to associate dimorphic species. Zoologischer Anzeiger 306: 90–107. https://doi.org/10.1016/j.jcz.2023.07.003
- Mertins JW (1980) Life history and behavior of *Laelius pedatus*, a gregarious bethylid ectoparasitoid of *Anthrenus verbasci*. Annals of the Entomological Society of America 73(6): 686–693. https://doi.org/10.1093/aesa/73.6.686
- Terayama M (2006) The Insects of Japan. Vol. I. Bethylidae (Hymenoptera). The Entomological Society of Japan. Touka Shobo. Japan.
- Vance AM (1932) Laelius anthrenivorus Trani, an interesting bethylid parasite of Anthrenus verbasci L. in France. Proceedings of the Entomological Society of Washington 34(1): 1–7.
- Vikberg V, Koponen M (2005) Contribution to the taxonomy of the Palaearctic species of the genus *Laelius* Ashmead, mainly from Finland and Sweden (Hymenoptera: Chrysidoidea: Bethylidae). Entomologica Fennica 16: 23–50. https://doi. org/10.33338/ef.84235
- Xu Z, He J, Terayama M (2003) A new species of the genus *Laelius* Ashmead, 1893 (Hymenoptera: Bethylidae) from China. Entomologie 73: 197–198.



Research Article

Four complete mitochondrial genomes of the subgenus *Pterelachisus* (Diptera, Tipulidae, *Tipula*) and implications for the higher phylogeny of the family Tipulidae

Yuetian Gao¹⁶, Wanxin Cai²⁶, Yupeng Li²⁶, Yan Li²⁶, Ding Yang¹⁶

1 Department of Entomology, College of Plant Protection, China Agricultural University, Beijing 100193, China

2 Key Laboratory of Economic and Applied Entomology of Liaoning Province, College of Plant Protection, Shenyang Agricultural University, Shenyang, Liaoning 110866, China

Corresponding authors: Yan Li (liyan2014@syau.edu.cn); Ding Yang (dyangcau@126.com)

Abstract

The complete mitochondrial genomes of Tipula (Pterelachisus) cinereocincta mesacantha Alexander, 1934, T. (P.) legalis Alexander, 1933, T. (P.) varipennis Meigen, 1818, and T. (P.) yasumatsuana Alexander, 1954 are reported, three of them being sequenced for the first time. The mitochondrial genome lengths of the four species are 15,907 bp, 15,625 bp, 15,772 bp, and 15,735 bp, respectively. All genomes exhibit a high AT base composition, with A + T content of 76.7%, 75.0%, 77.8%, and 75.4%, respectively. The newly reported mitogenomes herein show a general similarity in overall structure, gene order, base composition, and nucleotide content to those of the previously studied species within the family Tipulidae. Phylogenetic analyses were conducted to investigate the relationships within Tipulidae, using both Maximum Likelihood and Bayesian Inference approaches. The results show that the four target species of the subgenus T. (Pterelachisus) basically form a monophyletic group within Tipulidae, clustering with species of the Tipula subgenera T. (Lunatipula), T. (Vestiplex), and T. (Formotipula); however, the genus Tipula is not monophyletic. Moreover, neither the tipulid subfamily Tipulinae nor the family Limoniidae is supported to be a monophyletic group. The monophyly of the family Tipulidae, and the sister relationship between Tipulidae and Cylindrotomidae are reconfirmed. These research findings could contribute to deep insights into the systematic and evolutionary patterns of crane flies.

Key words: Comparative mitogenome, crane fly, phylogenetic analysis, Tipulinae

Introduction

The subgenus *Pterelachisus* Rondani, 1842, comprising approximately 200 species in the world, is one of the most speciose subgenera of the genus *Tipula* Linnaeus, 1758 belonging to Tipulidae, Tipuloidea, Diptera (Oosterbroek 2024). *Tipula (Pterelachisus)* is widely distributed in the Northern Hemisphere, primarily including the Palaearctic, Nearctic, and Oriental regions (Oosterbroek 2024). The adults of *T. (Pterelachisus)* are mostly medium- to large-sized, often gray, yellow, or brown, with pruinescence on the body, usually three or four darker stripes present on the prescutum of mesothorax, and more or less conspicuous



Academic editor: Pavel Starkevic Received: 10 March 2024 Accepted: 13 August 2024 Published: 27 September 2024

ZooBank: https://zoobank.org/ F06A209C-2CAC-4930-97B1-C77323CC226B

Citation: Gao Y, Cai W, Li Y, Li Y, Yang D (2024) Four complete mitochondrial genomes of the subgenus *Pterelachisus* (Diptera, Tipulidae, *Tipula*) and implications for the higher phylogeny of the family Tipulidae. ZooKeys 1213: 267–288. https://doi. org/10.3897/zookeys.1213.122708

Copyright: [©] Yuetian Gao 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). grayish or brownish markings on the wing. The larvae of *T*. (*Pterelachisus*) are believed to be detritivores or herbivores, commonly inhabit forests within humus-rich soil, decaying wood, or beneath moss on dead wood or rock (Brindle 1960; Alexander 2002; Polevoi and Pilipenko 2016; Kramer and Langlois 2019; Gorban and Podeniene 2022; Cai et al. 2023).

Though some revision work on the taxonomy of *T*. (*Pterelachisus*) had been done (Savchenko 1964; Alexander 1965; Theowald 1980; Salmela 2009; Pilipenko 2009), it was still difficult to define this subgenus. Due many similarities in both adults and larvae, the boundaries between *T*. (*Pterelachisus*) and *T*. (*Vestiplex*), *T*. (*Lunatipula*), and some other related subgenera of *Tipula*, are frequently confused, which make them loosely termed the "*Vestiplex-Lunatipula*" group of subgenera (Gelhaus 1986; 2005). Up to now, only a little phylogenetic work on the relationship within the group had been done based on morphological data (Savchenko 1979; Gelhaus 2005). However, questions about the monophyly and the delimitation of *T*. (*Pterelachisus*), as well as the evolutionary relationships between *T*. (*Pterelachisus*) and other subgenera remain unsolved.

Mitochondrial genomes typically exhibit a circular structure, with a size ranging from 15 to 18 kb, comprising multiple segments, including 13 protein-coding genes, two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes. The cytochrome c oxidase I (COI) gene has been widely employed as a barcoding marker for species identification (Hebert et al. 2003; Pilipenko et al. 2012; Men et al. 2017; Sharkey et al. 2021). Due to their relatively easy accessibility, stable gene content, relatively conserved gene arrangement, maternal inheritance, and infrequent recombination, mitochondrial genomes have shown significant value in resolving insect taxonomic study and reconstructing phylogenetic relationships over the past few decades (Wilson et al. 1985; Wei and Chen 2011; Cameron 2014). With the advancement of gene sequencing technology, mitochondrial genomes have been frequently used for insect systematic and evolutionary studies, not only at higher taxonomic levels (Timmermans and Vogler 2012; Li et al. 2017; Ding et al. 2019; Zhang et al. 2019c; Lorenz et al. 2021; Lin et al. 2022a, 2022b; Song et al. 2022; Liu et al. 2023; Zhang et al. 2023), but also on inter- or infraspecific groups (Du et al. 2019; 2021). Nevertheless, there has not been much research on crane flies in this area. Beckenbach (2012) was among the pioneers in releasing partial mitochondrial genomes of Tipulidae and using them to delineate phylogenetic relationships within the crane fly infraorder Tipulomorpha. Subsequently, Zhang et al. (2016) and Kang et al. (2023) used complete mitochondrial genomes to explore phylogenetic relationships within Tipulomorpha.

Before this study, only one species of *T*. (*Pterelachisus*), *T*. (*P*.) varipennis Meigen, 1818, had a partial mitogenome obtained from the whole-genome sequencing data (SRR1469981), which was updated into the NCBI database by Leerhoei in 2020 with the accession number MT410829. In this study, another three species of *Pterelachisus*, including *T*. (*P*.) *cinereocincta mesacantha* Alexander, 1934, *T*. (*P*.) *legalis* Alexander, 1933 and *T*. (*P*.) *yasumatsuana* Alexander, 1954 were sequenced by Next Generation Sequencing (NGS) technology. All complete mitochondrial genomes of the above four species were assembled and annotated. Nucleotide composition, codon use, transfer RNA secondary structure, evolutionary patterns among PCGs (protein-coding genes), and structural elements in the control region were analyzed. Based on these data, plus some previous mitogenomic data of other species, the phylogeny of Tipuloidea was reconstructed using both Bayesian Inference (BI) and Maximum Likelihood analysis (ML).

Materials and methods

Sampling, DNA extraction, and sequencing

All the specimens of the three species sequenced in this study were collected and identified by authors and the collecting information is summarized in Suppl. material 1. After collection, each specimen was immediately preserved in 95% ethanol, and later stored at -20 °C in the laboratory. The voucher specimen of *T.* (*P.*) *cinereocincta mesacantha* was deposited in the Entomological Museum of Shenyang Agricultural University (**SYAU**), while those of *T.* (*P.*) *legalis* and *T.* (*P.*) *yasumatsuana* were deposited in the Entomological Museum of China Agricultural University (**CAU**). Genomic DNA was extracted from the thoracic tissue of each specimen using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). The DNA concentration was quantified using an Agilent 5400 instrument.

For DNA library preparation, the NEB Next® UltraTM DNA Library Prep Kit was utilized, and paired-end sequencing was conducted on an Illumina NovaSeq 6000 platform, generating raw data with an insert size of 350 bp and a read length of 150 bp. Approximately 4 Gb of raw sequenced data was obtained. Novogene Biotechnology Company (Beijing, China) conducted the aforementioned processes. The paired raw reads for the whole mitogenome of *T*. (*P*.) varipennis was downloaded from NCBI under the accession number SRR11469981.

Mitochondrial genomes assembly, annotation, and analysis

The mitochondrial genomes of all species were assembled using IDBA-UD 1.1.3 (Peng et al. 2012), and circularization of resulting linear contigs was verified using the python script in MitoZ 2.3 software (Meng et al. 2019). Circular mitochondrial genomes were then submitted to the MITOS2 web service (Bernt et al. 2013) for annotation. The secondary structure of tRNA was determined using both the MITOS2 web service (Bernt et al. 2013) and the tRNAscan-SE 2.0 (Lowe and Chan 2016) web service. Annotated mitochondrial genomes underwent a comparative analysis with closely related species in Geneious 9.0.2, and manual corrections were applied. Subsequent analyses were conducted after error exclusion.

Gene maps of the mitochondrial genomes sequenced of four *T*. (*Pterelachisus*) species were generated using the Proksee web service (Grant et al. 2023). Basal composition and amino acid usage were calculated using PhyloSuite 1.2.3 (Zhang et al. 2020), with AT skew defined as [A - T] / [A + T] and GC skew defined as [G - C] / [G + C] (Perna and Kocher 1995). Ka and Ks values, along with nucleotide diversity (Pi), were obtained using DnaSP 6.12.03 (Rozas et al. 2017). Relative synonymous codon usage (RSCU) data were also acquired through PhyloSuite (Zhang et al. 2020), and Rscript was employed for graphical representation. Repeat segments in the control region (CR) were identified using the Tandem Repeats Finder 4.09 (Benson 1999).

Accurately annotated mitochondrial genomes, along with sequencing data, were deposited in the NCBI database under the BioProject PRJNA1067446.

Phylogenetic analysis

A total of 31 complete mitochondrial genomes were used for phylogenetic analysis in this study (Table 1). *Trichocera bimacula* Walker, 1848 and *Paracladura trichoptera* (Osten Sacken, 1877), members of the family Trichoceridae, were designated as outgroups, serving as the root of the phylogenetic tree. Twenty-nine species within four families of Tipuloidea were contained in the ingroups, which respectively include one species of Pediciidae, ten species within ten genera of Limoniidae, one species of Cylindrotomidae, and 17 species

Table 1	. Taxonomic	information,	GenBank	accession	numbers,	and	references	of	mitochondrial	genomes	used	in the
present	study.											

Family	Species	GenBank number	Reference				
Outgroup							
Trichoceridae	Paracladura trichoptera (Osten Sacken, 1877)	NC016173	(Beckenbach 2012)				
Trichoceridae	Trichocera bimacula Walker, 1848	NC016169	(Beckenbach 2012)				
Ingroup		· · · · · ·					
Pediciidae	Pedicia sp.	KT970062	(Zhang et al. 2016)				
Limoniidae	Conosia irrorata (Wiedemann, 1828)	NC057072	(Zhang et al. 2019a)				
Limoniidae	Dicranomyia modesta (Meigen, 1818)	MT628560	Direct submission				
Limoniidae	Epiphragma mediale Mao & Yang, 2009	NC057085	(Zhang et al. 2021)				
Limoniidae	Euphylidorea dispar (Meigen, 1818)	MT410841	Direct submission				
Limoniidae	Limonia phragmitidis (Schrank, 1781)	NC044484	(Ren et al. 2019c)				
Limoniidae	Metalimnobia quadrinotata (Meigen, 1818)	MT584154	Direct submission				
Limoniidae	Paradelphomyia sp.	KT970061	(Zhang et al. 2016)				
Limoniidae	Pseudolimnophila brunneinota Alexander, 1933	MN398932	(Ren et al. 2019a)				
Limoniidae	Rhipidia chenwenyoungi Zhang, Li &Yang, 2012	KT970063	(Zhang et al. 2016)				
Limoniidae	Symplecta hybrida (Meigen, 1804)	NC030519	(Zhang et al. 2016)				
Cylindrotomidae	Cylindrotoma sp.	KT970060	(Zhang et al. 2016)				
Tipuloidea	Nephrotoma flavescens (Linnaeus, 1758)	MT628586	Direct submission				
Tipuloidea	Nephrotoma quadrifaria (Meigen, 1804)	MT872674	Direct submission				
Tipuloidea	Nephrotoma tenuipes (Riedel, 1910)	MN053900	(Ren et al. 2019b)				
Tipuloidea	Nigrotipula nigra (Linnaeus, 1758)	MT483653	Direct submission				
Tipuloidea	Tanyptera hebeiensis Yang &Yang, 1988	NC053795	(Zhao et al. 2021)				
Tipuloidea	Tipula (Acutipula) cockerelliana Alexander, 1925	NC030520	(Zhang et al. 2016)				
Tipuloidea	Tipula (Dendrotipula) flavolineata Meigen, 1804	MT410828	Direct submission				
Tipuloidea	Tipula (Formotipula) melanomera gracilispina Savchenko, 1960	MK864102	(Zhang et al. 2019b)				
Tipuloidea	Tipula (Lunatipula) fascipennis Meigen, 1818	NC050319	Direct submission				
Tipuloidea	Tipula (Nippotipula) abdominalis (Say, 1823)	JN861743	(Beckenbach 2012)				
Tipuloidea	Tipula (Pterelachisus) legalis Alexander, 1933	PP209204	This study				
Tipuloidea	Tipula (Pterelachisus) cinereocincta mesacantha Alexander, 1934	PP209203	This study				
Tipuloidea	Tipula (Pterelachisus) varipennis Meigen, 1818	PP209205	This study				
Tipuloidea	Tipula (Pterelachisus) yasumatsuana Alexander, 1954	PP209206	This study				
Tipuloidea	Tipula (Tipula) paludosa Meigen, 1830	MT483696	Direct submission				
Tipuloidea	Tipula (Vestiplex) aestiva Savchenko, 1960	NC063751	(Gao et al. 2023)				
Tipuloidea	Tipula (Yamatotipula) nova Walker, 1848	NC057055	(Zhao et al. 2019)				

within 12 (sub)genera of Tipulidae. All data preprocessing was carried out using PhyloSuite 1.2.3. Mitochondrial genomes served as the basis for constructing four concatenated datasets: 1) 13PCG, including all three codon positions of 13 PCGs. 2) 13PCG + rRNA, including all three codon positions of 13 PCGs and two Ribosomal RNA genes. 3) 13PCG12, including the first and second codon positions of 13 PCGs. And 4) AA, including all amino acid of 13 PCGs. Prior to concatenation, all data underwent alignment using MAFFT (Katoh and Standley 2013), followed by manual correction in MEGA7 (Kumar et al. 2016) to eliminate gap regions. Model selection for optimal models was performed using PartitionFinder 2.1.1 (Lanfear et al. 2016).

AliGROOVE 1.08 (Kück et al. 2014) was used to offer the possibility to exclude taxa or gene partitions. Phylogenetic analysis for Maximum Likelihood (ML) trees utilized RAxML 8.2.12 (Stamatakis 2014) with specific parameters set as -m GTRGAMMA -x 1234 -p 12345 -# 1000. Bayesian analysis was conducted using MrBayes 2.3 (Ronquist et al. 2012) for 2,000,000 generations with the default settings. The resulting phylogenetic tree was visualized and enhanced for presentation using Figtree 1.4.4 and Adobe Photoshop 2022.

Result and discussion

Mitogenomic organization and base composition

The complete mitochondrial genomes of all four *T*. (*Pterelachisus*) species comprise 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and one non-coding region (A + T-rich control region) (Table 2; Fig. 1). These genes exhibit a ring structure and the tandem arrangement is consistent with previously published mitochondrial whole genome gene arrangements in species of Tipulidae. The total length of the four mitochondrial genomes ranges from 15,000 to 16,000 base pairs. Specifically, *T*. (*P*.) *cinereocincta mesacantha*, *T*. (*P*.) *legalis*, *T*. (*P*.) *varipennis*, and *T*. (*P*.) *yasumatsuana* have lengths of 15,907 bp, 15,625 bp, 15,772 bp, and 15,735 bp, respectively (Table 3). All mitochondrial genomes are notably AT-rich, with A + T base contents for *T*. (*P*.) *cinereocincta mesacantha*, *T*. (*P*.) *legalis*, *T*. (*P*.) *legalis*, *T*. (*P*.) *varipennis*, and *T*. (*P*.) *vasumatsuana* at 76.7%, 75.0%, 77.8%, and 75.4%, respectively (Table 3).

The mitochondrial genomes of the four species share similar, but not identical, intergenic regions and overlaps. The longest intergenic regions, found between *trnE* and *trnF* genes, measure 32 bp, 27 bp, 32 bp, and 27 bp for *T*. (*P*.) *cinereocincta mesacantha*, *T*. (*P*.) *legalis*, *T*. (*P*.) *varipennis*, and *T*. (*P*.) *yasumatsuana*, respectively. The longest overlaps, located between *trnW* and *trnC* genes, are consistent across all species at a length of 8 bp.

Protein-coding genes

All four mitochondrial genomes harbor 13 protein-coding genes, including *COX1*, *COX2*, *COX3*, *CYTB*, *ATP6*, *ATP8*, *ND2*, *ND3*, and *ND6* on the majority strand, and *ND4*, *ND4L*, *ND5*, and *ND1* on the minority strand (Fig. 1; Table 2). All species exhibit a pronounced AT richness, with A + T base content for *T*. (*P*.) *cinereocincta mesacantha*, *T*. (*P*.) *legalis*, *T*. (*P*.) *varipennis*, and *T*. (*P*.) *yasumatsuana* at 74.6%, 72.6%, 76.0%, and 73.0%, respectively. The AT richness is especially

Table 2. Mitochondrial genome structures of T. (P.) cinereocincta mesacantha Alexander, 1934, T. (P.) legalis Alexander
1933, T. (P.) varipennis Meigen, 1818, and T. (P.) yasumatsuana Alexander, 1954.

Gene	Strand	Position	Size	Codon	Intergenic nucleotides
trnl	Н	1-67/1-66/1-67/1-67	67/66/67/67	-	_
trnQ	L	65-133/64-132/65-133/65-133	69/69/69/69	-	-3/-3/-3/-3
trnM	Н	134-202/136-204/137-205/134-202	69/69/69/69	-	0/3/3/0
nad2	Н	203-1234/205-1236/206-1237/203-1234	1032/1032/1032/1032	ATT-TAA/ATT-TAA/ATT- TAA/ATT-TAA	_
trnW	Н	1245-1313/1248-1316/1236-1304/1233-1301	69/69/69/69	-	10/11/-2/-2
trnC	L	1306-1367/1309-1370/1297-1359/1294-1355	62/62/63/62	-	-8/-8/-8/-8
trnY	L	1369-1434/1374-1439/1361-1426/1358-1423	66/66/66/66	-	1/3/1/2
cox1	Н	1433-2968/1438-2973/1425-2960/1422-2957	1536/1536/1536/1536	TCG-TAA/TCG-TAA/TCG- TAA/TCG-TAA	-2/-2/-2/-2
trnL2	Н	2969-3032/2974-3037/2961-3024/2958-3021	64/64/64/64	-	_
cox2	Н	3041-3725/3046-3730/3033-3717/3030-3714	685/685/685/685	ATG-T/ATG-T/ATG-T/ ATG-T	8/8/8/8
trnK	Н	3726-3796/3731-3801/3718-3788/3715-3785	71/71/71/71	-	_
trnD	Н	3796-3861/3801-3866/3788-3853/3785-3851	66/66/66/67	-	-1/-1/-1/-1
atp8	Н	3862-4023/3867-4028/3854-4015/3852-4013	162/162/162/162	ATT-TAA/ATT-TAA/ATT- TAA/ATT-TAA	_
atp6	Н	4017-4694/4022-4699/4009-4686/4007-4684	678/678/678/678	ATG-TAA/ATG- TAA/ATG-TAA	-7/-7/-7/-7
cox3	Н	4697-5485/4702-5490/4689-5477/4687-5475	789/789/789/789	ATG-TAA/ATG- TAA/ATG-TAA	2/2/2/2
trnG	Н	5488-5553/5493-5558/5480-5543/5478-5543	66/66/64/66	-	2/2/2/2
nad3	Н	5554-5907/5559-5912/5544-5895/5544-5897	354/354/352/354	ATT-TAA/ATT-TAG/ATT-T/ ATT-TAA	_
trnA	Н	5907-5971/5911-5974/5896-5960/5898-5961	65/64/65/64	-	-1/-2/0/0
trnR	Н	5971-6033/5974-6038/5960-6023/5961-6023	63/65/64/63	-	-1/-1/-1/-1
trnN	Н	6036-6101/6040-6105/6026-6091/6024-6089	66/66/66/66	-	2/1/2/0
trnS1	Н	6102-6168/6106-6172/6092-6158/6090-6156	67/67/67	-	_
trnE	H	6169-6233/6173-6238/6159-6224/6157-6223	65/66/66/67	-	_
trnF	L	6266-6331/6266-6331/6257-6322/6251-6316	66/66/66	-	32/27/32/27
nad5	L	6332-8063/6332-8063/6323-8054/631/-8048	1/32/1/32/1/32/1/32	AIG-1/GIG-1/GIG-1/ GTG-T	_
trnH	L	8064-8129/8064-8129/8055-8120/8049-8114	66/66/66/66	-	-
nad4	L	8130-9465/8129-9466/8121-9456/8114-9451	1336/1338/1336/1338	ATG-T/ATG-TAA/ATG-T/ ATG-TAA	0/-1/0/-1
nad4L	L	9459-9755/9460-9756/9450-9746/9445-9741	297/297/297/297	ATG-TAA/ATG-TAA/ATG- TAA/ATG-TAA	-7/-7/-7/-7
trnT trnP	H	9758-9823/9759-9823/9749-9815/9744-9808 9824-9889/9824-9887/9816-9880/9809-9873	66/65/67/65 66/64/65/65		2/2/2/2
nad6	Н	9892-10419/9890-10417/9883-10410/9876- 10403	528/528/528/528	ATT-TAA/ATT-TAA/ATC- TAA/ATT-TAA	2/2/2/2
cytb	Н	10419-11555/10417-11553/10410- 11546/10403-11539	1137/1137/1137/1137	ATG-TAG/ATG-TAG/ATG- TAG/ATG-TAG	-1/-1/-1/-1
trnS2	Н	11554-11621/11552-11619/11545- 11612/11538-11605	68/68/68/68	-	-2/-2/-2/-2
nad1	L	11638-12579/11636-12577/11629- 12570/11622-12563	942/942/942/942	ATA-TAA/ATG-TAA/ATA- TAA/ATG-TAA	16/16/16/16
trnL1	L	12584-12647/12582-12645/12575- 12638/12568-12631	64/64/64/64	_	4/4/4/4
rrnL	L	12648-13966/12646-13966/12639- 13961/12632-13954	1319/1321/1323/1323	_	_
trnV	L	13967-14038/13967-14038/13962- 14033/13955-14026	72/72/72/72	-	_
rrnS	L	14039-14821/14039-14820/14034- 14815/14027-14809	783/782/782/783	-	_
control region		14822-15907/14821-15625/14816- 15772/14810-15735	1086/805/957/926	_	_

Yuetian Gao et al.: Complete mitochondrial genomes of Pterelachisus and phylogeny



Figure 1. Gene maps of the mitochondrial genomes of the four *T*. (*Pterelachisus*) species involved in this study. The transcriptional direction is indicated by arrows.

evident in third codon positions, all exceeding 80.0%, with *T*. (*P*.) varipennis having the highest value at 92.4%. The first and second codon positions have lower AT skewness values, all below 70.0%. The most frequently encoded amino acids in these four *T*. (*Pterelachisus*) mitogenomes are *Ser2*, *Leu2*, *Val*, *Gly*, *Pro*, *Thr*, *Arg*, and *Ala*, with the highest Relative Synonymous Codon Usage (RSCU) values (Fig. 2). The most common codons are UUA, AUU, UUU, and AUA, and the majority of codons are composed solely of A or T, reflecting the high AT content of protein-coding genes (PCGs).

For most PCGs, typical ATN start codons (ATT / ATG) are observed in both mitochondrial genomes, except for TCG in *COX1* genes. Stop codons for most PCGs are T + tRNA, while *CYTB* has a stop codon TAG (Table 2). The sliding window analysis reveals variable nucleotide diversity (Pi) among the 13 PCGs in the four mitochondrial genomes, with *ND2* exhibiting the highest Pi (0.308),

Species	Regions	Length (bp)	Т%	C%	A %	G%	A+T%	AT Skew	GC Skew
T. (P.) cinereocincta	Whole genome	15907	38.1	14.2	38.6	9.2	76.7	0.006	-0.214
mesacantha	PCGs	11205	43.0	12.3	31.6	13.1	74.6	-0.152	0.035
	1 st codon position	3735	36.4	11.9	32.2	19.5	68.6	-0.062	0.245
	2 nd codon position	3735	46.2	18.8	20.3	14.6	66.5	-0.389	-0.126
	3 rd codon position	3735	46.3	6.1	42.4	5.2	88.7	-0.044	-0.073
	tRNAs	1463	37.9	10.0	38.6	13.5	76.5	0.010	0.151
	rRNAs	2102	40.8	6.9	39.4	12.9	80.2	-0.018	0.308
	Control region	1086	46.8	5.8	43.9	3.5	90.7	-0.032	-0.247
T. (P.) legalis	Whole genome	15625	36.7	15.7	38.3	9.3	75.0	0.021	-0.257
	PCGs	11208	41.8	13.6	30.8	13.7	72.6	-0.152	0.004
	1 st codon position	3736	35.8	12.7	31.4	20.2	67.2	-0.066	0.229
	2 nd codon position	3736	46.0	19.3	20.3	14.5	66.3	-0.387	-0.144
	3 rd codon position	3736	43.7	9.0	40.7	6.6	84.4	-0.036	-0.151
	tRNAs	1461	37.9	9.9	39.3	12.9	77.2	0.018	0.135
	rRNAs	2103	40.8	7.0	38.3	13.9	79.1	-0.032	0.327
	Control region	805	47	6.1	44.5	2.5	91.5	-0.027	-0.419
T. (P.) varipennis	Whole genome	15772	39.0	13.1	38.8	9.1	77.8	-0.003	-0.182
	PCGs	11202	43.6	11.5	32.4	12.4	76.0	-0.147	0.040
	1 st codon position	3734	36.6	11.8	32.6	19.0	69.2	-0.059	0.237
	2 nd codon position	3734	46.3	18.8	20.4	14.5	66.7	-0.390	-0.127
	3 rd codon position	3734	48.0	3.9	44.4	3.7	92.4	-0.039	-0.028
	tRNAs	1464	38.5	9.7	38.9	12.8	77.4	0.005	0.139
	rRNAs	2105	40.9	6.9	39.5	12.7	80.4	-0.017	0.296
	Control region	957	47.5	5.3	43.9	3.2	91.4	-0.039	-0.247
T. (P.) yasumatsuana	Whole genome	15735	37.2	15.2	38.2	9.4	75.4	0.013	-0.235
	PCGs	11208	42.5	13.3	30.5	13.8	73.0	-0.164	0.019
	1 st codon position	3736	35.7	12.7	31.5	20.2	67.2	-0.063	0.227
	2 nd codon position	3736	45.9	19.5	20.2	14.4	66.1	-0.388	-0.149
	3 rd codon position	3736	45.9	7.6	39.8	6.7	85.7	-0.071	-0.062
	tRNAs	1463	38.1	10.2	38.7	13.1	76.8	0.008	0.124
	rRNAs	2106	40.9	7.0	38.5	13.5	79.4	-0.030	0.316
	Control region	926	46.7	5.4	44.7	3.2	91.4	-0.022	-0.256

Table 3. Nucleotide composition of mitochondrial genomes of the four T. (Pterelachisus) species.

followed by *ATP8* (0.276) and *ND6* (0.257). *ND5* shows the lowest Pi (0.151) (Fig. 3A). Further examination of the Ka / Ks ratio for each PCG indicates values less than 1, suggesting purification selection. *ND2* has a notably higher Ka / Ks ratio, indicating a higher evolutionary rate, while *COX1* undergoes the highest purification selection. The Ka / Ks ratio of *ND6* varies significantly among the four species, with *T*. (*P*.) *varipennis* having significantly higher values than the other species (Fig. 3B, Suppl. material 2).

Transfer RNA genes

All mitochondrial genomes encompass 22 tRNA genes, each capable of forming cloverleaf structures, with the exception of trnS1 (AGC), which has a dihydrouridine (DHU) arm forming a loop (Fig. 4). The length of the 22 tRNA genes ranges from 62 to 72 bp across the four mitochondrial genomes. The shortest trnC(GCA) genes are found in all species, with a length of 62 bp, except for trnC in *T*. (*P*.) varipennis, which is 63 bp. The longest tRNA genes are trnV (CAC) genes.





Thr Leu1 Glu

Met Arg Tyr

Gln His Asn Pro



Ala Ile

Asp Lys

Ser1 Ser2 Leu2 Cys

Figure 2. Relative synonymous codon usage (RSCU) in the mitogenomes of the four *T*. (*Pterelachisus*) species. Codes as follows: A: Ala; C: Cys; D: Asp; E: Glu; F: Phe; G: Gly; H: His; I: Ile; K: Lys; L: Leu; M: Met; N: Asn; P: Pro; Q: Gln; R: Arg; S: Ser; T: Thr; V: Val; W: Try; Y: Tyr.

Phe

Gly

Val

Trp



Figure 3. A The nucleotide diversity (Pi) of 13 protein-coding genes (PCGs) in four *T*. (*Pterelachisus*) species mitogenomes determined via sliding window analysis (sliding window: 100 bp; step size: 25 bp); the Pi value of each gene is shown under the gene name **B** evolutionary rates (ratios of Ka/Ks) of mitochondrial protein-coding genes of the four *T*. (*Pterelachisus*) species.

The tRNA genes in all four species exhibit significant AT richness, with A + T base content for *T*. (*P*.) *cinereocincta mesacantha*, *T*. (*P*.) *legalis*, *T*. (*P*.) *varipennis*, and *T*. (*P*.) *yasumatsuana* at 76.5%, 77.2%, 77.4%, and 76.8%, respectively (Table 3).

Ribosomal RNA genes and non-coding regions

All four mitochondrial genomes feature two ribosomal RNA genes, *rrnL* and *rrnS*, separated by *trnV*. The *rrnL* of all four species (1,319 bp -1,323 bp) is notably longer than the *rrnS* (782 bp -783 bp). The rRNA is significantly AT-rich in all species, with A + T base content for *T*. (*P*.) *cinereocincta mesacantha*, *T*. (*P*.) *legalis*, *T*. (*P*.) *varipennis*, and *T*. (*P*.) *yasumatsuana* at 80.2%, 79.1%, 80.4%, and 79.4%, respectively (Table 3).



Figure 4. Secondary structures of tRNAs of *T*. (*P*.) *varipennis*. All tRNAs are labeled with the abbreviations of their corresponding amino acids. The variable sites are indicated with the green coloration for *T*. (*P*.) *cinereocincta mesacantha*, blue coloration for *T*. (*P*.) *legalis* and pink coloration for *T*. (*P*.) *yasumatsuana*, respectively. A blank represents a missing base site.

The control region for all four species is situated between *rrnS* and *trnl* genes, with lengths ranging from 800 bp to 1,100 bp. *T*. (*P*.) *cinereocincta mesacantha* has the longest control region at 1,806 bp, while *T*. (*P*.) *legalis* has the shortest at 805 bp. The control regions of all four species exhibit significant AT richness, with *T*. (*P*.) *cinereocincta mesacantha*, *T*. (*P*.) *legalis*, *T*. (*P*.) *varipennis*, and *T*. (*P*.) *yasumatsuana* having A + T base content of 90.7%, 91.5%, 91.4%, and 91.4%, respectively (Table 3).

The control regions for all four species were analyzed using the Tandem Repeat Finder, revealing two or three tandem repeats of varying lengths (Fig. 5). *T*. (*P*.) *cinereocincta mesacantha* and *T*. (*P*.) *varipennis* have two sets of tandem repeats, while *T*. (*P*.) *legalis* and *T*. (*P*.) *yasumatsuana* have three sets. Tandem repeats between different species exhibit no obvious common features, displaying unique structural and evolutionary characteristics.

Phylogenetic analyses

Both Bayesian inference (BI) and Maximum Likelihood (ML) trees were reconstructed using four concatenated datasets (13PCG, 13PCG12, 13PCG + rRNA, and AA) of 31 mitochondrial genomes (Fig. 6, Suppl. material 3: figs S1–S6). The topologies of these trees show notable differences. Heterogeneity in pairwise sequence differences was examined and the results have shown the AA dataset with significantly lower heterogeneity compared to the other datasets (Fig. 7), which may be a major factor influencing the phylogenetic results.



Figure 5. Control region structure of the four *T*. (*Pterelachisus*) species. Orange, pink, and red coloration represent tandem repeats.

The four *T*. (*Pterelachisus*) species involved in this study are divided into two stable lineages in each phylogenetic tree above: *T*. (*P*.) *cinereocincta mesacantha* and *T*. (*P*.) *varipennis* form a sister group, while *T*. (*P*.) *legalis* and *T*. (*P*.) *yasumatsuana* form another one. Furthermore, almost all the trees, except those based on the AA dataset (Fig. 7, Suppl. material 3: figs S1, S2, S4, S5), have shown that the four *T*. (*Pterelachisus*) species compose a monophyletic lineage, but with variable support values among different trees, whereas both the ML and BI trees based on the AA dataset suggest *T*. (*Pterelachisus*) is a paraphyletic group (Suppl. material 3: figs S3, S6). Since the samples for the large subgenus *T*. (*Pterelachisus*) needs further study with more data.

The above "Vestiplex-Lunatipula" group of the Tipula subgenera are tentatively supported to be a monophyletic lineage by the phylogenetic results based on the 13PCG dataset (Fig. 6), but then the subgenus *T*. (Formotipula), unexpectedly, should be included in this group, in addition to *T*. (Vestiplex), *T*. (Lunatipula) and *T*. (Pterelachisus). These arguments largely agree with the phylogenetic results based on the 13PCG + rRNA dataset (Suppl. material 3: fig. S5) in the present study, as well as the previous research results of Gao et al. (2023), only with different topologies. On the contrary, the phylogenetic trees based on the AA (Suppl. material 3: figs S3, S6) and 13PCG12 (Suppl. material 3: figs S1, S4) datasets show that the "Vestiplex-Lunatipula" group is paraphyletic, and *T*. (Vestiplex) is a sister-group to the remaining Tipulidae. Morphologically, *T*. (Pterelachisus) and other related subgenera such as *T*. (Vestiplex), *T*. (Lunatipula), and other subgenera of *Tipula* share many similarities, making it



Figure 6. Phylogenetic trees of the selected species of Tipuloidea inferred from the datasets PCG under **A** ML and **B** BI methods. Numbers at the nodes are bootstrap values (ML tree) or posterior probabilities (BI tree). The two species of family Trichoceridae were set as the outgroups.

difficult to distinguish between them (Savchenko 1964; Gelhaus 1986; 2005). This supports their potential monophyly to some extent. However, it is challenging to explain the close phylogenetic relationship of *T*. (*Formotipula*) with





these subgenera. Obviously, in-depth research would be required to resolve the questions on its monophyly and relationships within the group. In addition, the genus *Tipula* is not supported to be a monophyletic lineage by any of the phylogenetic trees.

The monophyly of Tipulidae and the sister relationship between Tipulidae and Cylindrotomidae are strongly supported in all BI and ML trees constructed in this study, which are consistent with the previous phylogenetic studies of Ribeiro (2008), Petersen et al. (2010), Zhang et al. (2016), and Kang et al. (2017; 2023). Tipulidae was divided into three subfamilies, i.e., Ctenophorinae, Dolichopezinae, and Tipulinae (Kertesz 1902; Oosterbroek 2024). In the present phylogenetic study, Dolichopezinae is not included due to a lack of mitogenomic data on the subfam-

ily. Meanwhile, as the only representative of Ctenophorinae, *Tanyptera hebeiensis* Yang & Yang, 1998 is sister to some members of Tipulinae (i.e., *Tipula (Dendrotipula) flavolineata* Meigen, 1804, *Tipula (Vestiplex) aestiva* Savchenko, 1960, or *Nephrotoma* spp. in different topologies) and then clustered with some other members of Tipulinae, which indicates a para- or polyphyly of the subfamily Tipulinae.

Corroborating previous phylogenetic studies (Ribeiro 2008; Petersen et al. 2010; Zhang et al. 2016; Kang et al. 2023), Limoniidae is confirmed as a non-monophyletic group in this study. Among the four traditional subfamilies of Limoniidae established by Starý (1992), three are involved in the present phylogenetic study, i.e., Chioneinae, Limnophilinae, and Limoniinae. With relatively low supporting values, Symplecta hybrida (Meigen, 1804), the only representative of Chioneinae in this study, is sister to a clade of Cylindrotomidae + Tipulidae in almost all the trees except the BI one inferred from the dataset AA (Suppl. material 3: fig. S6). The traditional subfamily Limnophilinae is not supported as a monophyletic group, because one of its members, Epiphragma mediale Mao & Yang, 2019, has a relatively stable sister relationship with the clade of Limoniidae, instead of with other species of Limnophilinae, which is shown in all the BI trees and most ML trees except the one inferred from the dataset AA (Suppl. material 3: fig. S5). Similar results were also indicated in the previous studies by Kang et al. (2023) and Xu et al. (2023). Furthermore, the family Pediciidae is well supported to be sister to the remaining Tipuloidea, as all the phylogenetic analyses available on the Tipuloidea since Starý (1992).

Conclusions

In the present study, the complete mitochondrial genomes of four T. (Pterelachisus) species were newly assembled, annotated, and characterized. Tipula (P.) varipennis was first produced as a complete circle molecular structure based on previously published raw data (MT410829, 13,483bp), while another three were sequenced and reported upon for the first time. These four mitochondrial genomes show similarities in gene order, nucleotide composition, and codon usage with those of other known crane fly species. The phylogenetic results have reconfirmed the monophyly of the family Tipulidae, the sister relationship between Tipulidae and Cylindrotomidae, and the phylogenetic status of Pediciidae as sister group to the remaining Tipuloidea. On the other hand, the monophyly of the tipulid subfamily Tipulinae or the genus Tipula, as well as that of Limoniidae, have not been supported, while the limoniid subfamily Limnophilinae has been suggested as a polyphyletic group. The subgenus T. (Pterelachisus) might be a monophyletic lineage according to current mitogenome data, whereas it is not stable enough. Moreover, it has shown closer phylogenetic relationships between T. (Pterelachisus) and the subgenera T. (Formotipula), T. (Lunatipula), and T. (Vestiplex). The phylogenetic status of T. (Pterelachisus) in Tipulidae is under analysis using different mitogenomic datasets: both the ML and BI trees inferred from the AA dataset have shown more divergent topologies from other trees, probably due to the relatively lower heterogeneity of the dataset.

It is evident that the tiny number of samples is insufficient for a thorough phylogenetic analysis of the vast crane fly group. It is noteworthy to remember that, particularly in cases where the sample number is limited and replicates are few, mitochondrial genotyping may not be entirely successful in resolving deep phylogenetic relationships. This could lead to low support for particular evolutionary branches, which would impair the precision of the findings. However, this study provides new insights into the phylogenetic relationships within Tipulidae, particularly on *T.* (*Pterelachisus*). To better understand the phylogeny of crane flies, more samples covering a broader range of taxa will be necessary in the future study.

Acknowledgments

We would like to thank Dr. Xiao Zhang (Qingdao Agricultural University, Qingdao, China), Dr. Jinlong Ren (Xinjiang Agricultural University, Urumqi, China), Mr. Ruiyu Zhang, and Ms. Aidi Yang (Shenyang, China) for their assistance during collecting the specimens used in this study. We are also very grateful to Dr. Scott Williams and Dr. Yan Yan (Boston) for checking the manuscript and providing linguistic improvements.

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 supported by the National Natural Science Foundation of China (31970444; 31501880) and the Scientific Research Foundation for the Introduced Talent of Shenyang Agricultural University (880415013).

Author contributions

Yan Li and Ding Yang planned and designed the research. Yuetian Gao and Wanxin Cai performed experiments, and Yuetian Gao and Yupeng Li analyzed the data. Yuetian Gao wrote and other authors revised the manuscript. All authors have approved the manuscript for publication and agreed to be accountable for all aspects of the work.

Author ORCIDs

Yuetian Gao [©] https://orcid.org/0000-0003-2966-1745 Wanxin Cai [©] https://orcid.org/0000-0001-6604-4899 Yupeng Li [©] https://orcid.org/0009-0008-5381-2260 Yan Li [©] https://orcid.org/0000-0002-4896-5843 Ding Yang [©] https://orcid.org/0000-0002-7685-3478

Data availability

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

References

Alexander CP (1965) New subgenera and species of crane-flies from California (Diptera: Tipulidae). Pacific Insects 7: 333–386.

- Alexander KNA (2002) The invertebrates of living and decaying timber in Britain and Ireland: a provisional annotated checklist. English Nature Research 467: 1–142.
- Beckenbach AT (2012) Mitochondrial genome sequences of Nematocera (Lower Diptera): evidence of rearrangement following a complete genome duplication in a winter crane fly. Genome Biology and Evolution 4(2): 89–101. https://doi.org/10.1093/gbe/evr131
- Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Research 27(2): 573–580. https://doi.org/10.1093/nar/27.2.573
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69(2): 313–319. https://doi. org/10.1016/j.ympev.2012.08.023
- Brindle A (1960) The larvae and pupae of the British Tipulinae (Diptera: Tipulidae). Transactions of the Society for British Entomology 14: 63–114.
- Cai W, Gao Y, Li Y, Jiang L (2023) Morphological description of the last instar larva of *Tipula* (*Pterelachisus*) *cinereocincta mesacantha* Alexander 1934 (Diptera: Tipuloidea: Tipulidae), a newly recorded crane fly from China. Zoomorphology 142: 313–325. https://doi.org/10.1007/s00435-023-00603-w
- Cameron SL (2014) Insect mitochondrial genomics: implications for evolution and phylogeny. Annual Review of Entomology 59: 95–117. https://doi.org/10.1146/annurev-ento-011613-162007
- Ding S, Li W, Wang Y, Cameron SL, Murányi D, Yang D (2019) The phylogeny and evolutionary timescale of stoneflies (Insecta: Plecoptera) inferred from mitochondrial genomes. Molecular Phylogenetics and Evolution 135: 123–135. https://doi. org/10.1016/j.ympev.2019.03.005
- Du Z, Hasegawa H, Cooley JR, Simon C, Yoshimura J, Cai W, Sota T, Li H (2019) Mitochondrial genomics reveals shared phylogeographic patterns and demographic history among three periodical cicada species groups. Molecular Biology and Evolution 36(6): 1187–1200. https://doi.org/10.1093/molbev/msz051
- Du Z, Wu Y, Chen Z, Cao L, Ishikawa T, Kamitani S, Sota T, Song F, Tian L, Cai W, Li H (2021) Global phylogeography and invasion history of the spotted lanternfly revealed by mitochondrial phylogenomics. Evolutionary Applications 14(4): 915–930. https:// doi.org/10.1111/eva.13170
- Gao Y, Zhang B, Yang D, Li Y (2023) The mitochondrial genome of *Tipula* (*Vestiplex*) aestiva Savchenko, 1960 (Diptera: Tipulidae). Mitochondrial DNA Part B 8(2): 233–235. https://doi.org/10.1080/23802359.2023.2172975
- Gelhaus J (1986) Larvae of the crane fly genus *Tipula* in North America (Diptera: Tipulidae). The University of Kansas Science Bulletin 53: 121–182.
- Gelhaus J (2005) Systematics and biogeography of the desert crane fly subgenus *Tipula* (*Eremotipula*) Alexander (Diptera: Tipulidae). Memoirs of the American Entomological Society 46: 1–235.
- Gorban I, Podeniene V (2022) Dipteran (Bibionomorpha and Tipulomorpha) diversity in dead wood in Lithuania. Biodiversity Data Journal 10(e85034): 1–12. https://doi. org/10.3897/BDJ.10.e85034
- Grant JR, Enns E, Marinier E, Mandal A, Herman EK, Chen C, Graham M, Van Domselaar G, Stothard P (2023) Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Research 51(W1): W484–W492. https://doi.org/10.1093/ nar/gkad326
- Hebert PDN, Ratnasingham S, De Waard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the

Royal Society of London. Series B: Biological Sciences (Suppl.)270: S96–S99. https:// doi.org/10.1098/rsbl.2003.0025

- Kang Z, Zhang X, Ding S, Tang C, Wang Y, Jong HD, Cameron SL, Wang M, Yang D (2017) Transcriptomes of three species of Tipuloidea (Diptera, Tipulomorpha) and implications for phylogeny of Tipulomorpha. PLOS ONE 12(3): e0173207. https://doi. org/10.1371/journal.pone.0173207
- Kang Z, Xu Y, Wang G, Yang D, Zhang X (2023) First mitochondrial genomes of the crane fly tribe Elephantomyiini (Diptera, Tipuloidea, Limoniidae): comparative analysis and phylogenetic implications. Arthropod Systematics & Phylogeny 81: 731–746. https:// doi.org/10.3897/asp.81.e97946
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kertesz K (1902) Catalogus dipterorum huscuque descriptorum. Leipzig and Budapest 2: 1–359. https://doi.org/10.5962/bhl.title.58595
- Kramer J, Langlois D (2019) Craneflies (Diptera, Tipuloidea) of the ravin de valbois, France. Dipterists Digest 26: 73–85.
- Kück P, Meid SA, Groß C, Wägele JW, Misof B (2014) AliGROOVE visualization of heterogeneous sequence divergence within multiple sequence alignments and detection of inflated branch support. BMC Bioinformatics 15(294): 1–15. https://doi. org/10.1186/1471-2105-15-294
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772–773. https:// doi.org/10.1093/molbev/msw260
- Li H, Leavengood JM, Chapman EG, Burkhardt D, Song F, Jiang P, Liu J, Zhou X, Cai W (2017) Mitochondrial phylogenomics of Hemiptera reveals adaptive innovations driving the diversification of true bugs. Proceedings of the Royal Society B: Biological Sciences 284(1862): 20171223. https://doi.org/10.1098/rspb.2017.1223
- Lin X, Zhao Y, Yan L, Liu W, Bu W, Wang X, Zheng C (2022a) Mitogenomes provide new insights into the evolutionary history of Prodiamesinae (Diptera: Chironomidae). Zoo-logica Scripta 51(1): 119–132. https://doi.org/10.1111/zsc.12516
- Lin X, Liu Z, Yan L, Duan X, Bu W, Wang X, Zheng C (2022b) Mitogenomes provide new insights of evolutionary history of Boreheptagyiini and Diamesini (Diptera: Chironomidae: Diamesinae). Ecology and Evolution 12(5): e8957. https://doi.org/10.1002/ece3.8957
- Liu Q, Liu Y, Liu Q, Tian L, Li H, Song F, Cai W (2023) Exploring the mitogenomes of Mantodea: new insights from structural diversity and higher-level phylogenomic analyses. International Journal of Molecular Sciences 24(13): 10570. https://doi.org/10.3390/ ijms241310570
- Lorenz C, Alves JMP, Foster PG, Suesdek L, Sallum MAM (2021) Phylogeny and temporal diversification of mosquitoes (Diptera: Culicidae) with an emphasis on the Neotropical fauna. Systematic Entomology 46(4): 798–811. https://doi.org/10.1111/ syen.12489
- Lowe TM, Chan PP (2016) tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Research 44(W1): W54–W57. https://doi. org/10.1093/nar/gkw413

- Men Q, Xue G, Sun M, Sun Z (2017) Crane flies in the family Tipulidae (Diptera: Tipuloidea) from Dayaoshan National Nature Reserve, China, with description of one new species in the genus *Indotipula* and analysis of DNA barcodes. Entomotaxonomia 39(4): 251–264.
- Meng G, Li Y, Yang C, Liu S (2019) MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Research 47(11): e63. https://doi.org/10.1093/nar/gkz173
- Oosterbroek P (2024) Catalogue of the Craneflies of the World (Diptera, Tipuloidea: Pediciidae, Limoniidae, Cylindrotomidae, Tipulidae). Catalogue of the Craneflies of the World (Diptera, Tipuloidea: Pediciidae, Limoniidae, Cylindrotomidae, Tipulidae). https://ccw.naturalis.nl/ [accessed 12 January 2024]
- Peng Y, Leung HCM, Yiu SM, Chin FYL (2012) IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28(11): 1420–1428. https://doi.org/10.1093/bioinformatics/bts174
- Perna NT, Kocher TD (1995) Patterns of nueleotide composition at fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution 41(3): 353–358. https://doi.org/10.1007/BF01215182
- Petersen MJ, Bertone MA, Wiegmann BM, Courtney GW (2010) Phylogenetic synthesis of morphological and molecular data reveals new insights into the higher-level classification of Tipuloidea (Diptera). Systematic Entomology 35(3): 526–545. https://doi.org/10.1111/j.1365-3113.2010.00524.x
- Pilipenko VE (2009) A check list of craneflies (Diptera, Tipulidae) of the Central European territory of Russia. Zoosymposia 3(1): 203–220. https://doi.org/10.11646/ zoosymposia.3.1.17
- Pilipenko VE, Salmela J, Vesterinen E (2012) Description and DNA barcoding of *Tipula* (*Pterelachisus*) *recondita* sp. n. from the Palaearctic region (Diptera, Tipulidae). Zoo-Keys 192: 51–65. https://doi.org/10.3897/zookeys.192.2364
- Polevoi AV, Pilipenko VE (2016) The first records of the crane flies *Tipula apicispina* and *T. stenostyla* (Diptera: Tipulidae) from Russian Karelia with new data on their bionomics. Zoosystematica Rossica 25(2): 380–386. https://doi.org/10.31610/zsr/2016.25.2.380
- Ren J, Zhang B, Yang D (2019a) The mitochondrial genome of Chinese endemic species *Pseudolimnophila* (*Pseudolimnophila*) brunneinota (Diptera: Limoniidae). Mitochondrial DNA Part B 4(2): 3742–3743. https://doi.org/10.1080/23802359.2019.1681313
- Ren J, Zhang C, Chang W, Yang D (2019b) The mitochondrial genome of *Nephrotoma* tenuipes (Diptera Tipulidae). Mitochondrial DNA Part B 4(2): 3092–3093. https://doi. org/10.1080/23802359.2019.1667271
- Ren J, Yang Q, Gao S, Pan Z, Chang W, Yang D (2019c) The mitochondrial genome of *Limonia phragmitidis* (Diptera Limoniidae). Mitochondrial DNA Part B 4(1): 719–720. https://doi.org/10.1080/23802359.2018.1536478
- Ribeiro GC (2008) Phylogeny of the Limnophilinae (Limoniidae) and early evolution of the Tipulomorpha (Diptera). Invertebrate Systematics 22: 627–694. https://doi. org/10.1071/IS08017
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis

of large data sets. Molecular Biology and Evolution 34(12): 3299–3302. https://doi. org/10.1093/molbev/msx248

- Salmela J (2009) The subgenus *Tipula* (*Pterelachisus*) in Finland (Diptera, Tipulidae) – species and biogeographic analysis. Zoosymposia 3: 245–261. https://doi. org/10.11646/zoosymposia.3.1.21
- Savchenko EN (1964) Crane-flies (Diptera, Tipulidae), Subfam. Tipulinae, Genus *Tipula* L., 2. Fauna USSR, N.S. 89, Nasekomye Dvukrylye [Diptera], 2(4): 1–503.
- Savchenko EN (1979) Phylogenie und systematik der Tipulidae. Translated and revised by Br. Theowald and G. Theischinger. Tijdschrift voor Entomologie 122: 91–126.
- Sharkey MJ, Janzen DH, Hallwachs W, Chapman EG, Smith MA, Dapkey T, Brown A, Ratnasingham S, Naik S, Manjunath R, Perez K, Milton M, Hebert P, Shaw SR, Kittel RN, Solis MA, Metz MA, Goldstein PZ, Brown JW, Quicke DLJ, van Achterberg C, Brown BV, Burns JM (2021) Minimalist revision and description of 403 new species in 11 subfamilies of Costa Rican braconid parasitoid wasps, including host records for 219 species. ZooKeys 1013: 1–665. https://doi.org/10.3897/zookeys.1013.55600
- Song N, Xi Y, Yin X (2022) Phylogenetic relationships of Brachycera (Insecta: Diptera) inferred from mitochondrial genome sequences. Zoological Journal of the Linnean Society 196(2): 720–739. https://doi.org/10.1093/zoolinnean/zlab125
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/ bioinformatics/btu033
- Starý J (1992) Phylogeny and classification of Tipulomorpha, with special emphasis on the family Limoniidae. Acta Zoologica Cracoviensia 35: 11–36.
- Theowald B (1980) 15. Tipulidae. In: Lindner E (Ed.) Die Fliegen der palaearktischen Region, 3(5)1, Lief. 324: 437–538.
- Timmermans MJTN, Vogler AP (2012) Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). Molecular Phylogenetics and Evolution 63(2): 299–304. https://doi. org/10.1016/j.ympev.2011.12.021
- Wei S, Chen X (2011) Progress in research on the comparative mitogenomics of insects. Chinese Journal of Applied Entomology 48(6): 1573–1585.
- Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helmbychowski KM, Higuchi RG, Palumbi SR, Prager EM, Sage RD, Stoneking M (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnean Society 26(4): 375–400. https://doi.org/10.1111/j.1095-8312.1985.tb02048.x
- Xu Y, Zhang S, Chen Y, Wang G, Yang D, Zhang X (2023) Contribution to the knowledge of Dicranoptychini (Diptera, Tipuloidea, Limoniidae) in China, with the first mitochondrial genome of the tribe and its phylogenetic implications. Insects 14: 535. https://doi. org/10.3390/insects14060535
- Zhang X, Kang Z, Mao M, Li X, Cameron SL, Jong H de, Wang M, Yang D (2016) Comparative mt genomics of the Tipuloidea (Diptera: Nematocera: Tipulomorpha) and its implications for the phylogeny of the Tipulomorpha. PLOS ONE 11(6): e0158167. https://doi.org/10.1371/journal.pone.0158167
- Zhang B, Gao S, Yang D (2019a) The mitochondrial genome of *Conosia irrorata* (Diptera: Limoniidae). Mitochondrial DNA Part B 4(2): 2367–2368. https://doi.org/10.1080/2 3802359.2019.1631130
- Zhang B, Gao S, Cao Y, Chang W, Yang D (2019b) The mitochondrial genome of *Tipula* (*Formotipula*) *melanomera gracilispina* (Diptera: Tipulidae). Mitochondrial DNA Part B 4(1): 240–241. https://doi.org/10.1080/23802359.2018.1546136

- Zhang X, Kang Z, Ding S, Wang Y, Borkent C, Saigusa T, Yang D (2019c) Mitochondrial genomes provide insights into the phylogeny of Culicomorpha (Insecta: Diptera). International Journal of Molecular Sciences 20(3): 747. https://doi.org/10.3390/ijms20030747
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li W, Wang G (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhang B, Gao S, Yang D (2021) The mitochondrial genome of *Epiphragma* (*Epiphragma*) *mediale* (Diptera: Limoniidae). Mitochondrial DNA Part B 6(4): 1321–1323. https://doi.org/10.1080/23802359.2021.1907808
- Zhang X, Yang D, Kang Z (2023) New data on the mitochondrial genome of Nematocera (lower Diptera): features, structures and phylogenetic implications. Zoological Journal of the Linnean Society 197(1): 229–245. https://doi.org/10.1093/zoolinnean/zlac012
- Zhao C, Qian X, Wang S, Li Y, Zhang X (2019) The complete mitochondrial genome and phylogenetic analysis of *Tipula* (*Yamatotipula*) *nova* Walker, 1848 (Diptera, Tipulidae) from Qingdao, Shandong, China. Mitochondrial DNA Part B 4(2): 4211–4213. https://doi.org/10.1080/23802359.2019.1693305
- Zhao C, Kang Z, Xu Y, Zhang X (2021) *Tanyptera (Tanyptera) hebeiensis* Yang et Yang (Diptera: Tipulidae) newly recorded from Shandong, China: sequencing and phylogenetic analysis of the mitochondrial genome. Mitochondrial DNA Part B 6(1): 115–118. https://doi.org/10.1080/23802359.2020.1848478

Supplementary material 1

Information of the voucher specimens used for mitochondrial genomes sequencing in the present study

Authors: Yuetian Gao, Wanxin Cai, Yupeng Li, Yan Li, Ding Yang Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1213.122708.suppl1

Supplementary material 2

Synonymous and non-synonymous substitutional analysis of gene ATP6, ATP8, COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5, ND6

Authors: Yuetian Gao, Wanxin Cai, Yupeng Li, Yan Li, Ding Yang Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1213.122708.suppl2

Supplementary material 3

Phylogenetic trees

Authors: Yuetian Gao, Wanxin Cai, Yupeng Li, Yan Li, Ding Yang Data type: pdf

- Explanation note: figure S1. Phylogenetic trees of the selected species of Tipuloidea inferred from the datasets 13PCG12 under ML methods. Numbers at the nodes are bootstrap values. The two species of family Trichoceridae were set as the outgroups; figure S2. Phylogenetic trees of the selected species of Tipuloidea inferred from the datasets 13PCG+rRNA under ML methods. Numbers at the nodes are bootstrap values. The two species of family Trichoceridae were set as the outgroup; figure S3. Phylogenetic trees of the selected species of Tipuloidea inferred from the datasets AA under ML methods. Numbers at the nodes are bootstrap values. The two species of family Trichoceridae were set as the outgroups; figure S4. Phylogenetic trees of the selected species of Tipuloidea inferred from the datasets 13PCG12 under BI methods. Numbers at the nodes are posterior probabilities. The two species of family Trichoceridae were set as the outgroups; figure S5. Phylogenetic trees of the selected species of Tipuloidea inferred from the datasets 13PCG+rRNA under BI methods. Numbers at the nodes are posterior probabilities. The two species of family Trichoceridae were set as the outgroups; figure S6. Phylogenetic trees of the selected species of Tipuloidea inferred from the datasets AA under BI methods. Numbers at the nodes are posterior probabilities. The two species of family Trichoceridae were set as the outgroups.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1213.122708.suppl3


Research Article

Male-based key to the subfamilies and genera of Malagasy ants (Hymenoptera, Formicidae)

Manoa M. Ramamonjisoa¹⁰, Nicole Rasoamanana¹⁰, Brian L. Fisher²⁰

1 Madagascar Biodiversity Center, BP 6257, Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar

2 Entomology, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA

Corresponding author: Manoa M. Ramamonjisoa (ramamonjisoamanoa@gmail.com)

Abstract

The males of the family Formicidae of the Malagasy region, including the islands of the southwest Indian Ocean (Madagascar, Mauritius, Reunion, Comoros, and Seychelles) are reviewed. A male-based synopsis of each subfamily and genera are provided. A richly illustrated male-based key to the eight subfamilies and 72 genera for which males are known are provided. The key is specific to the ant genera and species of the Malagasy region. Terminologies for morphology and wing cells are also reviewed. The keys are a product of three decades of collecting across the region. Despite efforts to collect males for all genera, males from five genera (*Brachyponera, Chrysapace, Dicroaspis, Linepithema, Ochetellus*) were included in the keys based on males from species collected outside the region, and males from one genus (*Parvaponera*) are unknown globally and not included in the key.

Key words: Formicidae, identification, Malagasy region, male ants, morphology

Introduction

Most identification tools for ants are based on the worker female caste and neglect the male caste. Identifying males is important to understanding the life history, phenology, and reproductive biology of ants. In addition, some collecting methods like Malaise and light traps preferentially trap males and, without tools for their identification, limit the insights these methods can provide into ant community diversity and structure through time and space.

In the Malagasy region (Madagascar, Mauritius, Reunion, Comoros, and Seychelles), there has been a pioneering effort to develop the taxonomic tools to identify male ants to genus: Ponerinae (Yoshimura and Fisher 2007), Amblyoponinae (Yoshimura and Fisher 2012), Dolichoderinae (Yoshimura and Fisher 2011), Proceratiinae (Yoshimura and Fisher 2009), and Myrmicinae tribes (Ramamonjisoa et al. 2023). This body of work has greatly enriched our understanding of the diversity of ants in the region. Borowiec (2016) also provided an identification key for male Dorylinae from the African and Malagasy regions. Here, we update this previous work, provide additional characters and present keys to all genera, including the Myrmicinae for which males



Academic editor: Matthew Prebus Received: 8 February 2024 Accepted: 23 July 2024 Published: 27 September 2024

ZooBank: https://zoobank.org/ F121116A-A0B1-45A9-972F-E7B72DCDA82C

Citation: Ramamonjisoa MM, Rasoamanana N, Fisher BL (2024) Male-based key to the subfamilies and genera of Malagasy ants (Hymenoptera, Formicidae). ZooKeys 1213: 289–359. https://doi. org/10.3897/zookeys.1213.120531

Copyright: [©] Manoa M. Ramamonjisoa 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). are known. The newly proposed key uses a combination of morphological characters to create a navigational tool to identify the diversity of ant genera in the Malagasy region. The effectiveness of the key is enhanced by the integration of photographic illustrations, which provide a visual portal to the subtle intricacies that distinguish each genera. This study aims to increase the accessibility, accuracy, and applicability of ant genera identification in the Malagasy region.

Materials and methods

Morphological observations were carried out using Leica stereoscopic microscopes (MZ9.5). Digital color montage images were created using a JVC KY-F75 digital camera and Syncroscopy Auto-Montage software (v. 5.0), or a Leica DFC 425 camera in combination with the Leica Application Suite software (v. 3.8). These images are available online through AntWeb.org (2022) and are accessible using the unique specimen identifier code.

Terminology for general morphology follows Bolton (1994) and Boudinot (2013, 2015). The terminology for forewing venation follows Yoshimura and Fisher (2007) and for hindwing venation follows Yoshimura and Fisher (2011). When referring to the presence or absence of veins in the descriptions, a vein is considered present regardless of whether it is tubular, nebulous, or spectral (Mason 1986).

Subfamilies and genera of the Malagasy Region

The specimens used in this study are the product of a long-term effort to document the diversity of ants in the Malagasy region (Fisher 2005; Fisher and Peeters 2019). Males were collected by hand as part of colony series but also in light and Malaise traps. Despite these efforts, representative males have not been collected for all genera in the Malagasy region. Five genera (Brachyponera, Chrysapace, Dicroaspis, Linepithema, Ochetellus) have males known only from outside the Malagasy region. Males of Brachyponera (known from Mauritius), Dicroapsis (from Anjouan), Linepithema (from Reunion) and Ochetellus (from Reunion) are most likely absent because of the limited effort spent targeting these taxa on those islands. It is surprising that males have never been collected in the region for Chrysapace, a large Doryline present in northern Madagascar, despite the numerous Malaise and light traps placed throughout the range of the genus. Even more puzzling is the complete global absence of males of Parvaponera, as Parvaponera queens are regularly collected at black lights (Fig. 1). For a period of seven years, the Madagascar ant team directed efforts to collect males and locate colonies at sites where Parvaponera queens were present at lights. At one site, Nosy Faly in NW Madagascar, we located the first ground nest including workers for the genus in Madagascar. We set a series of yellow pan traps and Malaise traps during the period queens were present at black lights (Fig. 2), but no males were collected. Males of the genus remain unknown in Madagascar and globally. Parvaponera is the only genus in the Malagasy region absent from the key.

Manoa M. Ramamonjisoa et al.: Male-based key to the subfamilies and genera of Malagasy ants



Figure 1. Black light. Photographer Brian Fisher.



Figure 2. Yellow pan and Malaise trap. Photographer Brian Fisher.

Synoptic list of 73 ant genera of the Malagasy Region

For genera absent from Madagascar, the distribution is indicated in parentheses.

* Males unknown for the genus within the Malagasy region but included in keys based on males from outside the region.

+ Males unknown for genus globally and not included in key.

AMBLYOPONINAE Forel, 1893

- 1. Adetomyrma Ward, 1994
- 2. Mystrium Roger, 1862
- 3. Prionopelta Mayr, 1866
- 4. Stigmatomma Roger, 1859
- 5. Xymmer Santschi, 1914

DOLICHODERINAE Forel, 1878

- 1. Aptinoma Fisher, 2009
- 2. *Linepithema** Mayr, 1866 (Reunion)
- 3. Ochetellus* Shattuck, 1992 (Mauritius, Reunion)
- 4. Ravavy Fisher, 2009
- 5. Tapinoma Foerster, 1850
- 6. Technomyrmex Mayr, 1872

DORYLINAE Leach, 1815

- 1. Eburopone Borowiec, 2016
- 2. Chrysapace* Crawley, 1924
- 3. Lioponera Mayr, 1879
- 4. Lividopone Bolton & Fisher, 2016
- 5. Ooceraea Roger, 1862
- 6. Parasyscia Emery, 1882
- 7. Simopone Forel, 1891
- 8. Tanipone Bolton & Fisher, 2012

FORMICINAE Latreille, 1809

- 1. Anoplolepis Santschi, 1914 (Seychelles)
- 2. Brachymyrmex Mayr, 1868
- 3. Camponotus Mayr, 1861
- 4. Lepisiota Santschi, 1926
- 5. Nylanderia Emery, 1906
- 6. Paraparatrechina Donithorpe, 1947
- 7. Paratrechina Motschoulsky, 1863
- 8. Plagiolepis Mayr, 1861
- 9. Tapinolepis Emery, 1925

MYRMICINAE Lepeletier de Saint-Fargeau, 1835

- 1. Adelomyrmex Emery, 1897 (Seychelles)
- 2. Aphaenogaster Mayr, 1853
- 3. Calyptomyrmex Emery, 1887 (Comoros)

- 4. Cardiocondyla Emery, 1869
- 5. Carebara Westwood, 1840
- 6. Cataulacus Smith, 1853
- 7. Crematogaster Lund, 1831
- 8. Cyphomyrmex Mayr, 1862 (Reunion)
- 9. Dicroaspis* Emery, 1908 (Comoros)
- 10. Erromyrma Bolton & Fisher, 2016
- 11. Eurhopalothrix Brown & Kempf, 1961 (Comoros)
- 12. Eutetramorium Emery, 1899
- 13. Malagidris Bolton & Fisher, 2014
- 14. Melissotarsus Emery, 1877
- 15. Meranoplus Smith, 1853
- 16. Metapone Forel, 1911
- 17. Monomorium Mayr, 1855
- 18. Nesomyrmex Wheeler, 1910
- 19. Pheidole Westwood, 1839
- 20. Pilotrochus Brown, 1978
- 21. Pristomyrmex Mayr, 1866 (Mauritius)
- 22. Royidris Bolton & Fisher, 2014
- 23. Solenopsis Westwood, 1840
- 24. Strumigenys Smith, 1860
- 25. Syllophopsis Santschi, 1915
- 26. Terataner Emery, 1912
- 27. Tetramorium Mayr, 1855
- 28. Trichomyrmex Mayr, 1865
- 29. Vitsika Bolton & Fisher, 2014
- 30. Vollenhovia Mayr, 1865 (Seychelles)

PONERINAE Lepeletier de Saint-Fargeau, 1835

- 1. Anochetus Mayr, 1861
- 2. Bothroponera Mayr, 1862
- 3. Brachyponera* Emery, 1900 (Mauritius)
- 4. Euponera Forel, 1891
- 5. *Hypoponera* Santschi, 1938
- 6. Leptogenys Roger, 1861
- 7. Mesoponera Emery, 1900
- 8. Odontomachus Latreille, 1804
- 9. Parvaponera+ Schmidt & Shattuck, 2014
- 10. Platythyrea Roger, 1863
- 11. Ponera Latreille, 1804

PROCERATIINAE Emery, 1895

- 1. Discothyrea Roger, 1863
- 2. Probolomyrmex Mayr, 1901
- 3. Proceratium Roger, 1863

PSEUDOMYRMICINAE Smith, 1952

1. Tetraponera Smith, 1852

Male-based key to the subfamilies of the Malagasy Region



Figure 3. Portion of abdominal sternum IX **A** *Lioponera* sp. (CASENT0001042) **B** *Techno-myrmex* mg08 (CASENT0049527). Photographer Masashi Yoshimura.

- 2 Abdominal segment II nearly as large as or **longer than** segment III (postpetiole) in lateral view (Fig. 4A).....**3**
- Abdominal segment II much shorter than segment III in lateral view (Fig. 4B)......4



Figure 4. Abdominal segment II and III in lateral view **A** *Tetraponera longula* (CASENT0138661) **B** *Probolomyrmex curculiformis* (CASENT0050214). Photographers Dimby Raharinjanahary (**A**), April Nobile (**B**).

Ventral apex of meso- and metatibia, when viewed from the front with the femur at right angle to the body, with two spurs consisting of a large pectinate spur and a small simple spur (Fig. 5A)....... Pseudomyrmicinae
 Ventral apex of metatibia, when viewed from the front with the femur at

right angle to the body, with single simple spur or absent (Fig. 5B)......**Myrmicinae**



Figure 5. Metatibial spur **A** *Tetraponera* psw094 (CASENT0053316) **B** *Aphaenogaster swammerdami* (CASENT0000990). Photographers April Nobile (**A**), Masashi Yoshimura (**B**).

- Metatibia always with single ventroapical spur, cinctus always indistinct between abdominal segment III and abdominal segment IV (Fig. 6B)......7



Figure 6. Gaster in dorsal view, cinctus at abdominal segment IV level **A** *Euponera sikorae* (CASENT0065480) **B** *Technomyrmex albipes* (CASENT0055727). Photographer Michele Esposito.

- 5 Anal region of hind wing vestigial (Fig. 7A) and with the mesosoma in lateral view, oblique mesopleural furrow reaching pronotum close to pronotal posteroventral margin (Fig. 7C) Proceratiinae



Figure 7. Hindwings of male ants **A** *Discothyrea* mgm01 (CASENT0083649) **B** *Odontomachus coquereli* (CASENT0049797). Mesosoma in lateral view, showing oblique mesopleural furrow **C** *Proceratium* dr01 (CASENT0145100) **D** *Acropyga goeldii* (CASENT0903184). Photographers Erin Prado (**A**, **B**), Dimby Raharinjanahary (**C**), Ziv Lieberman (**D**).

6 Abdominal segment II broadly and dorsally attached to abdominal segment III; mandible long, falcate, curved inward and closed (Fig. 8A)

..... Amblyoponinae

Abdominal segment II narrowly and ventrally attached to abdominal segment III; mandible short, linear, subtriangular to triangular (Fig. 8B)......
 Ponerinae



Figure 8. Attachment of abdominal segment II to abdominal segment III **A** *Stigmatomma* mgm04 (CASENT0063981) **B** *Bothroponera perroti* (CASENT0135783). Photographers Erin Prado (**A**), Dimby Raharinjanahary (**B**).

- 7 With head in full-face view, scape short, not reaching posterior margin of head (Fig. 9A) Dolichoderinae



Figure 9. Head in full-face view showing the comparison of scape length **A** *Technomyrmex albipes* (CASENT0055727) **B** *Lepisiota canescens* (CASENT0906461). Photographers April Nobile (**A**), Cerise Chen (**B**).

AMBLYOPONINAE Forel, 1893

Diagnosis of male ants of the subfamily Amblyoponinae in the Malagasy region

- Antenna filiform, consisting of 13 segments.
- Scape not reaching posterior margin of head.
- Mesopleural oblique furrow usually vestigial, and when present, reaching pronotum far from pronotal posteroventral margin.

- Abdominal segment II broadly and dorsally attached to abdominal segment III.
- Abdominal segment II much smaller than segment III in lateral view.
- Protibia with one spur.
- Metatibia with one or two spurs.

Remarks. Our key includes five Amblyoponinae genera recorded from the Malagasy region. Key modified from Yoshimura and Fisher (2012).

Male-based key to genera of the subfamily Amblyoponinae

- 1 A single tibial spur present on metatibia (Fig. 10A). Mandible with apical and pre-apical teeth. Pterostigma reduced in size......*Prionopelta*



Figure 10. Tibial spur on metatibia **A** *Prionopelta subtilis* (CASENT0049809) **B** *Mystrium mirror* (CASENT0492154). Photographer Masashi Yoshimura.

- Constriction between abdominal segment II and abdominal segment III distinct in dorsal view. Cinctus between abdominal segment III and abdominal segment IV distinct and deep. On forewing, radial sector vein reaches costal margin and is connected with radius vein (Fig. 11B)3



Figure 11. Venation of forewing **A** *Adetomyrma caputleae* (CASENT0218013) **B** *Stigma-tomma* mg01 (CASENT0083104). Photographer Masashi Yoshimura.

- 3 Pygostyles present (Fig. 12A) Stigmatomma
- Pygostyles absent (Fig. 12B)......4



Figure 12. Posterior portion of abdomen in posterolateral view **A** *Stigmatomma* mgm01 (CASENT0007139) **B** *Xymmer* drm01 (CASENT0135825). Photographers April Nobile (**A**), Dimby Raharinjanahary (**B**).

- 4 Anterior margin of clypeus with tooth-like projections. Radial sector vein on forewing fully complete (Fig. 13A). Radius vein on hindwing present.... *Mystrium*



Figure 13. Venation of forewing **A** *Mystrium barrybressleri* (CASENT0078803) **B** *Xymmer* mgm04 (CASENT0113147). Photographer Masashi Yoshimura.

Adetomyrma Ward, 1994

Antenna with 13 segments. Frontal carinae absent. Anterior margin of clypeus with tooth-like projections. Mandible falcate with single apical tooth. Palpal formula 3,3/2,3/2,2. Notauli absent for some species or distinct in *Adetomyrma goblin*. Mesepimeron with or without epimeral lobe. Protibia with one spur. Mesotibia with two spurs. Metatibia with two spurs. In dorsal view, cinctus between abdominal segment III and abdominal segment IV indistinct. Pygostyles present. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1mcu present. Radial sector vein (Rs) between M+Rs and 2r-rs wholly or partially absent and fails to reach costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. The cross-vein cu-a proximal to junction between media and cubitus vein. Media (M) fused with radial sector vein to form Rs+M. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. 1rs-m absent. The median vein, proximally fused with cubital vein (M+Cu), following separation continuing as a free abscissa (M). M+Cu present. 1rs-m+M absent. Free section of cubitus present. Cross-vein cu-a present.

Mystrium Roger, 1862

Antenna with 13 segments. Frontal carinae present. Anterior margin of clypeus with tooth-like projections. Mandible falcate with single apical tooth. Palpal formula 4,3. Notauli absent for some but distinct in Mystrium rogeri, M. oberthueri, M. mysticum, and M. mirror. Mesepimeron with epimeral lobe. Protibial with one spur. Mesotibia with single or two spurs. Metatibia with two spurs. In dorsal view, cinctus between abdominal segment III and abdominal segment IV distinct and deep. Pygostyles absent. On forewing; pterostigma well developed; costal vein (C) present, cross-vein 1m-cu present. Radial sector vein (Rs) fully present. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a position variable located in line or proximal to junction between media and cubitus. Media (M) between Rs+M and 2rs-m completely present and after 2rs-m completely present. On hindwing, radius vein (R) present. Radial sector vein (Rs) present. 1rs-m present. The median vein (M), proximally fused with cubital vein (M+Cu), following separation continuing as a free abscissa (M) and joint apical to 1rs-m. M+Cu present. 1rs-m+M present. Free section of cubitus present. Cross-vein cu-a present.

Prionopelta Mayr, 1866

Antenna with 13 segments. Frontal carinae present. Anterior margin of clypeus with tooth-like projections. Mandible falcate with two sharp apical teeth. Palpal formula 2,2. Notauli present. Mesepimeron without epimeral lobe. Pro-, meso-, and metatibia with one spur. In dorsal view, cinctus between abdominal segment III and abdominal segment IV distinct and deep. Pygostyles present. On forewing, pterostigma reduced in size. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein distal to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) between Rs+M and 2rs-m completely present and after 2rs-m at least partially present. On hindwing, radius vein (R) present but absent in one species. Radial sector vein (Rs) present. 1rs-m present. The median vein (M), proximally fused with cubital vein (M+Cu), following separation continuing as a free abscissa (M) and joint apical to 1rs-m. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Stigmatomma Roger, 1859

Antenna with 13 segments. Frontal carinae absent. Anterior margin of clypeus with tooth-like projections. Mandible falcate with single apical tooth. Palpal formula 4,3/4,2/3,2. Notauli present. Mesepimeron with epimeral lobe. Protibia with one spur. Mesotibia with one or two spurs. Metatibia with two spurs. In dorsal view, cinctus between abdominal segment III and abdominal segment IV distinct and deep. Pygostyles present. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully present. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a located in line or proximal to junction between media and cubitus. Media (M) between Rs+M and 2rs-m completely present and after 2rs-m at least partially present. On hindwing, radius vein (R) present or absent. Radial sector vein (Rs) present. 1rs-m present. The median vein (M), proximally fused with cubital vein (M+Cu), following separation continuing as a free abscissa (M) and joint apical to 1rs-m. M+Cu present. 1rs-m+M present. Free section of cubitus present. Cross-vein cu-a present.

Xymmer Santschi, 1914

Antenna with 13 segments. Frontal carinae absent. Anterior margin of clypeus straight, without tooth-like projections. Mandible falcate with single apical tooth. Palpal formula 3,3 /3,2/4,3. Notauli present. Mesepimeron with epimeral lobe. Protibia with one spur. Mesotibia with one or without spur. Metatibia with two spurs. In dorsal view, cinctus between abdominal segment III and abdominal segment IV distinct and deep. Pygostyles absent. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) between Rs+M and 2rs-m completely present and after 2rs-m at least partially present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. 1rs-m absent. The median vein (M), proximally fused with cubital vein (M+Cu). Media (M) absent and not fused apical to 1rs-m. M+Cu present. 1rs-m+M absent. Free section of cubitus absent. Cross-vein cu-a present.

DOLICHODERINAE Forel, 1878

Diagnosis of male ants of the subfamily Dolichoderinae in the Malagasy region

- Antenna filiform, consisting of 12 to 13 segments.
- Scape short, not reaching the posterior margin of head.
- Mesopleural oblique furrow reaching pronotum far away from pronotal posteroventral margin.
- Notauli absent.
- Scuto-scutellar suture simple.
- Single, well-developed spur presents on pro-, meso-, and metatibia.
- Abdominal segment II much smaller than segment III in lateral view.
- Abdominal segment II narrowly or broadly attached to abdominal segment III.
- No constriction present between abdominal segments III and IV.
- Jugal lobe absent.
- Pygostyles present.
- Wing venation: Venation on forewing varies. Radius vein (R), Sc+R+Rs, Radial sector vein (Rs), cubitus (Cu), anal (A), 2r-rs, and cu-a present in all genera. Media (M) present between Rs+M and 2rs-m. 2rs-m present or continuous with media. On hindwing, R+Rs and anal present. Radius vein and media apical to rs-m absent. M+Cu, cubitus, 1rs-m, and cu-a variable. Clavus moderate in size, and jugum absent.

Remarks. Our key includes six genera of Dolichoderinae recorded from the Malagasy region. Key modified from Yoshimura and Fisher (2011). It is crucial to acknowledge that although male specimens of *Ochetellus glaber* have yet to be collected in the Malagasy region, they have been incorporated into this key based on morphological traits observed in *O. glaber* collected from Japan. Notably, the genus *Linepithema*, with its species *L. humile*, has been recently reported in the Malagasy region (Reunion: Nève de Mévergnies et al. 2024), and has been incorporated into this key based on morphological traits observed in specimens collected in California, USA.

Male-based key to genera of the subfamily Dolichoderinae

- 1 Masticatory margin of mandible with many serrate denticles (Fig. 14A) 2
- Masticatory margin of mandible with one to several large teeth (Fig. 14B)... 5



Figure 14. Mandible in full-face view **A** *Technomyrmex difficilis* (CASENT0049968) **B** *Ravavy miafina* (CASENT0474633). Photographer April Nobile.

- On hindwing, M+Cu present. In ventral view, telomere narrow, without a distinct ventral face (Fig. 15B)
 3



Figure 15. Telomere **A** *Technomyrmex* mg08 (CASENT0049527) **B** *Tapinoma* mg10 (CASENT0115650). Photographers Masashi Yoshimura (**A**), Erin Prado (**B**).



Figure 16. Head in full-face view showing the comparison of scape length **A** *Tapinoma* mg12 (CASENT0115678) **B** *Aptinoma mangabe* (CASENT0173594). Photographer April Nobile.

- 4 With head in full-face view, second funicular segment shorter than scape and first funicular segment more cylindrical (Fig. 17A) *Linepithema* (Reunion)



Figure 17. Head in full-face view, showing proportion of second funicular segment in relation to scape and form of first funicular segment **A** *Linepithema humile* (CASENT0724858) **B** *Aptinoma mangabe* (CASENT0173594) Photographers Wade Lee (**A**), April Nobile (**B**).

- Mandible triangular, with several stout teeth on distal apex (Fig. 18B). Abdominal segment II broadly attached to abdominal segment III
 Ochetellus (Mauritius, Reunion)



Figure 18. Mandible **A** *Ravavy miafina* (CASENT0179530) **B** *Ochetellus glaber* (CASENT0179489). Photographer Masashi Yoshimura.

Aptinoma Fisher, 2009

Antenna with 13 segments, scape shorter than 2+3 funicular segments, first funicular segment conical, second funicular segment straight. Medial hypostoma present. Mandible triangular, masticatory margin with serrate denticles. Palpal formula 6,3. Propodeal spiracle oval. Abdominal segment II not unusually expanded, narrowly attached to abdominal segment III. Abdominal segment III with a groove or indentation on anterior face. Pygostyles present. On forewing, pterostigma well developed; costal vein (C) and cross-vein 1m-cu present. Radial sector vein (Rs) partially absent between M+Rs and 2r-rs and reaches costal margin. Cross-vein 2r-rs connected to radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m present. On hindwing, radius vein (R) present. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu present. 1rsm+M absent. Free section of cubitus absent. Cross-vein cu-a present.

Linepithema Mayr, 1866

Antenna with 13 segments, scape shorter than second funicular segment, first funicular segment cylindrical, second funicular segment straight, last eight flagellar segments shorter. Mandible triangular, masticatory margin with serrate denticles. Basal margin of mandible smooth. Propodeal spiracle circular. Abdominal segment II squamiform and abdominal segment II narrowly attached to abdominal segment III. Pygostyles present. On forewing, pterostigma well developed; costal vein (C) and cross vein 1m-cu present. Radial sector vein (Rs) present between M+Rs and 2r-rs and reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Media before junction of radial sector vein (Rs) present. Cu-a proximal to junction between media and cubitus. On hindwing, radial sector vein (Rs) present, 1rs-m+M present, M+Cu present, free section of cubitus Cu present.

Ochetellus Shattuck, 1992

Antenna with 12 segments. Scape shorter than 2+4 funicular segments. First funicular segment barrel-shaped. Second funicular segment straight. Medial hypostoma present. Mandible triangular, basal margin of mandible without denticles and smooth, and masticatory margin with several stout teeth and minute denticles (Yoshimura and Fisher 2011). Palpal formula 6,4. Propodeal spiracle circular. Abdominal segment II expanded laterally and widened dorsally, broadly attached to abdominal segment III. Abdominal segment III without a groove. Pygostyles present. On forewing, pterostigma well developed. Costal vein (C) and 1m-cu present. Radial sector vein (Rs) between M+Rs and 2r-rs complete and reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely absent. On hindwing, radius vein (R) present. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu usually present. 1rs-m+M present. Free section of cubitus present. Cross-vein cu-a present.

Ravavy Fisher, 2009

Antenna with 13 segments (Fisher 2009). Scape shorter than 2+5 funicular segments. First funicular segment conical. Second funicular segment bent laterally. Medial hypostoma absent. Mandible broadly spatulate, edentate. Palpal formula 6,3. Propodeal spiracle circular. Abdominal segment II not unusually expanded and narrowly attached to abdominal segment III. Abdominal segment III with a groove or indentation on anterior face. Pygostyles present. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fused to M+Rs and reaches costal margin (Fisher 2009; Yoshimura and Fisher 2011). Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cu-a proximal to junction between media and cubitus. Media before junction with Rs present. On hindwing, radius vein (R) present. Radial sector vein (Rs) present. Media (M) absent. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Tapinoma Foerster, 1850

Antenna with 13 segments. Scape longer than 2+3 funicular segments but not exceeding posterior margin of head. First funicular segment conical. Second funicular segment straight. Medial hypostoma present. Mandible triangular, masticatory margin with or without serrate teeth. Palpal formula usually 6,4 but sometimes 6,3. Propodeal spiracle circular. Abdominal segment II not unusually expanded and narrowly attached to abdominal segment III. Abdominal segment III with a groove or indentation on anterior face. Pygostyles present. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely absent. On hindwing, radius vein (R) present. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu present. 1rs-m+M present. Free section of cubitus present. Crossvein cu-a present.

Technomyrmex Mayr, 1872

Antenna with 13 segments. Scape shorter than 2+5 funicular segments. First funicular segment conical. Second funicular segment straight. Medial hypostoma present. Mandible triangular, masticatory margin of mandible wholly covered with serrate denticles. Palpal formula 6,4. Propodeal spiracle circular. Abdominal segment II not unusually expanded and narrowly attached to abdominal segment III. Abdominal segment III with a groove or indentation on anterior face. Pygostyles present. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to

M+Rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m at least partially present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu absent. 1rs-m+M absent. Free section of cubitus absent. Cross-vein cu-a absent.

DORYLINAE Leach, 1815

Diagnosis of male ants of the subfamily Dorylinae in the Malagasy region

- Antenna filiform, consisting of 11–13 segments.
- Scape not reaching posterior margin of head.
- Scuto-scutellar suture usually longitudinally sculptured.
- Abdominal segment II attached to abdominal segment III ventrally.
- Abdominal segment II much smaller than segment III in lateral view.
- Two distinct, long, narrow spines present on the posterior portion of abdominal sternum IX.
- Pygostyles absent.
- Protibia with one spur.
- Girdling constriction between pre- and post-sclerites of abdominal segments V and VI absent.

Remarks. Our key includes eight Dorylinae genera recorded from the Malagasy region. Key modified from Borowiec (2016). It is important to note that while the males of *Chrysapace* are currently unknown in the Malagasy region, they have been included in this key based on examination of SE Asian specimens.

Male-based key to genera of the subfamily Dorylinae

- Antenna with 12 to13 segments2
- Maxillary palps short, never reaching occipital foramen, usually not visible without dissection and often with fewer than six segments (Fig. 19B)3



Figure 19. Maxillary palps **A** *Tanipone zona* (CASENT0168822) **B** *Lividopone* mg10 (CASENT0027622). Photographer Michele Esposito.

- 3 Cross vein 2rs-m present complete in forewing (Fig. 20A). Mesotibiae with two tibial spurs *Chrysapace*



Figure 20. Forewing showing cross vein 2rs-m **A** *Chrysapace sauteri* (CASENT0179567) **B** *Eburopone* dr03 (CASENT0138666).

- 4 Antenna with 12 segments. Mesotibiae without spurs (Fig. 21A)... Simopone
- Antenna with 13 segments. Mesotibiae with a single spur, which may be simple and inconspicuous (Fig. 21B)......5



Figure 21. Tibial spurs on mesotibia **A** *Simopone silens* (CASENT0740895) **B** *Lividopone* mg10 (CASENT0496142). Photographer Michele Esposito.

- 5 Costal vein (C) present in forewing (Fig. 22A)......**6**
- Costal vein (C) absent in forewing (Fig. 22B)......7



Figure 22. Forewing in lateral view showing costal vein (C) **A** *Eburopone* dr03 (CASENT0138666) **B** *Lioponera* mg06 (CASENT0138558). Photographer Michele Esposito.

6 Helcium circumference large and in profile dorsal surface of helcium arises from immediately below anterior dorsal angle of abdominal segment III (Fig. 23A). On forewing, radius vein (R) past pterostigma absent

Lividopone



Figure 23. Abdominal segment II and III in lateral view showing helcium circumference **A** *Lividopone* dr02 (CASENT0135633) **B** *Eburopone* dr03 (CASENT0138666). Photographer Michele Esposito.

- On forewing, complete and not reaching costal margin; radius vein (R) absent on costal margin (Fig. 24B). Parafrontal ridges present *Parasyscia*



Figure 24. Forewing showing Rs vein **A** *Lioponera* dr02 (CASENT0144823) **B** *Parasyscia imerinensis* (CASENT0117837). Photographer Michele Esposito.

Chrysapace Crawley, 1924

Antenna with 13 segments. Clypeus without cuticular apron. Parafrontal ridges present. Torulo-posttorular complex vertical. Maxillary palps unknown. Labial palps unknown. Mandibles triangular, masticatory margin edentate. Ventrolateral margins of head without lamella or ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen unknown. Pronotal flange separated from collar by distinct ridge. Notauli present. Transverse groove dividing mesopleuron present. Propodeal declivity with distinct dorsal edge or margin. Metapleural gland opening present. Propodeal spiracle present. Abdominal segment II anterodorsally marginate, dorsolaterally immarginate, and laterally above spiracle marginate. In profile dorsal surface of helcium arises some distance below anterodorsal angle of abdominal segment III. Prora forming a V-shaped protrusion. Spiracle openings of abdominal segments IV-VI circular. Mesotibia with two pectinate spurs. Metatibia with two pectinate spurs. Metatibial gland absent. Hind pretarsal claws with a tooth. On forewing, pterostigma broad. Costal vein (C) present. Radius vein (R) present. Radial sector vein (Rs) fully present between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs present and connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present (Borowiec 2016). Media (M) present, reaches wing margin. Cross-vein 1m-cu present. Cross-vein cu-a proximal to junction between media and cubitus. On hindwing, costal vein (C) absent. Radius vein (R) absent. Vein Sc+R present. Radial sector vein (Rs) present, not reaching wing margin. Cross-vein 1rs-m fused with M. Vein M+Cu present. Abscissa M present. Cross-vein cu-a present. Free section of cubitus present.

Eburopone Borowiec, 2016

Antenna with 13 segments. Clypeus with or without cuticular apron. Parafrontal ridges absent. Torulo-posttorular complex vertical. Maxillary palps 3- or 4-segmented. Labial palps 2- or 3-segmented. Mandibles triangular. Masticatory margin with teeth or falcate. Ventrolateral margins of head without lamella or ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen ventrally absent or present. Pronotal flange not separated from collar by distinct ridge. Notauli present at least anteriorly, very rarely absent. Transverse groove dividing mesopleuron absent or present. Propodeal declivity reduced, without distinct dorsal edge or margin. Metapleural gland opening absent. Propodeal spiracle present. Abdominal segment II anterodorsally immarginate or marginate, dorsolaterally immarginate, and laterally above spiracle immarginate. In profile dorsal surface of helcium arises some distance below anterodorsal angle of abdominal segment III. Prora simple, not delimited by carina. Spiracle openings of abdominal segments IV-VI circular. Mesotibia with single pectinate spur. Metatibia with single pectinate spur. Metatibial gland present as oval patch of whitish cuticle. Hind pretarsal claws simple. On forewing, pterostigma broad. Costal vein (C) present. Radius vein (R) present. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs present, forming base of "free stigma vein." Cross-vein 2rs-m absent. Media (M) reaches wing margin or not, rarely entirely absent. Cross-vein 1m-cu present or rarely absent. Cross-vein cu-a proximal to junction between media and cubitus. On hindwing, costal vein (C) absent. Radius vein (R) present, extending past Sc+R but not reaching distal wing margin. Vein Sc+R absent or present. Radial sector vein (Rs) absent or present, not reaching wing margin. Cross-vein 1rs-m fused with M or absent. Vein M+Cu absent or present. Abscissa M absent. Cross-vein cu-a absent or present. Free section of cubitus absent or present.

Lioponera Mayr, 1879

Antenna with 13 segments. Clypeus with cuticular apron. Parafrontal ridges absent. Torulo-posttorular complex vertical. Maxillary palps 3-segmented. Labial palps 2-segmented. Mandibles triangular. Masticatory margin edentate. Ventrolateral margins of head with or without cuticular ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen ventrally absent. Pronotal flange not separated from collar by distinct ridge. Notauli absent or present. Transverse groove dividing mesopleuron present. Propodeal declivity with distinct dorsal edge or margin. Metapleural gland opening present. Propodeal spiracle present. Abdominal segment II anterodorsally immarginate or marginate, dorsolaterally marginate, and laterally above spiracle marginate. In profile dorsal surface of helcium arises some distance below anterodorsal angle of abdominal segment III. Prora forming a simple U-shaped margin or protrusion. Spiracle openings of abdominal segments IV-VI circular. Mesotibia with single pectinate spur. Metatibia with single pectinate spur. Metatibial gland absent. Hind pretarsal claws simple. On forewing, pterostigma broad. Costal vein (C) absent. Radius vein (R) absent. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs most often present and forming base of "free stigma vein." Cross-vein 2rs-m absent. Media (M) fails to reach wing margin. Cross-vein 1m-cu present or more rarely absent. Cross-vein cu-a located close to junction between media and cubitus. On hindwing, costal vein (C) absent. Radius vein (R) absent. Vein Sc+R present. Radial sector vein (Rs) absent or present, not reaching wing margin. Cross-vein 1rs-m absent or present, approx. as long as M. Vein M+Cu absent or present. Abscissa M absent. Cross-vein cu-a absent or present. Free section of cubitus absent or present.

Lividopone Bolton & Fisher, 2016

Antenna with 13 segments. Clypeus with cuticular apron. Parafrontal ridges present. Torulo-posttorular complex vertical. Maxillary palps unknown. Labial palps unknown. Mandibles triangular. Masticatory margin edentate. Ventrolateral margins of head with cuticular ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen unknown. Pronotal flange separated from collar by distinct ridge. Notauli present. Transverse groove dividing mesopleuron present. Propodeal declivity with distinct dorsal edge or margin. Metapleural gland opening absent. Propodeal spiracle present. Abdominal segment II anterodorsally marginate, dorsolaterally immarginate, and laterally above spiracle marginate. In profile dorsal surface of helcium arises from immediately below anterior dorsal angle of abdominal segment III prora forming a U-shaped protrusion. Spiracle openings of abdominal segments IV-VI circular. Mesotibia with single pectinate spur. Metatibia with single pectinate spur. Metatibial gland absent. Hind pretarsal claws simple. On forewing, pterostigma broad. Costal vein (C) absent. Radius vein (R) absent. Radial sector vein (Rs) fully present between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs absent or present, forming base of "free stigma vein." Cross-vein 2rs-m absent. Media (M) absent or a stub. Cross-vein 1m-cu absent or present. Cross-vein cu-a proximal to junction between media and cubitus. On hindwing, costal vein (C) absent. Radius vein (R) absent. Vein Sc+R absent. Radial sector vein (Rs) absent or stub present. Cross-vein 1rs-m absent or present, approx. as long as M. Vein M+Cu absent or present. Abscissa M absent or present. Cross-vein cu-a absent. Free section of cubitus absent or present.

Ooceraea Roger, 1862

Antenna with 11 segments. Clypeus with cuticular apron. Parafrontal ridges absent. Torulo-posttorular complex vertical. Maxillary palps 5-segmented. Labial palps 3-segmented. Mandibles triangular. Masticatory margin edentate. Ventrolateral margins of head without lamella or ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen ventrally absent. Pronotal flange not separated from collar by distinct ridge, occasionally ridge marked on sides. Notauli present. Transverse groove dividing mesopleuron present. Propodeal declivity reduced, with or without distinct dorsal edge or margin. Metapleural gland opening absent. Propodeal spiracle present. Abdominal segment II anterodorsally immarginate, dorsolaterally immarginate, and laterally above spiracle marginate, inconspicuously in small species. In profile dorsal surface of helcium arises some distance below anterodorsal angle of abdominal segment III prora forming a simple U-shaped margin or a U-shaped margin with median ridge. Spiracle openings of abdominal segments IV-VI circular. Mesotibia with single pectinate spur. Metatibia with single pectinate spur. Metatibial gland present as oval patch of whitish cuticle. Hind pretarsal claws simple. On forewing, pterostigma broad. Costal vein (C) present or absent. Radius vein (R) absent. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs present, forming base of "free stigma vein." Cross-vein 2rsm absent. Media (M) fails to reach wing margin. Cross-vein 1m-cu absent or present. Cross-vein cu-a proximal to junction between media and cubitus. On hindwing, costal vein (C) absent. Radius vein (R) absent or present, extending past Sc+R but not reaching distal wing margin. Vein Sc+R absent, Vein Sc+R present. Radial sector vein (Rs) absent or present, not reaching wing margin. Cross-vein 1rs-m absent. Vein M+Cu absent or present. Abscissa M absent. Cross-vein cu-a absent or present. Free section of cubitus absent.

Parasyscia Emery, 1882

Antenna with 13 segments. Clypeus with cuticular apron. Parafrontal ridges present. Torulo- posttorular complex vertical. Maxillary palps 2-segmented. Labial palps 2-segmented. Mandibles triangular. Masticatory margin edentate. Ventrolateral margins of head without lamella or ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen ventrally absent. Pronotal flange separated from collar by distinct ridge mostly on sides or not separated. Notauli absent or present. Transverse groove dividing mesopleuron present. Propodeal declivity reduced, with or without distinct dorsal edge or margin. Metapleural gland opening absent.

Propodeal spiracle present. Abdominal segment II anterodorsally immarginate or marginate, dorsolaterally immarginate, and laterally above spiracle marginate. In profile dorsal surface of helcium arises some distance below anterodorsal angle of abdominal segment III. Prora forming a U-shaped margin with median ridge. Spiracle openings of abdominal segments IV-VI circular. Mesotibia with single pectinate spur. Metatibia with single pectinate spur. Metatibial gland absent. Hind pretarsal claws simple. On forewing, pterostigma broad. Costal vein (C) absent. Radius vein (R) absent. Radial sector vein (Rs) partially absent between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs present and connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Media (M) fails to reach wing margin. Cross-vein 1m-cu absent or present. Cross-vein cu-a located close to junction between media and cubitus. On hindwing, costal vein (C) absent. Radius vein (R) absent. Vein Sc+R absent. Radial sector vein (Rs) present, not reaching wing margin. Cross-vein 1rs-m present, approx. as long as M. Vein M+Cu present. Abscissa M absent or present. Cross-vein cu-a present. Free section of cubitus present.

Simopone Forel, 1891

Antenna with 12 segments. Clypeus without cuticular apron. Parafrontal ridges present. Torulo- posttorular complex horizontal. Maxillary palps 5- or 6-segmented. Labial palps 3- or 4-segmented. Mandibles triangular. Masticatory margin edentate. Ventrolateral margins of head without lamella or ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen ventrally absent. Pronotal flange separated from collar by distinct ridge. Notauli present. Transverse groove dividing mesopleuron absent. Propodeal declivity with distinct dorsal edge or margin. Metapleural gland opening absent. Propodeal spiracle present. Abdominal segment II anterodorsally marginate, dorsolaterally immarginate, and laterally above spiracle marginate. In profile dorsal surface of helcium arises some distance below anterodorsal angle of abdominal segment III. Prora forming a U-shaped protrusion. Spiracle openings of abdominal segments IV-VI circular. Mesotibia without spurs. Metatibia with single pectinate spur. Metatibial gland absent. Hind pretarsal claws with a tooth. On forewing, pterostigma broad. Costal vein (C) absent. Radius vein (R) absent. Radial sector vein (Rs) fully present between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs present and connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Media (M) reaches to wing margin. Cross-vein 1mcu present or absent. Cross-vein cu-a proximal to junction between media and cubitus. On hindwing, costal vein (C) absent. Radius vein (R) absent. Vein Sc+R present. Radial sector vein (Rs) absent. Cross-vein 1rs-m present, approx. as long as M, never tubular. Vein M+Cu present. Abscissa M present. Cross-vein cu-a present. Free section of cubitus present.

Tanipone Bolton & Fisher, 2012

Antenna with 13 segments. Clypeus without cuticular apron. Parafrontal ridges absent. Torulo- posttorular complex vertical. Maxillary palps 6-segmented.

Labial palps 4-segmented. Mandibles triangular. Masticatory margin edentate. Ventrolateral margins of head without lamella or ridge extending towards mandibles and beyond carina surrounding occipital foramen. Carina surrounding occipital foramen ventrally present. Pronotal flange separated from collar by distinct ridge or not. Notauli absent. Transverse groove dividing mesopleuron present. Propodeal declivity with distinct dorsal edge or margin. Metapleural gland opening absent. Propodeal spiracle present. Abdominal segment II anterodorsally immarginate, dorsolaterally immarginate, and laterally above spiracle marginate. In profile dorsal surface of helcium arises some distance below anterodorsal angle of abdominal segment III. Prora forming a simple U-shaped margin or U-shaped protrusion. Spiracle openings of abdominal segments IV-VI circular. Mesotibia without spurs. Metatibia with single pectinate spur. Metatibial gland absent. Hind pretarsal claws with a tooth. On forewing, pterostigma broad. Costal vein (C) absent. Radius vein (R) absent. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach to costal margin. Cross-vein 2r-rs absent or present and forming base of "free" stigmal vein. Cross-vein 2rs-m absent. Media (M) absent or present, reaches to wing margin. Cross-vein 1m-cu absent or present. Cross-vein cu-a proximal to junction media. On hindwing, costal vein (C) absent. Radius vein (R) absent. Vein Sc+R present. Radial sector vein (Rs) absent or present, reaching wing margin. Cross-vein 1rs-m absent or present, approx. as long as M. Vein M+Cu present. Abscissa M absent. Crossvein cu-a absent or present. Free section of cubitus present.

FORMICINAE Latreille, 1809

Diagnosis of male ants of the subfamily Formicinae in the Malagasy region

- Antenna filiform, consisting of 10–13 segments.
- Scape reaching or exceeding posterior margin of head.
- Mesopleural oblique furrow reaching pronotum far from pronotal posteroventral margin.
- Scuto-scutellar suture simple.
- Abdominal segment II attached to abdominal segment III ventrally.
- Abdominal segment II much smaller than segment III in lateral view.
- Apical portion of abdominal sternum IX not bi-spinose.
- Pygostyles well developed.
- Metatibia with one spur.

Remarks. Our article provides a guide highlighting nine genera of male Formicinae ants found in the Malagasy region.

Male-based key to genera of the subfamily Formicinae

1	Antenna with 10 segments, maxillary palp formula always 5,3 (Fig. 25A)
	Brachymyrmex
_	Antenna with 12 or 13 segments, maxillary palp formula 6,4 (Fig. 25B)2
2	Antenna with 12 segments
_	Antenna with 13 segments6



Figure 25. Maxillary palp **A** *Brachymyrmex cordemoyi* (CASENT0740909) **B** *Tapinolepis* mg01 (CASENT0763590). Photographer Veronica M. Sinotte.

- 3 Masticatory margin of mandible with 8 or 9 denticles (Fig. 26A)...... Anoplolepis (Seychelles)
- Masticatory margin of mandible with < 5 denticles (Fig. 26B)......4



Figure 26. Mandible showing the number of teeth on the masticatory margin **A** *Anoplolepis gracilipes* (CASENT0158950) **B** *Nylanderia amblyops* (CASENT0740913). Photographer Veronica M. Sinotte.

- 4 Funiculus longer than mesosoma length (Fig. 27A)......Tapinolepis
- Funiculus shorter than mesosoma length (Fig. 27B)......5
- First funicular segment length ~ 3× that of second funicular segment in medial view. Malar space extremely reduced, much narrower than scape width (Fig. 28B). Maxillary palp shorter than maximum eye length........... *Plagiolepis*



Figure 27. Body in lateral view comparing the length of the funiculus and mesosoma **A** *Tapinolepis* mg01 (CASENT0763590) **B** *Plagiolepis* mg02 (CASENT0179486). Photographers Veronica M. Sinotte (**A**), Erin Prado (**B**).



Figure 28. Head in lateral view showing the size of the malar space **A** *Lepisiota capensis* (CASENT0861517) **B** *Plagiolepis alluaudi* (CASENT0495472). Photographers Michele Esposito (**A**), Erin Prado (**B**).

- 6 Paired coarse setae absent from frons (Fig. 29A). Aroliae hypertrophied, conspicuous. Funiculus shorter than mesosomal length *Camponotus*



Figure 29. Head in full-face view showing setae disposition of frons **A** *Camponotus alamaina* (CASENT0481800) **B** *Nylanderia amblyops* (CASENT0066704). Photographers Erin Prado (**A**), Michele Esposito (**B**).

- 7 Scape with standing macrosetae (Fig. 30A)8
- Scape lacking standing macrosetae (Fig. 30B)......9



Figure 30. In full-face view, scape **A** *Nylanderia* jsl-galo (CASENT0370667) **B** *Paratrechina longicornis* (CASENT0137341). Photographers Michele Esposito (**A**), Erin Prado (**B**).

- 8 In lateral view, first funicular segment distinctly longer than second funicular segment (Fig. 31A)**Nylanderia**
- In lateral view, first funicular segment shorter than or equal to second funicular segment (Fig. 31B)......Paratrechina



Figure 31. Antennae in lateral view comparing length of first funicular segment and second funicular segment of funiculus **A** *Nylanderia bourbonica* (CASENT0160276) **B** *Paratrechina ankarana* (CASENT0701215). Photographer Michele Esposito.

- 9 Scape slightly shorter than head length (Fig. 32A). Maxillary palp longer than head length...... *Paraparatrechina*



Figure 32. Head in full-face view comparing length of scape and head **A** *Paraparatrechina glabra* (CASENT0497708) **B** *Paratrechina longicornis* (CASENT0244951). Photographers April Nobile (**A**), Michele Esposito (**B**).

Anoplolepis Santschi, 1914

Antenna with 12 segments. Scape distinctly longer than head. Scape lacking standing setae. First funicular segment slightly shorter than second funicular segment in medial view. Funiculus subequal in length to mesosoma. Mandibles well developed, masticatory margin of mandible with eight or nine denticles. Palpal formula 6,4; maxillary palp exceeding hypostomal margin, but not reaching occipital foramen. Frons lacking paired coarse setae. Malar space well developed, broader than maximum scape width. Propodeal spiracle slitshaped. Abdominal segment II lacking peduncle, node well developed. On forewing, pterostigma reduced in size. Costal vein (C) present. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) fails to reach wing margin. On hindwing, radius vein (R) present. Radial sector vein (Rs) present. 1rs-m absent. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a absent. Aroliae small, inconspicuous.

Brachymyrmex Mayr, 1868

Antenna with 10 segments. Aroliae small, inconspicuous. Mandibles reduced, spatulate to spiniform. Masticatory margin of mandible uni- to bidentate. Palpal formula 5,3. Maxillary palp approx. as long as maximum eye diameter. Frons lacking paired coarse setae. Scape shorter than head length. Scape lacking standing macrosetae. First funicular segment slightly longer than second funicular segment in medial view. Funiculus shorter than mesosoma length. Malar space well developed, approx. as long as scape is wide. Propodeal spiracle circular. Abdominal segment II lacking peduncle and node, very short anteroposteriorly. On forewing, pterostigma well developed. Costal vein (C) absent. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) fails to reach wing margin. On hindwing, veins present, 1rs-m incomplete.

Camponotus Mayr, 1861

Antenna with 13 segments. Aroliae hypertrophied, conspicuous. Mandibles well developed, lobate. Masticatory margin of mandible without or with one denticle. Palpal formula 6,4. Maxillary palp exceeding hypostomal margin, exceeding or occipital foramen or not. Frons lacking paired coarse setae. Scape subequal to longer than head length. Scape with or without standing setae. First funicular segment longer or shorter than second funicular segment in medial view. Funiculus shorter than mesosomal length. Malar space well developed, much broader than maximum scape width. Propodeal spiracle slit-shaped. Abdominal segment II lacking long peduncle, node well developed. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein

1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cu-a proximal to junction between media and cubitus. Media (M) fails to reach wing margin. On hind-wing, radius vein (R) absent. Radial sector vein (Rs) present. 1rs-m absent. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus present. Cross-vein cu-a present.

Lepisiota Santschi, 1926

Male description based on male of *Lepisiota capensis* Mayr, 1862. *Lepisiota bipartita* Smith, 1861is known from Réunion but males have not yet been collected.

Antenna with 12 segments. Aroliae small, inconspicuous. Ocelli placed close to occipital margin in front view. Anteromedian margin of clypeus straight. Mandibles well developed. Masticatory margin of mandible with four denticles. Palpal formula 6,4. Maxillary palp approx. as long as head length. Frons lacking paired coarse setae. Scape slightly longer than head length. Scape lacking standing macrosetae. First funicular segment subequal to or longer than second funicular segment in medial view. Funiculus shorter than mesosoma length. Malar space well developed, approx. as long as scape width. Propodeal spiracle oval. Abdominal segment II lacking peduncle and node, anteroposteriorly short. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cu-a proximal to junction between media and cubitus. Media (M) reaches wing margin. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. 1rs-m absent. Media (M) absent. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Nylanderia Emery, 1906

Antenna with 13 segments. Aroliae small, inconspicuous. Mandibles well developed. Masticatory margin of mandible with two denticles. Palpal formula 6,4. Maxillary palp longer than compound eye diameter and shorter than head length. Frons with paired coarse setae. Scape longer than head length but much shorter than mesosoma length. Scape usually with standing macrosetae. First funicular segment distinctly longer than second funicular segment in medial view. Funiculus longer than mesosoma length. Malar space very broad, approx. as long as first funicular segment. Propodeal spiracle circular. Abdominal segment II squamiform, posteriorly pedunculate. On forewing, pterostigma reduced in size. Costal vein (C) absent. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cu-a proximal to junction between media and cubitus. Media (M) fails to reach wing margin. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. 1rs-m absent. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus present. Cross-vein cu-a present.

Paraparatrechina Donithorpe, 1947

Antenna with 13 segments. Aroliae small, inconspicuous. Mandibles well developed, spatulate. Masticatory margin of mandible with single apical tooth. Palpal formula 6,4. Maxillary palp longer than head length. Frons with paired coarse setae. Scape slightly shorter than head length. Scape lacking standing macrosetae. First funicular segment shorter than second funicular segment in medial view from. Funiculus longer than mesosoma length. Malar space broader than scape width. Propodeal spiracle circular. Abdominal segment II squamiform, posteriorly pedunculate. On forewing, pterostigma reduced in size. Costal vein (C) absent. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) present and fails to reach wing margin. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu absent. 1rs-m+M present. Free section of cubitus absent. Crossvein cu-a present.

Paratrechina Motschoulsky, 1863

Paratrechina longicornis Latreille, 1802

Antenna with 13 segments. Aroliae small, inconspicuous. Mandibles well developed, spatulate. Masticatory margin of mandible with single apical tooth. Palpal formula 6,4. Maxillary palp approx. as long as head. Frons with paired coarse setae. Scape very long, longer than mesosoma. Scape lacking standing macrosetae. First funicular segment slightly shorter than second funicular segment in medial view. Funiculus longer than mesosoma length. Malar space very broad, approx. as long as first funicular segment. Propodeal spiracle circular. Abdominal segment II squamiform, posteriorly pedunculate. On forewing, pterostigma reduced in size. Costal vein (C) absent. Cross-vein 1mcu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cu-a proximal to junction between media and cubitus. Media (M) present and fails to reach wing margin. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu absent. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Paratrechina ankarana LaPolla & Fisher, 2014

Antenna with 13 segments. Aroliae small, inconspicuous. Mandibles well developed, spatulate. Masticatory margin of mandible with single apical tooth. Palpal formula 6,4. Maxillary palp approx. as long as head. Frons with paired coarse setae. Scape very long, longer than mesosoma. Scape usually with standing macrosetae. First funicular segment slightly shorter than second funicular segment in medial view. Funiculus longer than mesosoma length. Malar space very broad, approx. as long as first funicular segment. Propodeal spiracle circular. Abdominal segment II squamiform, posteriorly pedunculate. On forewing, pterostigma reduced in size. Costal vein (C) absent. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) fails to reach wing margin. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu absent. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Paratrechina antsingy LaPolla & Fisher, 2014 the male is not known

Plagiolepis Mayr, 1861

Antenna with 12 segments. Aroliae small, inconspicuous. Mandibles well developed. Masticatory margin of mandible with two or three teeth. Palpal formula 6,4. Maxillary palp slightly longer than compound eye. Frons lacking paired coarse setae. Scape slightly longer than head. Scape lacking standing macrosetae. First funicular segment ~ 2× length of second funicular segment in medial view. Funiculus shorter than mesosoma length. Malar space reduced, shorter than scape width. Propodeal spiracle circular. Abdominal segment II anteroposteriorly short, posteriorly pedunculate. On forewing, pterostigma reduced in size. Costal vein (C) absent. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) fails to reach wing margin. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu absent. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Tapinolepis Emery, 1925

Antenna with 12 segments. Aroliae small, inconspicuous. Mandibles well developed. Masticatory margin of mandible with four denticles. Palpal formula 6,4. Maxillary palp slightly shorter than head length. Frons lacking paired coarse setae. Scape slightly shorter than head length. Scape lacking standing macrosetae. First funicular segment shorter than second funicular segment in medial view. Funiculus longer than mesosoma. Malar space well developed, approx. as long as scape width. Propodeal spiracle circular. Abdominal segment II squamiform, lacking peduncle and with short node. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus. Media (M) fails to reach wing margin. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. 1rs-m absent. Media (M) absent. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

MYRMICINAE Lepeletier de Saint-Fargeau, 1835

Diagnosis of male ants of the subfamily Myrmicinae in the Malagasy region

- Antenna filiform, consisting of 10 to 13 segments.
- Abdominal segment II attached to abdominal segment III ventrally.
- Abdominal segment II nearly as large or longer than III in lateral view
- Apical portion of abdominal sternum IX not bi-spinose.
- Pygostyles well developed.
- Front tibial with or without spur.
- Metatibia with one or spur absent.

Remarks. Our key includes thirty genera of male myrmicinae recorded from the Malagasy region. Males for *Dicroaspis* are not yet known from the Malagasy region and the diagnosis is based on males from the Afrotropics.

Male-based key to genera of the subfamily Myrmicinae



Figure 33. In profile view showing occipital carina **A**, **C** *Aphaenogaster bressleri* (CASENT0495103). In dorsal view showing mesoscutellum **B**, **D** *Cyphomyrmex minutus* (CASENT0264488). Photographers April Nobile (**A**, **C**), Michele Esposito (**B**, **D**).

- 2 In profile, posterodorsal margin of head almost straight from base of lateral ocelli to midpoint of occipital carina (Fig. 34A).......**3 (Tribe Attini, part 1)**
- In profile, posterodorsal margin of head gradually rounded from base of lateral ocelli to midpoint of occipital margin (Fig. 34B)...........5 (Tribe Attini, part2)



Figure 34. Head in profile view **A** *Strumigenys chilo* (CASENT0145240) **B** *Tetramorium silvicola* (CASENT0494732). Photographers Dimby Raharinjanahary (**A**), Erin Prado (**B**).

- 3 Mandible with 3 teeth. Scape long, distinctly exceeding posterior margin of head in full-face view (Fig. 35A)..... *Cyphomyrmex* (Reunion)



Figure 35. Scape length in profile view **A** *Cyphomyrmex minutus* (CASENT0264488) **B** *Eurhopalothrix* km01 (CASENT0146071). Photographers Michele Esposito (**A**), Erin Prado (**B**).

- 4 Radial sector vein on forewing is curved toward costal margin and reaches costal margin (Fig. 36A)..... *Eurhopalothrix* (Comoros)



Figure 36. Forewing in lateral view showing radial sector vein **A** *Eurhopalothrix* km01 (CASENT0146071) **B** *Strumigenys dicomas* (CASENT0135118). Photographer Erin Prado.

- 5 Cross vein 2rs-m present on forewing (Fig. 37A)...... Pheidole
- Cross vein 2rs-m absent on forewing (Fig. 37B)......6



Figure 37. Forewing in lateral view showing cross vein 2rs-m **A** *Pheidole* mgs006 (CASENT0135889) **B** *Carebara* drm03 (CASENT0143975). Photographer Dimby Raharinjanahary.

- Mandible normal to reduced; masticatory margin edentate to multidentate with many acute teeth which decrease in size from apex to base; without denticle between teeth (Fig. 38B)



Figure 38. Mandible in full-face view **A** *Pilotrochus besmerus* (CASENT0083498) **B** *Malagidris sofina* (CASENT0906626). Photographers Michele Esposito (**A**), Estella Ortega (**B**).

- In lateral view, anterior margin of promesonotum interrupted by an impressed pronotal furrow that breaks outline (Fig. 39B) or mesonotum strongly produced anterodorsally (Fig. 39C)...12 (Tribe Crematogastrinii)



Figure 39. Head and mesosoma in profile view **A** *Monomorium termitobium* (CASENT0460162) **B** *Meranoplus mayri* (CASENT0062813) **C** *Crematogaster hazolava* (CASENT0317643). Photographers April Nobile (**A**, **B**), Estella Ortega (**C**).

8 Antenna with 12 segments...... Solenopsis

- Antenna with 13 segments......9



Figure 40. Head in full-face view showing first funicular segment, mandible, and postero-median margin of clypeus **A** *Erromyrma latinodis* (CASENT0788835) **B** *Syllophopsis modesta* (CASENT0143818). Photographers Michele Esposito (**A**), Dimby Raharinjanahary (**B**).

- Forewing with four closed cells, 1m-cu cross-vein absent (Fig. 41B).
 In profile, petiolar peduncle absent or shorter than postpetiolar length (Fig. 41D)



Figure 41. Forewing. Abdominal segment II and abdominal segment III in lateral view showing 1m-cu cross-vein and peduncular length **A, C** *Syllophopsis modesta* (CASENT0135642) **B** *Monomorium termitobium* (CASENT0135673) **D** *Monomorium termitobium* (CASENT0135952). Photographer Dimby Raharinjanahary.

- With head in full-face view, antennal scape long reaching occipital margin; mandible short and spatulate, basal margin linear, unidentate (Fig. 42B)..
 Adelomyrmex (Seychelles)



Figure 42. Head in full-face view showing form of mandible and scape length **A** *Monomorium madecassum* (CASENT0209350) **B** *Adelomyrmex* sc01 (CASENT0160764). Photographer Michele Esposito.

- 12 Antennal scrobe runs below eyes (Fig. 43A)..... Cataulacus
- Antennal scrobe absent or runs above eyes (Fig. 43B)13



Figure 43. Head in lateral view showing position of antennal scrobe **A** *Cataulacus oberthueri* (CASENT0435930) **B** *Strumigenys ambatrix* (CASENT0135807). Photographers April Nobile (**A**), Dimby Raharinjanahary (**B**).

13	Protibia without spur (Fig. 44A)	Melissotarsus
-	Protibia with single spur (Fig. 44B)	14



Figure 44. Protibia in ventral view **A** *Melissotarsus insularis* (CASENT0804569) **B** *Terataner* fhg22 (CASENT0429745). Photographer Michele Esposito.
- 14 In lateral view, mesonotal suture extends downward from transverse suture to upper margin of mesopleuron, ending higher than highest point of wing insertion (Fig. 45A)......*Terataner*
- In lateral view, mesonotal suture situated at same level or lower than highest point of wing insertion (Fig. 45B)......15



Figure 45. Mesosoma in lateral view showing position of mesonotal suture relative to point of wing process **A** *Terataner alluaudi* (CASENT0496102) **B** *Malagidris dulcis* (CASENT0135071). Photographers Erin Prado (**A**), Estella Ortega (**B**).

- 15 Abdominal segment III attached dorsally to abdominal segment IV (Fig. 46A). Scape and remaining segments same size (Fig. 46C)**Crematogaster**



Figure 46. Abdominal segment III attachment to abdominal segment IV **A** *Crematogaster maina* (CASENT0132785) **B** *Pilotrochus besmerus* (CASENT0083498). Size comparison of scape and remaining segments **C** *Crematogaster agnetis* (CASENT0101760) **D** *Carebara jajoby* (CASENT0494540). Photographers Estella Ortega (**A**), April Nobile (**B**–**D**)



Figure 47. Abdominal segment II and III in lateral view showing the peduncular length **A***Eutetramorium mocquerysi* (CASENT0495192) **B***Meranoplus mayri* (CASENT0062813). Photographer April Nobile.



Figure 48. Antennae in lateral view showing the length of second funicular segment **A** *Tetramorium mars* (CASENT0134555) **B** *Pilotrochus besmerus* (CASENT0057183). Photographers Dimby Raharinjanahary (**A**), Michele Esposito (**B**).

- 18 Notauli present (Fig. 49A)...... Tetramorium
- Notauli absent (Fig. 49B)



Figure 49. Promesonotum in dorsal view **A** *Tetramorium kelleri* (CASENT0133425) **B** *Dicroaspis* sp. (CASENT0389458). Photographers Erin Prado (**A**), Michele Esposito (**B**).

- 19 With head in full-face view, occipital carina visible (Fig. 50A).... Malagidris
- 20 Antennal scrobe clearly present (Fig. 51A) Metapone
- Antennal scrobe reduced to absent (Fig. 51B)......21

- Antenna with 13 segments......23



Figure 50. Head in full-face view showing occipital carina **A** *Malagidris alperti* (CASENT0248385) **B** *Calyptomyrmex* km01 (CASENT0136409). Photographers Michele Esposito (**A**), April Nobile (**B**).



Figure 51. Head in full-face view showing antennal scrobe **A** *Metapone emersoni* (CASENT0113799) **B** *Nesomyrmex angulatus* (CASENT0147245). Photographers Michele Esposito (**A**), Erin Prado (**B**).

- Cross-vein 1m-Cu absent. Propodeum unarmed and round (Fig. 52B)......
 Pristomyrmex (Mauritius)
- 23 Propodeal spines distinctly present (Fig. 53A) Cardiocondyla



Figure 52. Propodeum in lateral view **A** *Calyptomyrmex* km01 (CASENT0136409) **B** *Pristomyrmex bispinosus* (CASENT0055726). Photographer April Nobile.



Figure 53. Propodeal spines in lateral view A Cardiocondyla emeryi (CASENT0082706) B Vollenhovia piroskae (CASENT0101658). Photographers Michele Esposito (A), April Nobile (B).

- Radial sector vein on forewing is downcurved and never reaches costal margin (Fig. 54B). Occipital carina is unclear or very weakly present, vertex slopes to occiput gently and gradually and is not divided by a carina27



Figure 54. Forewing showing Rs reaching the costal margin **A** *Carebara* drm03 (CASENT0143975) **B** *Monomorium exiguum* (CASENT0135614). Photographer Dimby Raharinjanahary.



Figure 55. Abdomen in lateral view showing the attachment of abdominal segment III **A** *Carebara jajoby* (CASENT0494540) **B** *Nesomyrmex hafahafa* (CASENT0053313). Photographer April Nobile.

26	Mandible edentate (Fig. 56A)	Meranoplus
-	Mandible with 3-5 teeth which decrease in size from	apex to base
	(Fig. 56B)	Nesomyrmex
27	Mandible edentate (Fig. 57A) Vollenhov	ia (Seychelles)
_	Mandible distinctly toothed (Fig. 57B)	



Figure 56. Mandible in full-face view **A** *Meranoplus mayri* (CASENT0062813) **B** *Nesomyrmex tamatavensis* (CASENT0496295). Photographers April Nobile (**A**), Erin Prado (**B**).



Figure 57. Mandible in full-face view **A** *Vollenhovia piroskae* (CASENT0159914) **B** *Monomorium madecassum* (CASENT0209350). Photographer Michele Esposito.



Figure 58. Promesonotum in dorsal view **A** *Trichomyrmex destructor* (CASENT0787666) **B** *Royidris notorthotenes* (CASENT0002249) Photographers Michele Esposito (**A**). April Nobile (**B**).



Figure 59. Mandible in full-face view and forewing fringe features in profile view **A**, **C** *Vitsika crebra* (CASENT0050262) **B**, **D** *Royidris peregrina* (CASENT0206165). Photographers April Nobile (**A**, **C**), Estella Ortega (**B**, **D**).

Adelomyrmex Emery, 1897

Mandible edentate. Palp formula unknown. Antennal scrobe absent. Antenna with 13 segments. First funicular segment not globular, shorter than scape. Scape very long, extending to margin of head. Length of first funicular segment is equal to first flagellar segment. In full-face view, eye located above of base of clypeus. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed and round. Abdominal segment II with a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle absent. Pubescence short, dense over most of body. On forewing, pterostigma reduced in size. Costal vein (C) present. Media between Rs+M and 2r-rs completely absent. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M absent. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu absent. Free section of cubitus absent.

Aphaenogaster Mayr, 1853

Mandible with 3-6 teeth which decrease in size from apex to base. Palp formula 3,2. Antennal scrobe absent. Antenna with 13 segments. First funicular segment not globular, shorter than scape. Scape not short, reaching lower edge of margin of lateral ocelli. Eyes large, at or in front of midlength of sides. Ocelli placed well below occipital margin in front view. Occipital carina strongly developed, forming a nuchal collar. With head full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eves is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed, sometimes with short teeth/denticles. Abdominal segment II with a long anterior peduncle, spiracle located at apex of peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally so that 1m-cu arises from Rs+M not from M. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Calyptomyrmex Emery, 1887

Mandible triangular and distinctly dentate, with five or six teeth which decrease in size from apex to base. Palp formula 2,2. Antennal scrobe reduced. Antenna with 12 segments. First funicular segment not globular, shorter than scape. Scape short, not reaching lower edge of margin of lateral ocelli. Eyes large, at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With in head full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum punctate. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum armed, projects at a low angle. Abdominal segment II with a long anterior peduncle, spiracle located at apex of peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle absent. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus absent.

Remarks. The Malagasy species, *Calyptomyrmex* km01 does not have notauli, in contrast to the descriptions by Ito et al. (2023) and Emery (1922).

Cardiocondyla Emery, 1869

Ergatoid males of *Cardiocondyla* are easily distinguished by having long, toothless, and saber-shaped mandibles for *Cardiocondyla wroughtonii* but worker-like mandibles have been observed in *Cardiocondyla emeryi* and *Cardiocondyla shuckardi*, and reduced black pigmentation (leading to a pale yellowish-brown overall coloration), decreased eye size, and partially or completely reduced ocelli (Seifert 2003).

In winged males, mandible reduced, short, and narrow, with only five teeth. Palp formula 2,2. Antennal scrobe reduced. Antenna with 13 segments. First funicular segment not globular, shorter than scape. Scape short, not reaching lower edge of margin of lateral ocelli. In full-face view, eye located above base of clypeus. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum punctate. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum armed. Abdominal segment II with a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle absent. Pubescence short, dense over most of body. On forewing, pterostigma reduced in size. Costal vein (C) absent. Media between Rs+M and 2r-rs completely absent. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M absent. Radius vein (R) absent. Crossvein cu-a absent. Cu absent. Free section of cubitus absent.

Carebara Westwood, 1840

Mandible reduced, with three or four teeth which decrease in size from apex to base. Palp formula 3,2. Antennal scrobe absent. Antenna with 13 segments. First funicular segment not globular, shorter than scape. Scape shorter than second funicular segment. Eyes large, at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent with a longitudinal median carina that is narrowly bifurcated anteriorly. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed and round. Abdominal segment II with a short, stout anterior peduncle and a short but relatively high node. Abdominal segment III broadly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pubescence short, dense over most of body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) reaches costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1mcu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Cataulacus Smith, 1853

Mandible triangular with denticles which decrease in size from apex to base. Palp formula 4,2. Antennal scrobe running below eyes. Antenna with 11 segments (Emery 1922; Bolton 1974). Length of first funicular is equal to that of second funicular segment + third funicular segment. Scape short, not reaching lower edge of margin of lateral ocelli. In full-face view, eye located in front of midlength of head capsule. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum striate. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II without a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere visible. Pygostyle absent. Pilosity simple throughout body. On forewing, pterostigma reduced in size. Costal vein (C) absent. Media between Rs+M and 2r-rs completely absent. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M merge with Rs. Radius vein (R) absent. Cross-vein cu-a absent. Cu absent. Free section of cubitus absent.

Crematogaster Lund, 1831

Mandible triangular edentate or dentate with one or two teeth. Palp formula 3,2; 5,3. Antennal scrobe is absent. Antenna with 11 or 12 segments. First funicular segment subglobular, shorter than scape. Scape shorter than 1+2 flagellar segment. Eyes large, at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed and round. Abdominal segment II and Abdominal segment III are equal in size. Abdominal segment III dorsally attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) between Rs+M and 2rs-m and after 2rs-m completely present. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Rs+M present. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus absent.

Cyphomyrmex Mayr, 1862

Mandible triangular with three teeth. Palp formula 2,2. Antennal scrobe running above eyes. Antenna with 13 segments. First funicular segment not globular, shorter than scape. Eyes large, at or in front of midlength of sides Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in fullface view, width excluding eyes is distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior margin of eyes is nearly twice as wide as that at level of mandible insertions. Pronotum anterodorsally sharply marginate, with sharp, dentate corners. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum armed or angle projects as a low, obtuse tooth. Abdominal segment II with a short peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere visible. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma reduced in size. Costal vein (C) present. Media between Rs+M and 2r-rs completely absent. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M merge with Rs. Radius vein (R) present. Cross-vein cu-a absent. Cu absent. Free section of cubitus absent.

Dicroaspis Emery, 1908

Mandible triangular with seven teeth. Antennal scrobe running above eyes. Antenna with ten segments. First funicular segment not globular, shorter than scape. Scape very long, extending to margin of head. Eyes large, at or in front of midlength of sides. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Pronotum anterodorsally sharply marginate, with sharp, dentate corners. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed and round. Abdominal segment II with a long peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere visible. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M merge with Rs. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu absent. Free section of cubitus absent.

Erromyrma Bolton & Fisher, 2016

Mandible triangular (Fisher and Bolton 2016; Ramamonjisoa et al. 2023), short, and narrow, with only four or five teeth. Palp formula 5,3. Antennal scrobe absent. Antenna with 13 segments. First funicular segment subglobular, same size as scape. Eyes large, at or in front of midlength of sides. Ocelli placed close to occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed and round. Abdominal segment II with a short peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere visible. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein

2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Eurhopalothrix Brown & Kempf, 1961

Mandible triangular edentate. Palp formula 2,2. Antennal scrobe running above eyes. Antenna with 13 segments. First funicular segment not globular, shorter than scape. Eyes large, at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior margin of eyes is nearly twice as wide as that at level of mandible insertions. Mesoscutum punctate. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum angle projects as a low, obtuse tooth. Abdominal segment II with a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma reduced in size. Costal vein (C) absent. Media between Rs+M and 2r-rs completely absent. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M merge with Rs. Radius vein (R) present. Cross-vein cu-a absent. Cu absent. Free section of cubitus absent.

Eutetramorium Emery, 1899

Mandible stoutly triangular, with seven teeth. Palp formula 4,3. Antennal scrobe is absent. Antenna with 13 segments. SI 31. First funicular segment long but not globular, ~ 25% longer than length of second funicular segment. In fullface view, eye located in front of midlength of head capsule. Ocelli placed well below occipital margin in front view. Occipital carina sharp but not forming a raised crest. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Anterior mesoscutum between notauli arms, with a longitudinal median carina that is narrowly bifurcated anteriorly. Notauli weakly present, anterior arms forming a V-shape. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur simple. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed, spiracle low on side and in front of midlength of sclerite; propodeal lobes conspicuous, rounded. Abdominal segment II with a short, stout anterior peduncle and a short but relatively high node, spiracle approx. level with base of anterior face of node. Abdominal segment III greatly elongated, in profile almost twice length of abdominal segment II. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle present. Denser upright pilosity. On forewing, pterostigma reduced in size. Costal

vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Malagidris Bolton & Fisher, 2014

Mandible triangular and strongly dentate, with nine sharp teeth. Palp formula 3,2. Antennal scrobe is reduced. Antenna with 13 segments. First funicular segment short, not globular, ~ 1/2 length of second funicular segment. In fullface view, eye located in front of midlength of head capsule. Ocelli placed near occipital margin in front view. Occipital carina sharp, forming a distinct crest. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum convex in profile, mesoscutum and mesoscutellum elevated, much higher than propodeal dorsum, which is depressed and slopes downward posteriorly. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur simple. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed, spiracle high on side and at approx. midlength, or slightly in front of midlength, of sclerite; propodeal lobes conspicuous, rounded. Abdominal segment II with a long anterior peduncle and a low node, spiracle at or behind midlength of peduncle, but in front of level of node. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity scarce. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Melissotarsus Emery, 1877

Mandible triangular edentate or dentate with one or two teeth. Palp formula 0,1. Antennal scrobe is reduced. Antenna with 12 segments. First funicular segment short, not globular, ~ 1/2 length of second funicular segment. In full-face view, eye located in front of midlength of head capsule. Ocelli placed close to occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum convex in profile, mesoscutum and mesoscutellum elevated, much higher than propodeal dorsum, which is depressed and slopes downward posteriorly. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia without tibial spur. Mesotibia tibial spur simple. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed and round. Abdominal segment II without a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle absent. Pilosity simple throughout body. On forewing, pterostigma reduced in size. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) present. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M present. Radius vein (R) present. Cross-vein cu-a present. Cu absent. Free section of cubitus absent.

Meranoplus Smith, 1853

Mandible reduced, short, and narrow, with only one tooth. Palp formula 5,3. Antennal scrobe absent. Antenna with 13 segments. First funicular segment short, not globular, ~ 1/2 length of second funicular segment. In full-face view, eye located in front of midlength of head capsule. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur simple. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed and round. Abdominal segment II without a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere visible. Pygostyle absent. Pilosity long throughout body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu absent. Free section of cubitus absent.

Metapone Forel, 1911

Mandible triangular and distinctly dentate with four teeth. Palp formula 1,2. Antennal scrobe running above eyes. Antenna with 12 segments. First funicular segment short, not globular, about same size as second funicular segment. In full-face view, eye located in front of midlength of head capsule. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in fullface view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum striate. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed. Abdominal segment II without peduncle. In profile, petiolar node rectangular nodiform; both waist segments strongly sculptured. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle absent. Pilosity long, erect to suberect. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Crossvein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Monomorium Mayr, 1855

Mandible triangular with three or four teeth. Palp formula 5,3. Antennal scrobe absent. Antenna with 13 segments. First funicular segment short, not globular. In full-face view, eye located in front of midlength of head capsule. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed and round. Abdominal segment II without peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Rs+M absent. Radius vein (R) absent. Crossvein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Nesomyrmex Wheeler, 1910

Mandible triangular and distinctly dentate, with five teeth. Palp formula 5,3. Antennal scrobe reduced. Antenna with 13 segments. First funicular segment not globular, shorter than scape. In full-face view, eye located in front of midlength of head capsule Ocelli placed well below occipital margin in front view. Occipital carina sharp but not forming a raised crest. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II with a long anterior peduncle and a low node, spiracle at or behind midlength of peduncle, but in front of level of node. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle absent. Sparse pilosity. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M merge with Rs. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu absent. Free section of cubitus absent.

Pheidole Westwood, 1839

Mandible with 4-7 teeth which decrease in size from apex to base. Palp formula 5,3. Antennal scrobe is absent. Antenna with 13 segments. First funicular segment globular, shorter than scape. In full-face view, eye located in front of midlength of head capsule. Ocelli placed close to occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II with a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle present. Sparse pilosity. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Pilotrochus Brown, 1978

Mandible with 4–7 teeth. Palp formula 5,3. Antennal scrobe is reduced. Antenna with 13 segments. First funicular segment globular, shorter than scape. In full-face view, eye located in front of midlength of head capsule. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior margin of eyes is nearly twice as wide as that at level of mandible insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II with a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle present. Sparse pilosity. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Pristomyrmex Mayr, 1866

Mandible edentate. Palp formula 2,2. Antennal scrobe reduced. Antenna with 12 segments. First funicular segment short, not globular, about a third length of second funicular segment. In full-face view, eye located above of base of clypeus. Ocelli placed close to occipital margin in front view. Occipital carina invisible. With head full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II with a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M merge with Rs. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu absent. Free section of cubitus absent.

Royidris Bolton & Fisher, 2014

Mandible triangular and distinctly dentate, with two or three teeth. Palp formula 4,3. Antennal scrobe absent. Antenna with 13 segments. SI 30-52. First funicular segment short and globular. Eves large, located at or in front of midlength of sides. Ocelli placed close to occipital margin in front view. Occipital carina sharp but not forming a raised crest. With head full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli variably developed, from vestigial to having anterior arms present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur simple. Metatibia tibial spur simple. Aroliae small. Propodeum usually unarmed and rounded, but in some posterodorsal angle is reinforced by a carina, or angle projects as a low, obtuse tooth; propodeal lobes rounded. Abdominal segment II with an anterior peduncle, spiracle at, or slightly in front of, midlength of peduncle, well in front of level of low, rounded node. Abdominal segment II in profile slightly longer than Abdominal segment III. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus absent.

Solenopsis Westwood, 1840

Mandible with two or three teeth. Palp formula 5,3. Antennal scrobe is reduced. Antenna with 12 segments. First funicular segment globular, shorter than scape. Eyes large, located at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II with a short peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1mcu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Strumigenys Smith, 1860

Mandible edentate. Palp formula 5,3. Antennal scrobe is absent. Antenna with 13 segments. First funicular segment not subglobular, same size as scape. Eyes large, located at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior margin of eyes is nearly twice as wide as that at level of mandible insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum angle projects as a low, obtuse tooth. Abdominal segment II with a short peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle present. Sparse pilosity. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) absent. Radial sector vein (Rs) never reaching costal margin.

Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M absent. Radius vein (R) absent. Cross-vein cu-a absent. Cu absent. Free section of cubitus absent.

Syllophopsis Santschi, 1915

Mandible with three teeth. Palp formula 5,3. Antennal scrobe reduced. Antenna with 13 segments. First funicular segment short, not globular. Eyes large, located at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II with a short peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Terataner Emery, 1912

Mandible triangular and distinctly dentate, with five or six teeth. Palp formula 4,3. Antennal scrobe absent. Antenna with 13 segments. First funicular segment globular, shorter than scape. Eyes large, at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Pronotum anterodorsally sharply marginate, with sharp, dentate corners. Notauli absent. With mesopleuron in lateral view, anterodorsal portion is higher than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur simple. Aroliae small. Propodeum unarmed. Abdominal segment II with a long anterior peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity long, erect to suberect. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) present. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Tetramorium Mayr, 1855

Mandible triangular and distinctly dentate, with 4–7 teeth. Palp formula 5,3. Antennal scrobe reduced. Antenna with 10-13 segments. First funicular segment is more distinctly elongated than ors: length is nearly or more than twice as long as third funicular segment. Eyes large, at or in front of midlength of sides. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur simple. Aroliae small. Propodeum armed or angle projects as a low, obtuse tooth. Abdominal segment II with a short peduncle. Abdominal segment III narrowly attaches to Abdominal segment IV. Paramere small. Pygostyle present. Pilosity long, erect to suberect. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Trichomyrmex Mayr, 1865

Mandible reduced, short, and narrow, with only two or three teeth. Palp formula 5,3. Antennal scrobe absent. Antenna with 13 segments. First funicular segment subglobular. Eyes large, at or in front of midlength of sides. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II with a short peduncle. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere small. Pygostyle absent. Sparse pilosity. On forewing, pterostigma well developed. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M merge with Rs. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus absent.

Vitsika Bolton & Fisher, 2014

Mandible triangular and distinctly dentate, with 5–7 teeth. Palp formula 4,3. Antennal scrobe reduced. Antenna with 13 segments. SI 30-52. First funicular segment short but not globular. Eyes large, located at or in front of midlength of sides. Ocelli placed near occipital margin in front view. Occipital carina sharp but not forming a raised crest. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli variably developed, from vestigial to having anterior arms present. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum usually unarmed and rounded. Abdominal segment II with an anterior peduncle, spiracle at, or slightly in front of, midlength of peduncle, well in front of level of low, rounded node. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. Pilosity simple throughout body. On forewing, pterostigma well developed. Costal vein (C) present. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally so that 1m-cu arises from Rs+M not from M. Radius vein (R) absent. Cross-vein cu-a proximal to junction between media and cubitus. Cu present. Free section of cubitus present.

Vollenhovia Mayr, 1865

Mandible edentate. Palp formula 2,2. Antennal scrobe absent. Antenna with 13 segments. First funicular equal in size to scape, not globular. Ocelli placed well below occipital margin in front view. Occipital carina invisible. With head in full-face view, width excluding eyes is not distinctly narrowed anteriorly from level of posterior margin of eyes: width at level of posterior edge of eyes is not twice as wide as that at level of mandibular insertions. Mesoscutum in profile strongly overhangs pronotum, latter not visible in dorsal view. Notauli absent with a longitudinal median carina that is narrowly bifurcated anteriorly. With mesopleuron in lateral view, anterodorsal portion lower than highest point of wing process. Protibia with pectinate tibial spur. Mesotibia tibial spur absent. Metatibia tibial spur absent. Aroliae small. Propodeum unarmed. Abdominal segment II without peduncle, in profile petiolar node rectangular nodiform. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle absent. Pilosity long, erect to suberect. On forewing, pterostigma reduced in size. Costal vein (C) absent. Media between Rs+M and 2r-rs completely absent. Media (M) absent. Radial sector vein (Rs) never reaching costal margin. Cross-vein 2r-rs present, forming base of "free stigma vein." Cross-vein 2rs-m absent. Cross-vein 1m-cu absent. Rs+M absent. Radius vein (R) absent. Cross-vein cu-a absent. Cu absent. Free section of cubitus absent.

PONERINAE Lepeletier de Saint-Fargeau, 1835

Diagnosis of male ants of the subfamily Ponerinae in the Malagasy region

- Antenna filiform, consisting of 13 segments.
- Scape not reaching posterior margin of head.
- Mesopleural oblique furrow reaching pronotum far from pronotal posteroventral margin.
- Scuto-scutellar suture usually longitudinally sculptured.
- Abdominal segment II much smaller than segment III in lateral view.
- Abdominal segment II with distinct front, top, and posterior faces in lateral view.
- Abdominal segment II attachment to abdominal segment III narrow and strongly constricted in lateral view.
- Abdominal segment III is nearly as large as abdominal segment IV.
- Cinctus between the segments III and IV distinct and deep.
- Apical portion of abdominal sternum IX not bi-spinose.
- Pygostyles well developed.
- Metatibia with one or two spurs.

Remarks. Our key includes ten Ponerinae genera recorded from the Malagasy region. Overall key modified from Yoshimura and Fisher (2007). Males of *Parvaponera* are unknown were not included in this genera key. *Mesoponera* is known to be paraphyletic (Schmidt and Shattuck 2014). The two species in the Malagasy region, *Mesoponera ambigua* and *Mesoponera melanaria macra* are keyed out separately.

Male-based key to genera of the subfamily Ponerinae

- 1 Wings absent Hypoponera punctatissima
- 2 Mandibles stout and fully developed, masticatory margins overlap completely when mandibles are fully closed (Fig. 60A). Antennal scrobe well defined and extends as long as length of antennal scape*Platythyrea*



Figure 60. Mandible in full-face view **A** *Platythyrea arthuri* (CASENT0442287) **B** *Mesoponera ambigua* (CASENT0052325). Photographer April Nobile.

- 3 Pretarsal claw multidentate to pectinate (Fig. 61A)Leptogenys
- Pretarsal claw edentate or with at most two preapical teeth (Fig. 61B) ...4



Figure 61. Pretarsal claw **A** *Leptogenys mangabe* (CASENT0496777) **B** *Bothroponera cambouei* (CASENT0497079). Photographer April Nobile.

- 4 Hind wing with jugal lobe (Fig. 62A)5
- Hind wing without jugal lobe (Fig. 62B).....11



Figure 62. Hind wing **A** *Odontomachus coquereli* (CASENT0740610) **B** *Leptogenys mangabe* (CASENT0496777). Photographers Isabella Muratore (**A**) April Nobile (**B**).

- 5 Notauli present on mesoscutum (Fig. 63A)6



Figure 63. Notauli on mesoscutum **A** *Anochetus goodmani* (CASENT0147683) **B** *Bothroponera wasmannii* (CASENT0134532). Photographer Dimby Raharinjanahary.

- 6 Mesometapleural suture deep and sculptured, dorsal margin of abdominal segment II, in frontal view, usually showing two apices (Fig. 64A)...... *Anochetus goodmani*
- Mesometapleural suture deep but not sculptured, dorsolateral corner of abdominal segment II, in frontal view, not showing two apices (Fig. 64B)7
- abdominal tergum VIII not forming a spine (Fig. 65D)...*Mesoponera ambigua*



Figure 64. Dorsolateral corner of abdominal segment II in rear view **A** *Anochetus good-mani* (CASENT0147683) **B** *Mesoponera ambigua* (CASENT0108325). Photographer Michele Esposito.



Figure 65. Abdominal segment II in profile view showing the subpetiolar process; apical portion of abdominal tergum VIII **A**, **C** *Mesoponera melanaria macra* (CASENT0272313) **B**, **D** *Mesoponera ambigua* (CASENT0135592). Photographers Michele Esposito (**A**, **C**), Dimby Raharinjanahary (**B**, **D**).

- 8 Apical portion of abdominal tergum VIII not forming a spine (Fig. 66A).....
- Anochetus Apical portion of abdominal tergum VIII forming a distinct spine (Fig. 66B).....9



Figure 66. Apical portion of abdominal tergum VIII **A** *Anochetus madagascarensis* (CASENT0442379)**B***Odontomachuscoquereli*(CASENT0049797). PhotographerAprilNobile.

- Dorsal margin of abdominal segment II, in frontal view, without single sharp apex (Fig. 67B).....10



Figure 67. Abdominal segment II in frontal view **A** *Odontomachus coquereli* (CASENT0049797) **B** *Bothroponera cambouei* (CASENT0497079). Photographers Masashi Yoshimura (**A**), April Nobile (**B**).



Figure 68. Abdominal segment II form **A** *Bothroponera wasmannii* (CASENT0147642) **B** *Brachyponera sennaarensis* (https://www.antweb.org/specimen.do?code=SAM-HYM-C002312). Photographer Michele Esposito.

- Apical portion of abdominal tergum VIII with downcurved spine (Fig. 69B).....12
- 12 Ventral apex of meso- and metatibia, when viewed from front with femur at right angle to body, with single spur, spur large and pectinate (Fig. 70A)... Ponera



Figure 69. Apical portion of abdominal tergum VIII**A***Hypoponera* mg016 (CASENT0466110) **B** *Euponera vohitravo* (CASENT0740617). Photographer Michele Esposito.



Figure 70. Tibial spur on metatibia **A** *Hypoponera* mg057 (CASENT0430684) **B** *Euponera vohitravo* (CASENT0740617). Photographers April Nobile (**A**), Michele Esposito (**B**).

Anochetus Mayr, 1861

All males winged. Antennal scrobe absent. Mandible reduced. Basal cavity of mandible extending to front face, visible in full-face view. Antenna with 13 segments. Notauli absent except for Anochetus goodmani. Mesepimeron with epimeral lobe. In most cases, each dorsolateral corner of abdominal segment II in anterior view with distinct projection. Dorsal margin of abdominal segment II, in anterior view, usually showing two apices. Apical margin of abdominal tergum VIII not projecting into sharp spine. Jugal lobe of hind wing present. Mesotibia and metatibia with two spurs. Claws simple, not multidentate or pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1mcu present. Radial sector vein (Rs) complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m present. Media (M) usually present. M+Cu present. 1rs-m+M absent. Free section of cubitus present. Cross-vein cu-a present.

The presence of notauli is known for *Anochetus* in the Asian region, including in Vietnam *Anochetus mixtus*, *Anochetus princeps* and in Indonesia *Anochetus filicornis*, but only the *goodmani* species exhibits this feature in the Malagasy region.

Bothroponera Mayr, 1862

Males winged. Antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli never impressed on mesoscutum. Mesepimeron with epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view not projecting. Dorsal margin of abdominal segment II, in frontal view, rounded and in profile view, abdominal segment II surmounted by a thick node. Apical margin of abdominal tergum VIII projecting into sharp spine. Jugal lobe of hind wing present. Mesotibia and metatibiae with two spurs. Claws simple, never multidentate or pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m present. Media (M) absent. M+Cu present. 1rs-m+M present. Free section of cubitus present. Cross-vein cu-a present.

Brachyponera Emery, 1900

Males winged. Antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli never impressed on mesoscutum. Mesepimeron with epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view not projecting. Dorsal margin of abdominal segment II, in frontal view, rounded and in profile view, petiolar node generally scale-like and thin. Apical margin of abdominal tergum VIII projecting into sharp spine. Jugal lobe of hind wing present. Mesotibia and metatibiae with two spurs. Claws simple, never multidentate or pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a located in line to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) absent. Cross-vein 1rs-m present. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Euponera Forel, 1891.

Males winged. Antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli present or absent. Mesepimeron with epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view not projecting. Dorsal margin of abdominal segment II, in frontal view, rounded. Apical margin of abdominal tergum VIII projecting into sharp spine. Jugal lobe of hind wing absent. Mesotibia and metatibiae with two spurs. Claws simple, never multi-dentate or pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a located in line to junction between media and cubitus vein. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) absent. Free section of cubitus absent. Cross-vein cu-a present.

Hypoponera Santschi, 1938

Ergatoid males of Ponerinae are easily distinguished by having: (1) abdominal segment III as large as segment IV; and (2) a distinct constriction between abdominal segments III and IV.

In winged males, antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli never impressed on mesoscutum. Mesepimeron without epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view lacking distinct projection. Dorsal margin of abdominal segment II, in anterior view, without a conical or pointed apex. Apical margin of abdominal tergum VIII without spine. Jugal lobe of hind wing absent. Mesotibia and metatibia with single spur. Claws simple, never multidentate or pectinate. On forewing, pterostigma reduced in size. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein distal to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus vein. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) absent. Cross-vein 1rs-m present. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Leptogenys Roger, 1861

Males winged. Antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli impressed on mesoscutum in most species. Mesepimeron with epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view without distinct projections. Dorsal margin of abdominal segment II in anterior view gently rounded, not forming a conical or pointed apex. Apical margin of abdominal tergum VIII occasionally featuring downcurved projection. Jugal lobe of hindwing absent in most species. Mesotibia and metatibia with two spurs. Pretarsal claw multidentate to pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) absent. Cross-vein 1rs-m present. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Mesoponera Emery, 1900

Mesoponera ambigua André, 1890. Males winged. Antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli impressed on mesoscutum. Mesepimeron with epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view not projecting. Dorsal margin of abdominal segment II, in frontal view, rounded. Subpetiolar process in profile view subtriangular. Apical portion of abdominal tergum VIII without downcurved spine. Jugal lobe of hind wing present. Mesotibia and metatibiae with two spurs. Claws simple, never multidentate or pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a located in line to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m present. Media (M) present. M+Cu present. Free section of cubitus present. Cross-vein cu-a present.

Mesoponera melanaria macra Emery, 1894. Males winged. Antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli impressed on mesoscutum. Mesepimeron with epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view not projecting. Dorsal margin of abdominal segment II, in frontal view, rounded. Subpetiolar process in profile view convex ventrally. Apical portion of abdominal tergum VIII with downcurved spine. Jugal lobe of hind wing present. Mesotibia and metatibiae with two spurs. Claws simple, never multidentate or pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) present. Radial sector vein (Rs) present. Cross-vein 1rs-m present. Media (M) present. M+Cu present. Free section of cubitus present. Cross-vein cu-a present.

Odontomachus Latreille, 1804

Males winged. Antennal scrobe absent. Mandible reduced. Basal cavity of mandible extending to front face and visible in full-face view. Antenna with 13 segments. Notauli never impressed on mesoscutum. Mesepimeron with

epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view not projecting. Dorsal margin of abdominal segment II in anterior view more or less conical, with a narrowly rounded or pointed apex. Apical margin of abdominal tergum VIII projecting into a sharp spine. Jugal lobe of hind wing present. Mesotibia and metatibia with two spurs. Claws simple, never multidentate to pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) absent. Cross-vein 1rs-m present. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

Parvaponera Schmidt & Shattuck, 2014

While the male of this species remains unknown worldwide, the analysis of wing venation and morphological characteristics based on the gyne might be helpful to identify the male of this species in the future.

Queen: Antenna with 12 segments. Mesotibia and metatibia with two spurs. Claws simple, never multidentate to pectinate. On forewing (Fig. 71), pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a distal to junction between media and cubitus. Media between Rs+M and 2rs-m completely present.

Platythyrea Roger, 1863

Males winged. Antennal scrobe distinct. Mandible large, stout, triangular, with many teeth on masticatory margin, and masticatory margins completely overlap when mandibles are fully closed. Basal cavity of mandible invisible in full-face view. Antenna with 13 segments. Notauli impressed on mesoscutum. Mesepimeron with epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view lacking distinct projection. Dorsal margin of abdominal segment II, in anterior view, broadly or narrowly rounded. Apical margin of abdominal tergum VIII does not project strongly into sharp spine. Jugal lobe of hind wing may or may not be present. Mesotibia and metatibiae with two spurs. Claws simple, never multidentate or pectinate. Body surface sparsely punctate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m present. Cross-vein cu-a located in line to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) absent. Cross-vein 1rs-m present. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.



Figure 71. Forewing venation in queen caste. *Parvaponera darwinii madecassa* (CASENT0410199). Photographer Cerise Chen.

Ponera Latreille, 1804

Males winged. Antennal scrobe absent. Mandible reduced in size. Basal cavity of mandible extending to front face, visible in full-face view. Antenna with 13 segments. Notauli never impressed on mesoscutum. Mesepimeron without epimeral lobe. Dorsolateral corner of abdominal segment II in anterior view lacking distinct projection. Dorsal margin abdominal segment II, in anterior view, without narrowly rounded or pointed apex. Apical margin of abdominal tergum VIII strongly projecting into a sharp spine. Jugal lobe of hind wing absent. Mesotibia and metatibiae with single spur. Claws simple, never multidentate or pectinate. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu present. Radial sector vein (Rs) fully complete between M+Rs and 2r-rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rsm present. Cross-vein cu-a proximal to junction between media and cubitus. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) absent. Cross-vein 1rs-m present. Media (M) present. M+Cu present. 1rs-m+M present. Free section of cubitus absent. Cross-vein cu-a present.

PROCERATIINAE Emery, 1895

Diagnosis of male ants of the subfamily Proceratiinae in the Malagasy region

- Antenna filiform, consisting of 13 segments.
- Scape not reaching posterior margin of head.
- Mesopleural oblique furrow reaching pronotum close to pronotal posteroventral margin.
- Scuto-scutellar suture usually longitudinally sculptured.
- Abdominal segment II attached to abdominal segment III ventrally.
- Abdominal segment II much smaller than segment III in lateral view.

- Abdominal segment II broadly and dorsally attached to abdominal segment III.
- Apical portion of abdominal sternum IX not bi-spinose.
- Pygostyles absent or present.
- Metatibia with one spur.

Remarks. Our key includes three Proceratiinae genera recorded from the Malagasy region. Key modified from Yoshimura and Fisher (2009).

Male-based key to genera of the subfamily Proceratiinae



Figure 72. Head in full-face view showing the frontal carinae **A** *Proceratium* mgm09 (CASENT0081854) **B** *Probolomyrmex curculiformis* (CASENT0080551). Photographer April Nobile.



Figure 73. Forewing venation **A** *Discothyrea* mgm01 (CASENT0083649) **B** *Probolomyrmex curculiformis* (CASENT0050214). Photographers Erin Prado (**A**), April Nobile (**B**).

Discothyrea Roger, 1863

Mandible smaller than in conspecific worker, but also triangular to subtriangular. Frontoclypeal region projecting dorsally. Frontal carinae merged into a single median carina. Antennal sockets opening posteriorly. Antenna with 12–13 segments. Labrum bilobed apically. Second segment of maxillary palp not hammer-shaped. Pro-, meso-, and metatibia with a single spur. Pygostyles present. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu absent. Radial sector vein (Rs) fused to M+Rs. Radial sector vein (Rs) reaches to costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus vein. Media between Rs+M and 2rs-m completely absent. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu absent. 1rs-m+M absent. Free section of cubitus absent. Cross-vein cu-a absent.

Probolomyrmex Mayr, 1901

Mandible smaller than in conspecific worker, but also triangular to subtriangular. Frontoclypeal region projecting dorsally. Frontal carinae merged into single median carina. Antennal socket opening posteriorly. Antenna with 13 segments. Labrum bilobed apically. Second segment of maxillary palp hammer-shaped. Pro-, meso-, and metatibia with a single spur. Pygostyles absent. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1m-cu absent. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs present, forming base of "free stigma vein." Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus vein. Media between Rs+M and 2rs-m completely absent. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m absent. Media (M) absent. M+Cu absent. 1rs-m+M absent. Free section of cubitus absent. Cross-vein cu-a absent.

Proceratium Roger, 1863

Mandible smaller than in conspecific worker, but also triangular to subtriangular. Frontoclypeal region not projecting dorsally. Frontal carinae separated, not merged into single median carina. Antennal sockets opening dorsally. Antenna with 13 segments. Labrum bilobed apically. Second segment of maxillary palp hammer-shaped. Pro-, meso-, and metatibia with a single spur. Pygostyles absent. On forewing, pterostigma well developed. Costal vein (C) present. Cross-vein 1mcu absent. Radial sector vein (Rs) absent between M+Rs and 2r-rs. Radial sector vein (Rs) fails to reach costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein cu-a proximal to junction between media and cubitus vein. Media between Rs+M and 2rs-m completely present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m present. Media (M) usually present. M+Cu present. 1rs-m+M present. Free section of cubitus present. Cross-vein cu-a proximal.

PSEUDOMYRMICINAE Smith, 1952

Diagnosis of male *Tetraponera* in the subfamily Pseudomyrmicinae in the Malagasy region.

- Antenna filiform, consisting of 12 segments.
- Abdominal segment II nearly as large as segment III in lateral view.
- Mesopleural oblique furrow reaching pronotum far from pronotal posteroventral margin.
- Apical portion of abdominal sternum IX not bi-spinose.
- Pygostyles present.
- Protibia with one spur.
- Mesotibia with two spurs.
- Metatibia with two spurs.

Mandible triangular and distinctly dentate. Masticatory margin with 2-6 teeth. Anterior margin of clypeus straight to broadly convex, rarely emarginate. Palp formula 6,4. Antennal scrobe absent. Antenna with 12 segments. First funicular segment short and globular. Eyes large, located at or in front of midlength of sides. Ocelli conspicuous. Occipital carina sharp but not forming a raised crest. Promesonotal suture visible in profile or dorsally. Notauli absent. Protibia with pectinate tibial spur. Meso- and metatibiae with two tibial spurs. Aroliae small. Propodeum usually unarmed and rounded. Propodeal spiracle rounded. Abdominal segment III narrowly attaches to abdominal segment IV. Paramere large. Pygostyle present. On forewing, pterostigma well developed but not pigmented. Costal vein (C) absent. Media (M) fused with Rs+M. Media (M) never reaching costal margin. Radial sector vein (Rs) reaches costal margin. Cross-vein 2r-rs connected with radial sector vein posterior to pterostigma. Cross-vein 2rs-m absent. Cross-vein 1m-cu present. Fusion of Rs+M extended distally, so that 1m-cu arises from Rs+M, not from M. R present. Cu-a proximal to junction between media and cubitus vein. Cu present. Free section of cubitus present. On hindwing, radius vein (R) absent. Radial sector vein (Rs) present. Cross-vein 1rs-m present. Media (M) present. M+Cu absent. 1rsm+M present. Free section of cubitus absent. Cross-vein cu-a absent.

Acknowledgements

We would like to express our sincere gratitude to everyone at the Madagascar Biodiversity Center, especially Team Vitsika, for giving us the opportunity to work on this project. We are particularly grateful to Balsama Rajemison, Jean Claude Rakotonirina, Jean Jacques Rafanomezantsoa, Chrislain Ranaivo, Claver Randrianandrasana, and Miranto Razafindranaivo for their support. We would also like to thank Michele Esposito, Cerise Chen, Veronica M. Sinotte, Ziv Lieberman, April Nobile, Erin Prado, Estella Ortega, Isabella Muratore, Masashi Yoshimura, Wade Lee, and Dimby Raharinjanahary for kindly imaging the specimens needed for the key.

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 supported in part by a series of National Science Foundation awards to BLF with the most recent being DEB 1655076 MAMI, DEB 1932469 AoW, DEB 1856400 CanBe.

Author contributions

All authors have contributed equally.

Author ORCIDs

Manoa M. Ramamonjisoa IIII https://orcid.org/0009-0005-7805-4433 Nicole Rasoamanana IIII https://orcid.org/0000-0001-9702-7231 Brian L. Fisher IIIII https://orcid.org/0000-0002-4653-3270

Data availability

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

References

- AntWeb.org (2024) AntWeb, California Academy of Sciences, San Francisco, California, USA. http://www.antweb.org [16 August 2024]
- Bolton B (1974) A revision of the Palaeotropical arboreal ant genus *Cataulacus* F. Smith (Hymenoptera: Formicidae). Bulletin of the British Museum (Natural History). Entomology 30: 1–105. https://doi.org/10.5962/bhl.part.24939
- Bolton B (1994) Identification guide to the ant genera of the world. Harvard University Press, Cambridge, Mass., 222 pp.
- Borowiec ML (2016) Generic revision of the ant subfamily Dorylinae (Hymenoptera, Formicidae). ZooKeys 608: 1–280. https://doi.org/https://doi.org/10.3897/zookeys.608.9427
- Boudinot BE (2013) The male genitalia of ants: Musculature, homology, and functional morphology (Hymenoptera, Aculeata, Formicidae). Journal of Hymenoptera Research 30: 29–49. https://doi.org/https://doi.org/10.3897/jhr.30.3535
- Boudinot BE (2015) Contributions to the knowledge of Formicidae (Hymenoptera, Aculeata): A new diagnosis of the family, the first global male-based key to subfamilies, and a treatment of early branching lineages. European Journal of Taxonomy 120(120): 1–62. https://doi.org/https://doi.org/10.5852/ejt.2015.120
- Emery C (1922) Hymenoptera. Fam. Formicidae. Subfam. Myrmicinae. Genera Insectorum 174C: 207–397.
- Fisher BL (2005) A new species of *Discothyrea roger* from Mauritius and a new species of *Proceratium roger* from Madagascar (Hymenoptera: Formicidae). Proceedings of the California Academy of Sciences 56 (35): 657–667.
- Fisher BL (2009) Two new dolichoderine ant genera from Madagascar: Aptinoma gen. n. and Ravavy gen. n. (Hymenoptera: Formicidae). Zootaxa 2118: 37–52. https://doi. org/10.11646/zootaxa.2118.1.3
- Fisher BL, Bolton B (2016) Ants of the world. Ants of Africa and Madagascar. A guide to the genera. University of California Press, Berkeley, 274–276. https://doi. org/10.1525/9780520962996
- Fisher BL, Peeters C (2019) Ants of Madagascar: a guide to the 62 genera. Association Vahatra, Antananarivo, Madagascar, 260 pp.

- Ito F, Luong PTH, Yamane S (2023). Specialized predation on arthropod eggs in the myrmicine ant *Calyptomyrmex rectopilosus* collected in northern Vietnam, with a description of new species of *Calyptomyrmex* from Bogor, West Java, Indonesia. Tropics 32 (2): 65–72. https://doi.org/10.3759/tropics.MS22-08
- Mason WRM (1986) Standard drawing conventions and definitions for venation and other features of wings of Hymenoptera. Proceedings of the Entomological Society of Washington 88: 1–7.
- Nève de Mévergnies T, Carval D, Haran J, Bourel M, Ramage T, Chailleux A (2024) First record of the Argentine ant, *Linepithema humile* (Mayr, 1868) (Hymenoptera: Formicidae), from the Malagasy region. Annales de la Société entomologique de France (N.S.) International Journal of Entomology 60(2): 166–174. https://doi.org/10.1080/ 00379271.2024.2311163
- Ramamonjisoa MM, Rasoamanana N, Fisher BL (2023) Description of the male of *Erromyrma* Bolton & Fisher, 2016 (Hymenoptera, Formicidae). ZooKeys 1163: 61–77. https://doi.org/10.3897/zookeys.1163.95696
- Schmidt CA, Shattuck SO (2014) The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of ponerine ecology and behavior. Zootaxa 3817 (1): 1–242. https://doi.org/10.11646/zootaxa.3817.1.1
- Seifert B (2003) The ant genus Cardiocondyla (Insecta: Hymenoptera: Formicidae) a taxonomic revision of the C. elegans, C. bulgarica, C. batesii, C. nuda, C. shuckardi, C. stambuloffii, C. wroughtonii, C. emeryi, and C. minutior species groups. Annalen des Naturhistorischen Museums in Wien. B, Botanik, Zoologie 104: 203–338.
- Yoshimura M, Fisher BL (2007) A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): Key to subfamilies and treatment of the genera of Ponerinae. Zootaxa 1654(1): 21–40. https://doi.org/10.11646/zootaxa.1654.1.2
- Yoshimura M, Fisher BL (2009) A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): key to genera of the subfamily Proceratiinae. Zootaxa 2216: 1–21. https://doi.org/10.11646/zootaxa.2216.1.1
- Yoshimura M, Fisher BL (2011) A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): key to genera of the subfamily Dolichoderinae. Zootaxa 2794: 1–34. https://doi.org/10.11646/zootaxa.2794.1.1
- Yoshimura M, Fisher BL (2012) A revision of male ants of the Malagasy Amblyoponinae (Hymenoptera: Formicidae) with resurrections of the genera *Stigmatomma* and *Xymmer*. PLoS ONE 7: e33325. https://doi.org/10.1371/journal.pone.0033325